Elevated Levels of Volatile Organic Carcinogen and Toxicant Biomarkers in Chinese Women Who Regularly Cook at Home

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Abstract

Background: Epidemiologic studies associate lung cancer in nonsmoking Chinese women with Chinese-style wok cooking. Our goal was to quantify carcinogen and toxicant biomarkers in Chinese women who reported regularly doing home cooking compared with women randomly selected from the Singapore Chinese Health Study as controls.

Methods: Biomarkers were quantified by high-performance liquid chromatography–mass spectrometry, high-performance liquid chromatography with fluorescence detection, and gas chromatography–mass spectrometry.

Results: Compared with controls, women who engaged in regular home cooking had significantly higher levels of mercapturic acids of acrolein {geometric mean, 1,959 pmol/mg creatinine [95% confidence interval (95% CI), 1,554-2,467] versus 1,370 (95% CI, 1,077-1,742); $P = 0.038$}, crotonaldehyde [geometric mean, 232 pmol/mg creatinine (95% CI, 193-277) versus 142 (95% CI, 118-171); $P = 0.0004$], and benzene [geometric mean, 0.58 pmol/mg creatinine (95% CI, 0.44-0.78) versus 0.18 (95% CI, 0.14-0.24); $P < 0.0001$]. No significant differences were found in levels of mercapturic acids of 1,3-butadiene, metabolites of pyrene and phenanthrene, or acetaldehyde-leukocyte DNA adduct levels between the groups. Levels of the ethylene oxide mercapturic acid were significantly higher in the controls.

Conclusions: The higher levels of the mercapturic acid of benzene, a multiorgan carcinogen, in the women who cooked are particularly notable. Overall, the results showing increased exposure to the volatile toxicants and carcinogens acrolein, crotonaldehyde, and benzene in Chinese women who regularly cook provide a plausible lead for further investigating the role of volatile compounds generated during high-temperature cooking with oils as causes of lung cancer.

Impact: A new direction for research on lung cancer etiology is suggested. *Cancer Epidemiol Biomarkers Prev; 19(5); 1185–92. ©2010 AACR.*

Introduction

Chinese women in Asia have higher rates of lung cancer than in many other places in the world. In China, the age-standardized incidence rate for female lung cancer is 19.9 per 100,000, and in Singapore, it is 17.5, whereas rates are far lower (1.5-9.2) in countries such as Vietnam, Indonesia, India, and Russia (1, 2). Cigarette smoking is definitely not the major risk factor for lung cancer in these women, nor is there strong evidence implicating secondhand smoke exposure (3). In some parts of China, use of solid fuels for heating in poorly ventilated spaces is considered a cause of lung cancer (4). However, a more common cause may be Chinese-style cooking. Multiple epidemiologic studies document an association between lung cancer in Chinese women and Chinese-style cooking with oils heated at high temperatures (3-11). In one recent study, a strong link was observed between lung cancer risk and cooking dish-years, a measure of lifetime cooking by stir frying, deep frying, or frying in a wok, common in Chinese households (3). A working group of the IARC concluded that emissions from high-temperature frying are "probably carcinogenic to humans (Group 2A)" (4).

Several studies have analyzed fumes generated during high-temperature cooking with various oils. A variety of carcinogens and toxicants have been detected in the fumes, including acetaldehyde, acrolein, various higher aldehydes, benzene, 1,3-butadiene, ethylene oxide, and polycyclic aromatic hydrocarbons (PAH; refs. 12-16). Benzene, 1,3-butadiene, ethylene oxide, and a representative

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PAH, benzo[a]pyrene, are considered carcinogenic to humans by IARC, whereas acetaldehyde is classified as possibly carcinogenic to humans (17-21). Acrolein, a well-known toxicant and cilia-toxic agent, is rated as not classifiable for carcinogenicity (22).

