Urinary Biomarkers in Charcoal Workers Exposed to Wood Smoke in Bahia State, Brazil

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Abstract

Charcoal is an important source of energy for domestic and industrial use in many countries. Brazil is the largest producer of charcoal in the world, with ~350,000 workers linked to the production and transportation of charcoal. To evaluate the occupational exposure to wood smoke and potential genotoxic effects on workers in charcoal production, we studied urinary mutagenicity in *Salmonella* YG1041 +S9 and urinary levels of 2-naphthol and 1-pyrenol in 154 workers of northeastern Bahia. Workers were classified into three categories according to their working location, and information about sociodemographic data, diet, alcohol consumption, and smoking was obtained using a standard questionnaire. Spot urine samples were collected to evaluate urinary mutagenicity and urinary metabolites. Urinary mutagenicity increased significantly with exposure to wood smoke and was modified by smoking. The prevalence odds ratio was 5.31, and the 95% confidence interval was 1.85; 15.27 for urinary mutagenicity in the highly exposed group relative to the nonexposed group. The levels of urinary metabolites increased monotonically with wood smoke exposure and were associated with the *GSTM1* null genotype, which was determined previously. The prevalence odds ratio (95% confidence interval) for higher levels of 2-naphtol among the highly exposed was 17.13 (6.91; 42.44) and for 1-hydroxyprene 11.55 (5.32; 25.08) when compared with nonexposed workers. Urinary 2-naphthol was the most sensitive indicator of wood smoke exposure. This is the first reported measurement of internal exposure to wood smoke among charcoal workers, and the results showed that these workers receive a systemic exposure to genotoxic compounds. (Cancer Epidemiol Biomarkers Prev 2004;13(6):1005–12)

Introduction

Approximately 40,000 million tons of charcoal are produced annually in the world (1). In many sub-Saharan countries, the charcoal is destined mainly for domestic use (2). In Brazil as well as in Malaysia, Zambia, and the Philippines, charcoal is used mainly for industrial purposes (1, 3). With more than 12 million tons produced per year, Brazil is the world's largest charcoal producer and is responsible for ~30% of the world's production. Approximately 350,000 workers are linked to the production and transportation of charcoal in Brazil (4).

Numerous iron and steel industries in Brazil depend on charcoal for their production (4). In most regions of the country, industrial-scale charcoal production is a poorly mechanized process based on workers' empirical knowledge. The production process has been described in detail (5). Briefly, the charcoal-making process consists of two main activities: tree cutting and carbonization of wood in kilns. Trees are cut down, divided into logs of appropriate size for the kilns, left to dry, and later transported to the carbonization area. After a 4- to 7-day combustion process, charcoal is removed manually from the kiln and piled up nearby. Later, trucks are loaded to bring the product to iron or steel industries. The process goes on year-round, and the working conditions are such that workers are exposed to wood smoke and charcoal dust during this process.

Previous studies of wood burned in experimental conditions have found a variety of chemical classes in the emissions, including benzene, toluene, naphthalenes, substituted naphthalenes, and oxygenated monoaromatics (6-10). In addition to benzene and naphthalene, many of the heavier polycyclic aromatic hydrocarbons (PAH), which are present in small amounts, are suspected to be carcinogenic/mutagenic to humans (11). Pimenta et al. (12) showed the mutagenicity of some of the wood smoke fractions. However, there are only a few studies on the health of workers engaged in charcoal production (13-15), and none have evaluated molecular end points about exposure to wood smoke among charcoal workers.

To characterize the occupational exposure to wood smoke and potential health effects on workers engaged in

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charcoal production, we examined urinary mutagenicity and urinary elimination of 2-naphthol (2-NAP) and 1-pyrenol (1-OHP), of which the parent compounds, naphthalene and pyrene, have been found in wood smoke (6). These biomarkers have been used previously to characterize occupational exposures to other complex mixtures (16-20). The urinary mutagenicity assay provides a measure of exposure to a mixture of mutagenic compounds present in the environment, whereas urinary metabolite analysis indicates exposure to a specific chemical or a class of compounds. In addition, we compared the results to factors determined previously (5) that might influence the levels of these biomarkers, including genetic polymorphisms of glutathione *S*-transferase (*GST*) and cytochrome P450 (*CYP1A1*), diet, and smoking.