There are no reports of biomarker studies to assess carcinogen and toxicant uptake in Chinese women who regularly cook at home. Therefore, in this study, we determined levels of biomarkers of exposure to acrolein, crotonaldehyde, benzene, 1,3-butadiene, ethylene oxide, acetaldehyde, and PAH in nonsmoking, nonalcohol drinking Chinese women from Singapore who reported doing home cooking ≥5 days per week and in Chinese women who were randomly chosen among the participants of the population-based Singapore Chinese Health Study.

Materials and Methods

Study design. Forty-two women volunteers who enrolled in a study on food mutagens (23) constituted one group of "regular cooks" in this study. The subjects were identified using a combination of purposive sampling and "snowballing." Criteria for participation were that they should be Singapore residents, of Chinese ethnicity, nonsmokers, and that they cooked regularly at home ≥5 days a week. All women were asked to consume two to four cups of coffee the preceding afternoon and to abstain from painkillers and chocolate for 2 days to allow measurement of the caffeine metabolic ratio, which was used in a separate study (23). When research staff visited the home, a structured questionnaire was administered and a 20-mL sample of blood was drawn by venipuncture. This sample collected from 42 nonsmoking and nondrinking women was used for the acetaldehyde-leukocyte DNA adduct analysis.

Because the above group had consumed coffee, which we thought could interfere with the urinary biomarker analyses of exposure to PAH, we recruited a second group of women who regularly did wok cooking, and collected single-void spot urine samples at randomly timed points from these women. Using the same recruitment and sampling method as the first group, we recruited 54 women in August 2009. The criteria for the present study were Chinese ethnicity, citizens or permanent residents of Singapore who cooked regularly at home ≥5 days a week, but neither smoked cigarettes nor drank coffee or alcoholic beverages.

As the comparison group (general population controls) for the present study, 50 women were randomly chosen from the subset of nonsmoking, nonalcohol drinking female participants of the Singapore Chinese Health Study, a population-based cohort study of Chinese adults initiated in 1993. The cohort included 63,257 Chinese men and women (representing 85% of eligible subjects) belonging to the two major dialect groups (Cantonese and Hokkien) of Chinese in Singapore. The subjects, who were 45 to 74 years of age and resided in government-built housing estates, were enrolled between April 1, 1993 and December 31, 1998. At the time of recruitment, each subject was interviewed in person by a trained interviewer using a structured questionnaire that requested information on demographics, lifetime use of tobacco, current consumption of alcoholic beverages, current physical activity, menstrual and reproductive histories (women only), occupational exposure, medical history, and family history of cancer. Information about dietary habits during the past 12 months was obtained using a validated food frequency questionnaire (24). We requested blood (or buccal cells if blood donation was refused) and single-void spot urine specimens from a random 3% sample of cohort participants between April 1994 and December 1999. Beginning in January 2000, request for biospecimens was extended to all surviving members of the cohort. During the same time, a follow-up survey conducted by telephone brought up-to-date subjects' histories on use of tobacco and alcohol, medical and medication histories, and, for women, menopausal status and lifetime use of replacement hormones. By April 2005, all surviving cohort subjects had been contacted for biospecimen donation. Samples were obtained from 32,535 participants, representing 61% of eligible subjects. These 50 women were chosen among the 12,600 nonsmoking, nonalcohol drinking women who had donated both blood and urine samples.

Singapore is a small city-state country, located one degree north of the equator and has tropical weather year around. Therefore, there is no need for the use of heating systems in houses or apartments. Windows, especially those in the kitchen, are usually open for ventilation during cooking.

Both buffy coat and urine specimens from each chosen subject were retrieved from storage in −80°C freezers at the National University of Singapore and shipped in boxes packed with dry ice to the University of Minnesota, where the laboratory measurement took place. The Institutional Review Boards at the University of Minnesota and the National University of Singapore approved the study.

Biomarker analyses. Urinary mercapturic acid metabolites of the following were quantified: for acrolein [3-hydroxypropyl mercapturic acid (HPMA)], for crotonaldehyde [4-hydroxybut-2-yl mercapturic acid (HBMA)], for benzene [S-phenyl mercapturic acid (SPMA)], for 1,3-butadiene [1-hydroxy-2-(N-acetylcysteinyl)-3-butene and 1-(N-acetylcysteinyl)-2-hydroxy-3-butene, collectively called monohydroxybutyl mercapturic acid (MHBMA)], and for ethylene oxide [(N-acetylcysteinyl)ethanol, also called 2-hydroxyethyl mercapturic acid (HEMA)]. Urinary 1-hydroxypyrene (1-HOP) and r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), metabolites of the PAH pyrene and phenanthrene, were also quantified. As a measure of exposure to acetaldehyde, leukocyte DNA adducts were measured. The analyses for the mercapturic acids (25), 1-HOP (26), and PheT (27, 28) were carried out essentially as described previously.

The analysis for the acetaldehyde-DNA adduct N^2-ethylidene-dG (as N^2-ethyl-dG) was similar to that described previously (29) but modified for analyzing small amounts of DNA. The buffy coat samples were diluted with PBS buffer (1:3, v/v) to 1 mL and stored in two plastic straws (each with 0.5 mL) in a −80°C freezer until use. DNA isolation was carried out using the Puregene DNA purification protocol (Qiagen) with modifications. Buffy coat samples were thawed at room temperature and then transferred into 15-mL centrifuge tubes, followed by addition of 3.0 mL of RBC lysis solution into each tube. The cell lysate was centrifuged and the supernatant was discarded. The WBC pellet was suspended by vortexing, and 300 μL of cell lysis solution and 1 μL of proteinase K (20 mg/mL) were added to the mixture. One microliter of RNase I (4 mg/mL) was added to the cell lysate on the next day, and the mixture was incubated for 2 hours at room temperature. After adding 250 μL of protein precipitation solution, the mixture was centrifuged to remove protein. DNA was precipitated from the supernatant by adding 750 μL of ice-cold isopropanol. The clumped DNA was washed with 100% ethanol and dried under a stream of N_2. The DNA was stored at −30°C until analysis.

DNA samples were dissolved in 400 μL of 10 mmol/L Tris/5 mmol/L $MgCl_2$ buffer containing [$^{15}N_5$]N^2-ethyl-dG (50 fmol) and $NaBH_3CN$ (10 mg). The pH was adjusted to 7 with 10 μL of 0.1 N HCl, and the DNA was digested overnight at room temperature with 650 units of DNase I (type II, from bovine pancreas). Then, to the resulting mixture were added 650 additional units of DNase I, 0.03 unit of phosphodiesterase I (type II, from *Crotalus adamanteus* venom), and 240 units of alkaline phosphatase. The mixture was incubated at 37°C for 70 minutes and then allowed to stand overnight at room temperature. Enzymes were removed by centrifugation using a Centrifree MPS device (molecular weight cutoff of 30,000; Amicon). The hydrolysate, after removal of a 10 μL aliquot for dG analysis, was desalted and purified using a solid-phase extraction cartridge (Strata-X, 33 μm, 30 mg/1 mL; Phenomenex). The cartridge was washed with H_2O, 10% CH_3OH, and 1 mL of 70% CH_3OH in H_2O. The 70% CH_3OH fraction was collected and evaporated to dryness, dissolved in H_2O, and purified using a mixed mode, anion exchange reversed-phase extraction cartridge (Oasis MAX, 30 mg/cartridge; Waters). The pH of the sample was adjusted to >12 by addition of 20 μL of 0.2 N KOH and applied to the cartridge, which had been equilibrated with 0.2 N KOH. The cartridge was washed with 0.01 N KOH, 0.01 N KOH in CH_3OH, H_2O, 1 mol/L ammonium acetate (pH 6.8), H_2O, and 10% CH_3OH. Adducts were eluted with 70% CH_3OH, and the solution was evaporated to dryness. The residue was dissolved in 20 μL of H_2O and analyzed by liquid chromatography–electrospray ionization–tandem mass spectrometry (MS). We used an Agilent 1100 high-performance liquid chromatography system with a 150 mm × 0.5 mm 4-μm Polar RP column (Phenomenex) and a Discovery Max (Thermoelectron) triple-quadrupole mass spectrometer. Solvent A was 15 mmol/L ammonium acetate and solvent B was methanol. The column temperature was maintained at 50°C. The gradient started with 5% B and increased to 22% B in 20 min and then was ramped to 80% in 5 minutes. The flow rate was 10 μL/min. The column was flushed with 100% B for 10 minutes and then equilibrated under the initial conditions for 15 minutes. The electrospray ionization source was operated in the positive ion mode, and the MS parameters were set as follows: voltage, 3.8 kV; current, 3 μA; and heated ion transfer tube, 275°C. The collision energy was 12 eV and the argon collision gas pressure was 1.0 mTorr.