Methods

Subjects. This study received the approval of the Institutional Review Board of the University of North Carolina, School of Public Health, the Bioethics Committee of the Centro Estadual de Oncologia in Brasil, and the Human Subjects Research Review Official of the U.S. Environmental Protection Agency. Subjects provided informed consent. In the northeastern region of Bahia, Brazil, inspections by the Ministry of Labor and Employment identified 11 charcoal companies with a total of 400 workers in the year 2000 (21). During the period of August 2001 to June 2002, we visited eight of these companies and identified 250 workers based on information provided by company supervisors. From them, 169 were contacted, and 162 agreed to participate after a clarification session and signed the informed consent. However, some were not able to provide all the biological specimens; the collaboration frequency was 95.1% for first-time urine samples. There is no reason to think that the participants were different from the workers at any other companies that were not part of the study in terms of exposure to wood smoke, age, ethnicity, educational level, salary, eating and drinking habits, or health status.

Information about socio-demographic data, occupational history, health status, smoking, and drinking habits as well as perception of occupational risk factors was obtained by interview as described previously (5). Briefly, the cohort consisted entirely of males, ranging from 19 to 65 years, with a mean ± SD of 34.05 ± 10.47 years. Half of them were nonsmokers, and only 10 workers (7%) smoked more than 10 cigarettes/day. Only 9% reported being nondrinkers (Table 1). The average alcohol ingestion per day for a month was 19.26 ± 23.89 g ethanol/day. Their diet consisted mostly of sweet coffee, bread or crackers, beans cooked with meat, rice or spaghetti, and cassava meal. Cooked or fried salted beef and stewed chicken were the types of meat reported most frequently. Fresh vegetables and fruit were not often included in their meals.

Definition of Exposure to Wood Smoke. Jobs with similar tasks were grouped together and classified further into three categories about exposure to wood smoke: no-, low-, and high-exposure subgroups. Workers who executed their tasks at woodcutting sites (lumberjacks, helpers, log carriers) were not exposed to wood smoke generated in the kilns. Tractor or truck drivers and helpers might have had peak exposures to wood smoke while unloading logs from trucks or tractors in the kiln area. They were classified along log carriers and lumberjacks that worked in the kiln area, as well as bricklayers in the intermediate group. Kiln workers comprised the highest exposed group; they inhaled smoke coming from the surroundings while loading a kiln (Fig. 1). They also inhaled smoke while carrying the charcoal out of the kilns. Although the exposure to wood smoke might have varied, kiln workers spent all their work hours close to the kilns and part of their time covered with charcoal dust. All tasks were conducted outdoors with the exception of the period when workers unloaded kilns, which was a semi-confined space.

As we showed previously (5), occupational exposure to wood smoke was not significantly associated with age, smoking or alcohol consumption, meat intake, body mass index, enzyme polymorphisms, or with the fact that workers slept at work (Table 1). A preliminary quantitative assessment of the exposure of kiln workers (n = 6) showed concentrations of 11.50 (1.54) $\mu g/m^3$ for naphthalene geometric mean (geometric SD) in the gaseous phase and 0.81 (1.61) $\mu g/m^3$ for pyrene (5). The average naphthalene/pyrene ratio in these samples was 14.4. Also as part of the preliminary tests, material collected at work sites was assayed for mutagenicity. Tests with strains TA98, TA100, YG1041, and YG1042 of *Salmonella* were negative for the organic material extracted from charcoal, *Eucalyptus* wood, and charcoal kiln residue samples.[6]

Collection and Processing of Urine Samples. Urine samples were collected after the third day of the workweek. Some workers were not able to donate enough volume of urine to be used in all analyses, yielding 156 samples for first-time metabolite analyses and 133 for mutagenicity testing. There was no indication that the workers who did not provide samples differed from those who did with respect to exposure status. From a subgroup of workers, more than one spot urine sample was obtained so that the variability due to the exposure and the metabolism of the subject was taken into account during analysis. The number of samples obtained was not related to exposure status, smoking, or age. Two samples were obtained from 57 workers, 3 samples from 31 subjects, and 4 replicates from 13 individuals, totaling 265 samples. Samples were kept in the dark at −20°C until processed.

Preliminary experiments were done with pooled urine samples from exposed workers to evaluate the effects of enzymatic or acid hydrolyses as well as a fractionation procedure on urinary mutagenicity. In the absence of hydrolysis, the organics were extracted as described (16). Briefly, urine was passed through washed C18 resin columns (M. Baker, Phillipsburg, NJ) at 25 ml of urine/column; the organics were eluted with methanol.