Quantitation of dG was done by high-performance liquid chromatography using a 250 mm × 0.5 mm 5-μm Luna, C18(2) column (Phenomenex). Solvent A was H_2O and solvent B was CH_3OH. The gradient started with 5% B and increased to 22% B in 20 minutes and then was ramped to 80% B in 5 minutes. The flow rate was 10 μL/min. The column was then equilibrated under the initial conditions for 15 minutes.

Buffer blanks containing internal standard were processed as above and analyzed to check the baseline and possible contamination. Calf thymus DNA (50 μg) with internal standard added as above was used as a positive control to determine interday precision and accuracy. Each set of samples was run together with buffer blanks and positive controls. Of the 42 samples, the assay failed on three samples. Therefore, we reported results of N^2-ethylidene-dG on 39 women cooks only.

Creatinine was assayed by the University of Minnesota Medical Center (Fairview) Diagnostic Laboratories (Minneapolis, MN) using Vitros CREA slides.

Statistical analyses. The χ^2 test and the t test were used to compare the distributions of selected variables between Chinese women who did home cooking ≥5 days per week ("daily cooks") and a random sample of Chinese women from the Singapore Chinese Health Study ("population controls"). Urinary levels of PheT, 1-HOP, and the individual mercapturic acids HPMA, HBMA, SPMA, MHBMA, and HEMA were expressed in units of pmol/mg creatinine to correct for varying water contents of individual spot urine samples, whereas the level of the acetaldehyde-DNA adduct N^2-ethylidene-dG (measured as N^2-ethyl-dG) was in fmol/μmol dG. We also summed all measured mercapturic acid metabolites (i.e., HPMA, HBMA, SPMA, MHBMA, and HEMA) to represent the overall level of exposure to the volatile carcinogens and toxicants through home cooking. The summed values were analyzed in the same way as the individual mercapturic acid biomarkers. In addition, we created another unweighted summed index for the overall level of exposure to reduce the dominant effect of one biomarker with high concentration (i.e., the acrolein metabolite—HPMA) over other biomarkers due to their huge difference in urinary concentrations. For this unweighted index, we first assigned to each of the five mercapturic acid metabolites a rank score to each individual among all women: the smallest rank score

Table 1. Geometric means of biomarker levels in women who did frequent home cooking (daily cooks) versus women randomly selected from the Singapore Chinese Health Study (random controls)

A

Urinary biomarker	Source	Geometric mean, pmol/mg creatinine (N)		P
		Daily cooks	Random controls	
HPMA	Acrolein	1,959 (54)	1,370 (50)	0.038
HBMA	Crotonaldehyde	232 (54)	142 (50)	0.0004
SPMA	Benzene	0.58 (53)	0.18 (50)	<0.0001
MHBMA	1,3-Butadiene	19.3 (48)	20.2 (50)	0.70
HEMA	Ethylene oxide	20.1 (54)	28.3 (50)	0.02
Summed mercapturic acids*	All above	2,476 (47)	1,622 (50)	0.016
1-HOP	Pyrene	1.00 (54)	0.94 (50)	0.56
PheT	Phenanthrene	1.94 (54)	2.40 (50)	0.11

B

Leukocyte DNA biomarker	Source	Geometric mean, pmol/mg creatinine (N)		P
		Daily cooks	Random controls	
N^2-ethylidene-dG	Acetaldehyde	10,996 (39)[†]	15,299 (50)	0.22

NOTE: Adjusted for age at sample collection. The urinary biomarkers and leukocyte DNA biomarkers were obtained from different groups of daily cooks, but the random controls were the same for both (see Materials and Methods).
*The sum of HPMA, HBMA, SPMA, MHBMA, and HEMA.
[†]Three samples could not be analyzed due to technical problems.

(i.e., 1) was assigned to an individual with the lowest concentration of a given biomarker, whereas the largest score was assigned to the one with the highest concentration of the biomarker. We then added the rank scores over the five mercapturic acid metabolites for a given individual, which gave an equal weight to each of the five mercapturic acid metabolites regardless of their actual values. The distributions of all biomarkers measured were markedly skewed toward high values, which were corrected to a large extent by transformation to logarithmic values. Therefore, formal statistical testing was done on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented. The analysis of covariance method (30) was used to examine the difference in biomarker levels between the daily cooks and the population controls with age as a covariate, whereas the difference in unweighted summed indices between the two groups was examined using the Mann-Whitney test (30). We further assessed the association between home cooking and elevated biomarker levels by means of the odds ratio (OR) and its corresponding 95% confidence interval (95% CI) and P value using the logistic regression method (31). Women were grouped into tertiles according to the distribution of a given biomarker or summed indices among the population controls, except for HBMA and SPMA. The cutoffs of tertiles for these

two biomarkers were based on their distributions of both cooks and population controls to have sufficient number of subjects in each tertile for the calculation of OR because cooks had much higher levels of HBMA and SPMA in their urine. The lowest tertile level was defined as the reference category (OR, 1.0). For a given biomarker, elevated ORs (i.e., >1.0) associated with the second and third tertiles would indicate that women who cooked daily were more likely to exhibit high levels of the biomarker relative to their general population counterparts.

Statistical analyses were carried out using SAS software version 9.1 (SAS Institute). All P values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

Results

The mean age (±SD) of the 54 cooking women who provided samples for the urinary biomarkers was 62.1 (±8.4) years. The corresponding figure for the 50 general population controls was 61.8 (±7.8) years. The mean body mass indices of these daily cooking and control women were 23.7 (±3.5) and 23.6 (±3.3), respectively. These daily cooks had a higher level of education (42.6% with secondary school or higher) than the random control women (30.0% with secondary school or higher). The mean age

of the 39 women cooks who gave blood samples was 51.7 (±10.3) years, and their mean body mass index was 24.8 ± 4.4. The mean body mass index was comparable with the comparison women, whereas the mean age was younger than the control group. They had a higher level of education (64.3% with secondary school or higher) than the randomly selected control women.

The results of the biomarker analyses, adjusted for age at sample collection, are summarized in Table 1. Statistically significant higher levels of HPMA from acrolein [geometric mean, 1,959 pmol/mg creatinine (95% CI, 1,554-2,467) versus 1,370 (95% CI, 1,077-1,742); $P = 0.038$], crotonaldehyde [geometric mean, 232 pmol/mg creatinine (95% CI, 193-277) versus 142 (95% CI, 118-171); $P = 0.0004$], and benzene [geometric mean, 0.58 pmol/mg creatinine (95% CI, 0.44-0.78) versus 0.18 (95% CI, 0.14-0.24); $P < 0.0001$] were observed in the urine of the daily cooks compared with the random controls.

Women who did frequent home cooking also showed statistically significantly elevated levels of summed mercapturic acid metabolites [geometric mean, 2,476 pmol/mg creatinine (95% CI, 1,940-3,158) versus 1,622 pmol/mg creatinine (95% CI, 1,281-2,053); $P = 0.016$] and elevated summed rank scores (median score, 284 versus 224; $P = 0.002$). There were no differences in levels of the urinary biomarkers MHBMA from 1,3-butadiene, 1-HOP from pyrene, PheT from phenanthrene, or N^2-ethylidene-dG from acetaldehyde. Levels of urinary HEMA from ethylene oxide were significantly lower in the daily cooks than in the random controls [20.1 pmol/mg creatinine (95% CI, 18.4-24.7) versus 28.3 (95% CI, 23.1-34.7); $P = 0.02$].