The acid hydrolysis has been described previously (22). Briefly, 5 mL of 6 mol/L HCl were added per 30 ml of urine and incubated at 70°C for 6 hours. This hydrolysate was then neutralized by the addition of 6 mol/L NaOH and $NaHCO_3$, and the organics were extracted as described by C18/methanol. An enzymatic hydrolysis was done by adding 5 ml of acetate buffer (pH 5.0) per

[6] Umbuzeiro G, Rego MAV, Kato M, Unpublished observations.

Table 1. Prevalence of some characteristics among charcoal workers in Bahia (2001-2002) according to wood smoke exposure

Characteristic*	No exposure *N* (%)	Low exposure *N* (%)	High exposure *N* (%)	Total *N* (%)
Older than 32 years of age	19 (46.3)	24 (41.4)	32 (59.3)	75 (49.0)
CYP1A1 positive genotype	10 (29.4)	26 (50.52)	23 (50.0)	59 (45.4)
GSTM1 null genotype	13 (38.2)	18 (36.7)	13 (28.3)	44 (34.1)
GSTT1 null genotype	8 (23.5)	17 (34.7)	7 (15.2)	32 (24.8)
Body mass index >25	5 (13.5)	8 (14.6)	3 (5.56)	16 (11.0)
Sleep at work site	22 (55.0)	39 (67.2)	35 (64.8)	96 (63.2)
Smokers	17 (42.5)	29 (50.9)	29 (54.7)	75 (50.0)
No alcohol drinkers	4 (10.0)	5 (8.9)	4 (7.8)	13 (8.9)
No meat yesterday	1 (2.5)	3 (5.2)	4 (7.4)	8 (5.3)

Abbreviations: GM, geometric mean; GSD, geometric standard deviation.
*Differences among groups were not significantly different ($P > 0.05$); data from ref. (5).

25 ml of urine as well as 20 units of β-glucuronidase (Sigma Chemical Co., St. Louis, MO) and 5 units of sulfatase (Sigma) per milliliter of urine, and incubating for 16 hours at 37°C. The organics were then eluted as described by C18/methanol. These extracts were suitable both for urinary mutagenicity (18) and metabolite analysis (23, 24). For use in the mutagenicity assay, the organics were solvent exchanged under a stream of nitrogen into DMSO at a concentration of 150×.

Urinary Concentrations of 2-NAP, 1-Hydroxyprene, and Creatinine. Determination of the major hydroxy-metabolites of pyrene and naphthalene, 1-OHP (Aldrich Chemical Co., Milwaukee, WI), and 2-NAP (Aldrich Chemical), respectively, was done using a Waters high-performance liquid chromatograph (Millipore, Milford, MA) equipped with an auto-sampler, a gradient pump, a fluorescence detector, and integration software. The method was adapted from Hollender et al. (23) and Kim et al. (24). C18/methanol extracts corresponding to 20 ml of urine were concentrated to 2 mL under a gentle nitrogen stream, filtered, and stored at −20°C until analysis. An aliquot of 50 μL of each sample was injected in a 150-mm reverse-phase column under a programmed acetonitrile/water mobile-phase flow, and concentration was measured by a fluorescence detector. The gradient of acetonitrile/water changed from 5:95 to 95:5 gradually over 65 minutes, ending with a 15-min equilibration phase. The flow rate was 1 mL/minute. The retention time was 29.88 minutes for 2-NAP and 50.03 minutes for 1-OHP. The calibration was conducted with methanol standards, and the linear calibration curve range was 0.35 to 17.80 μg/mL for 2-NAP and 1.20 to 60.65 ng/mL for 1-OHP. The excitation/emission wavelengths for 2-NAP were 227/355 and 212/388 nm for 1-OHP. The limit of detection of the method was 0.02 μg/mL for 2-NAP and 0.01 μg/mL for 1-OHP. The average coefficient of variation of the analysis ($n = 5$), including sample preparation with the C18 column, was 8.6% for 1-NAP and 12.9% for 1-OHP.