The data are presented by tertiles of the study variables in Table 2. Statistically significant trends of increasing biomarker levels with daily cooking were observed for all of the biomarkers for which significant differences were seen in Table 1. Relative to the general population controls,

Table 2. ORs (95% CIs) of women who did frequent home cooking relative to women in a random sample of the Singapore Chinese Health Study associated with elevated biomarker levels by tertiles

A

Urinary biomarker	Source	OR (95% CI), no. cooks/no. controls			P for trend
		1st tertile	2nd tertile	3rd tertile	
HPMA	Acrolein	1.00 (reference) (7/17)	3.26 (1.10-9.64) (23/17)	3.65 (1.23-10.79) (24/16)	0.028
HBMA*	Crotonaldehyde	1.00 (reference) (5/30)	13.51 (4.02-45.43) (24/12)	21.60 (6.06-77.00) (26/8)	<0.0001
SPMA*	Benzene	1.00 (reference) (10/25)	2.48 (0.91-6.75) (17/18)	11.10 (3.48-35.41) (27/7)	<0.0001
MHBMA	1,3-Butadiene	1.00 (reference) (13/17)	1.87 (0.71-4.96) (24/17)	0.90 (0.31-2.58) (11/16)	0.89
HEMA	Ethylene oxide	1.00 (reference) (29/17)	0.45 (0.18-1.14) (13/17)	0.43 (0.16-1.13) (12/16)	0.066
Summed mercapturic acids†	All above	1.00 (reference) (6/17)	3.15 (1.00-9.86) (19/17)	3.90 (1.26-12.11) (22/16)	0.024
1-HOP	Pyrene	1.00 (reference) (19/17)	0.69 (0.26-1.82) (13/17)	1.23 (0.49-3.08) (22/16)	0.65
PheT	Phenanthrene	1.00 (reference) (24/17)	0.96 (0.39-2.40) (23/17)	0.31 (0.10-0.92) (7/16)	0.051

B

Leukocyte DNA biomarker	Source	OR (95% CI), no. cooks/no. controls			P
		1st tertile (cooking/random)	2nd tertile (cooking/random)	3rd tertile (cooking/random)	
N^2-ethylidene-dG	Acetaldehyde	1.00 (reference) (13/17)	1.54 (0.48-5.01) (16/17)	0.82 (0.24-2.82) (10/16)	0.78

NOTE: Adjusted for age at sample collection.

*The tertile cutoff values were based on both cooks and controls combined. For all other biomarkers, the tertile cutoff values were based on control subjects only.

†The sum of HPMA, HBMA, SPMA, MHBMA, and HEMA.

daily cooks were significantly more likely to fall into the highest tertile category of urinary HPMA, HBMA, and SPMA. The association between the summed values or rank scores of all five mercapturic acid metabolites and frequent home cooking also was statistically significant. When compared with the lowest tertile of summed rank score, ORs (95% CIs) for the second and third tertile of summed rank scores associated with doing frequent home cooking were 5.91 (1.41-24.7) and 9.75 (2.45-38.91), respectively (P for trend = 0.001). A slightly inverse relationship was observed for PheT, and none of the other biomarkers was significantly associated with cooking status.

Discussion

The results of this study provide a plausible lead for understanding epidemiologic studies that show a higher risk of lung cancer in nonsmoking women who regularly cook with high-temperature oils (3-11). Significantly higher levels of mercapturic acid biomarkers of the volatile organic carcinogens and toxicants acrolein, crotonaldehyde, and benzene were observed in the women who cooked compared with controls. These results provide an initial biochemical link between previous studies showing the presence of these compounds in cooking oil fumes (12-16) and the epidemiologic studies of lung cancer. Many other volatile organic compounds, some of which may have toxic properties, have been detected in cooking oil fumes (13, 14), and our results suggest that uptake of these volatiles might also be increased during wok cooking, although biomarkers for most of them are not available.

Our finding of elevated levels of SPMA, a biomarker of benzene uptake, in the urine of women who cooked is particularly interesting. Levels of this biomarker were approximately three times greater in the women who cooked than in controls. Benzene is considered carcinogenic to humans by the U.S. Department of Health and Human Services and by the IARC (32, 33). The most common finding in epidemiologic studies of benzene exposure is increased risk for leukemia, and benzene is considered a known human leukemogen. However, one large study of industrial exposure carried out in China showed an increased risk for lung cancer in those exposed to benzene (34). Benzene is a multipotent carcinogen in laboratory animals, inducing a variety of types of tumors, including lung tumors (32, 33).

We also found elevated levels of HPMA and HBMA, biomarkers of uptake of acrolein and its homologue crotonaldehyde, respectively. Both acrolein and crotonaldehyde are weak carcinogens, and neither is known to target the lung (22, 35). However, acrolein is cilia toxic and thus may impede the clearance of toxicants and other foreign substances from the lung (36). Acrolein is highly reactive with proteins and may be involved in inflammation (37, 38). Both acrolein and crotonaldehyde are products of oxidative damage, and both are known to react with DNA, producing adducts, some of which have miscoding properties (39-42). These adducts have

been detected in various *in vitro* systems and in the human lung (41, 43, 44). There is a background level of acrolein and crotonaldehyde-DNA adducts in humans, which may be related to oxidative damage and lipid peroxidation (41, 43, 44). Our results suggest that such damage may be exacerbated by exposure to fumes from wok cooking, and this should be examined in future studies. Acrolein-DNA adducts have also been associated with mutations in the *p53* tumor suppressor gene, as seen in lung cancer (45). Collectively, these results suggest that the increased exposure to acrolein and crotonaldehyde observed here may play some role in lung cancer induction associated with wok cooking.

The increases in levels of mercapturic acids of benzene, acrolein, and crotonaldehyde observed in this study were not nearly as great as seen in smokers (25). In a recent study, we found that levels of these mercapturic acids decreased by 7- to 8-fold 3 days after smokers stopped smoking to levels that were generally consistent with those observed in the nonsmoking women in our study (25). Cigarette smoking provides a far greater exposure to these compounds than does wok cooking, so the smaller increases seen in this study are fully plausible (14, 25). The high biomarker levels seen in smokers are consistent with the known amounts of benzene, acrolein, and crotonaldehyde in cigarette smoke and with the far higher risk for lung cancer from smoking compared with indoor fume exposure.

We did not observe increases in biomarkers of exposure to 1,3-butadiene or PAH. Levels of 1,3-butadiene in fumes from heated oils are far lower than those of several other volatile compounds such as acrolein (14). Therefore, this result is not unexpected. Although PAHs have been identified in cooking oil fumes (12), they are high molecular weight, nonvolatile compounds. Thus, their uptake under normal cooking circumstances would be less likely than that of the more volatile compounds such as benzene, acrolein, and crotonaldehyde.

Leukocyte N^2-ethylidene-dG is a biomarker of acetaldehyde exposure, which is moderately increased by both cigarette smoking and alcohol consumption (29, 46). However, all subjects analyzed to date have appreciable levels of this biomarker even if they do not smoke or drink because there are multiple sources of acetaldehyde exposure. The average levels of N^2-ethylidene-dG observed here were considerably higher than in our previous studies (29, 46), which may have diminished our ability to detect a change in this biomarker due to exposure to acetaldehyde, a known constituent of cooking fumes. The basis for the high DNA adduct levels observed here requires further investigation. One possibility is exposure from vegetation fires in Indonesia, which may release volatiles that were carried to Singapore by prevailing winds.

One curious finding was the lower level of HEMA, a biomarker of ethylene oxide exposure, in the women who cooked than in controls. Ethylene oxide has been identified as a constituent of cooking fumes (15). However,

it could also be formed endogenously by oxidation of ethylene (47). The endogenous formation of ethylene oxide could lead to considerable variation in HEMA levels in a relatively small study such as ours. Further studies are required to investigate this finding.

One previous study carried out in Chinese restaurant workers examined the effects on biomarkers of exposure to cooking oil fumes (16). Pan et al. found that urinary levels of 1-HOP and 8-hydroxy-dG, a biomarker of oxidative damage, were significantly higher in kitchen staff than in service staff. They concluded that oxidative DNA damage was associated with exposure of Chinese restaurant workers to cooking oil fumes and also observed that the response was greater in female than in male restaurant workers.

There are some limitations to this study. First, the size was quite small, with only 42 to 54 exposed subjects and 50 general population controls. Second, we do not have information on the cooking habits of the general population controls. Third, we do not know the exact half-lives of the biomarkers studied. Therefore, a spot urine collected at randomly timed points would not be optimal for the assessment of biomarkers with short half-lives. In spite of these limitations, we observed significant differences in three carcinogen and toxicant biomarkers, which supports the hypothesis that exposure to cooking fumes is a cause of lung cancer in Chinese women.

In summary, the results of this study show elevated levels of volatile organic compound biomarkers in Chinese women who regularly cook at home. These biomarkers reflect increased exposure to volatile carcinogens and toxicants such as benzene, acrolein, and crotonaldehyde. Although larger studies are needed to confirm these results, the present data, together with multiple epidemiologic studies, show the urgent need for preventive measures, such as improved ventilation, to efficiently remove cooking oil fumes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Correction: Elevated Levels of Volatile Organic Carcinogen and Toxicant Biomarkers in Chinese Women Who Regularly Cook at Home

In this article (Cancer Epidemiol Biomarkers Prev 2010;19:1185–92), which was published in the May 2010 issue of *Cancer Epidemiology, Biomarkers & Prevention* (1), the authors apologize for an error in which standard curve values from a previous study were accidentally and inappropriately applied to the present one. Therefore, some of the values in Table 1 are incorrect. The corrected Table 1 is shown below, with revised values for HBMA, MHBMA, HEMA, and summed mercapturic acids. The statistical analysis and conclusions of the article are unchanged.

Table 1. Geometric means of biomarker levels in women who did frequent home cooking (daily cooks) versus women randomly selected from the Singapore Chinese Health Study (random controls)

A. Urinary biomarker	Source	Geometric mean (N), pmol/mg creatinine		*P*
		Daily cooks	Random controls	
HPMA	Acrolein	1,959 (54)	1,370 (50)	0.038
HBMA	Crotonaldehyde	1,158 (54)	711 (50)	0.0004
SPMA	Benzene	0.58 (53)	0.18 (50)	<0.0001
MHBMA	1,3-Butadiene	9.66 (48)	10.1 (50)	0.70
HEMA	Ethylene oxide	10.1 (54)	14.2 (50)	0.02
Summed mercapturic acids[a]	All above	3,630 (47)	2,233 (50)	0.005
1-HOP	Pyrene	1.00 (54)	0.94 (50)	0.56
PheT	Phenanthrene	1.94 (54)	2.40 (50)	0.11

B. Leukocyte DNA biomarker	Source	fmol/μmol dG		*P*
		Daily cooks	Random controls	
*N*2-Ethylidene-dG	Acetaldehyde	10,996 (39)[b]	15,299 (50)	0.22

NOTE: Adjusted for age at sample collection. The urinary biomarkers and leukocyte DNA biomarkers were obtained from different groups of daily cooks but the random controls were the same for both (see Materials and Methods).
[a]The sum of HPMA, HBMA, SPMA, MHBMA, and HEMA.
[b]Three samples could not be analyzed because of technical problems.

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1. Hecht SS, Seow A, Wang M, Wang R, Meng L, Koh W, et al. Elevated levels of volatile organic carcinogen and toxicant biomarkers in Chinese women who regularly cook at home. Cancer Epidemiol Biomarkers Prev 2010;19:1185–92.

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