Urinary Mutagenicity. Urinary mutagenicity was assessed with the *Salmonella* plate-incorporation mutagenicity assay (25). Preliminary experiments were done with pooled urine samples from exposed workers to identify the most sensitive strain of *Salmonella*. The strains tested were the frameshift strains TA98 (*hisD3052*, *rfa*, Δ*uvrB*, pKM101) and its homologue YG1041, which contains acetyltransferase and nitroreductase activities (26), as well as the base-substitution strain TA100 (*hisG46*, *rfa*, Δ*uvrB*, pKM101) and its homologue YG1042. These preliminary experiments were done without and with Aroclor 1254-induced Sprague-Dawley rat liver S9 (Moltox, Boone, NC) mix at 1 mg of S9 protein/plate. Doses of urine extract

Figure 1. Charcoal kiln being loaded showing wood smoke emissions by neighboring kilns.

were 0.3 to 15 mL-equivalents (eq) per plate. Plates were incubated for 3 days, and revertants (rev) were counted with an automatic colony counter.

Mutagenic potencies (rev/mL-eq) were calculated from the linear portion of the dose-response curves and were adjusted for creatinine (rev/mol creatinine) to compensate for variations in urinary flow (16). Creatinine was determined using a kit (Sigma Diagnostics, St. Louis, MO) that was based on a colorimetric method developed by Heinegard and Tiderstrom (27). Cytotoxicity was assessed by noting the lowest mL-eq/plate that caused a reduction in the number of rev/plate relative to the previous doses or the control values.

Statistical Analysis. The main dependent variables, urinary mutagenicity and metabolite levels, were adjusted for creatinine excretion, and the values were log-transformed to reduce dispersion; their distribution parameters were expressed as the geometric mean and the geometric SD. The comparison between continuous variables was conducted with generalized linear models and mixed models. These variables also were dichotomized in log-scale for the estimation of prevalence odds ratios (POR). The PORs of having levels higher than the median for urinary mutagenicity were estimated using unconditional logistic regression. For the metabolites, a generalized estimating equation method was used to account for the repeated samples. Statistical significance was defined if the *P* value was less than 5% or if the 95% confidence interval (95% CI) did not include the null value.

Potential confounders of the urinary markers, represented by age, smoking behavior, alcohol consumption, meat intake, genetic polymorphisms, obesity, and diet, were assessed by stratification using categorical analysis (Mantel-Haenszel or Cochran-Mantel-Haenszel tests) and multivariate modeling. Statistical analyses were conducted using SAS software version 8 (SAS Institute, Cary, NC).

Results

Urinary 2-NAP and 1-OHP. Ninety percent of the urinary levels of 2-NAP and 1-OHP were between 0.07 and 53.20 μmol/mol creatinine and 0.00 and 0.46 μmol/mol creatinine, respectively. Samples with the highest 5% of the 2-NAP values belonged to exposed workers. However, both exposed and nonexposed workers provided urine samples with the highest 5% of the 1-OHP values. Levels of 2-NAP and 1-OHP were not detectable in 8 (3.2%) and in 11 (4.4%) of 253 and 250 samples, respectively, and these were considered the limits of detection for statistical analysis. Geometric mean values of urinary 2-NAP and 1-OHP both increased monotonically with the level of exposure to wood smoke and with smoking (Table 2). The linear correlation between the log-transformed urinary levels of 2-NAP and 1-OHP was moderate, with a Pearson correlation coefficient of 0.53 ($P < 0.01$) for the study population and 0.59 ($P < 0.01$) if only nonsmokers were considered.

In multivariate analysis (Table 3), the POR for having high excretion levels of 2-NAP and 1-OHP increased substantially with exposure to wood smoke and with *GSTM1* null genotype, which was determined previously (5). The effect of wood smoke exposure was more evident at lower levels among nonsmokers.

Urinary Mutagenicity. Preliminary experiments showed that the urine extracts from exposed workers were more mutagenic in the YG strains than in the TA strains (data not shown), which indicated the presence of nitroarenes and/or aromatic amines in the urine (26). In a test with pooled samples of exposed workers conducted with YG1041 and YG1042, the addition of S9 increased the response, and both strains yielded similar results, with YG1041 giving somewhat higher results (data not shown). This suggested that the urine extracts contained more frameshift- than base substitution–type mutagenic activity.

Preliminary experiments with pooled urine extracts that were not hydrolyzed yielded lower mutagenic responses than the acid-hydrolyzed samples; practically no difference was observed between acid hydrolysis and enzymatic hydrolysis (data not shown). This indicated that the hydrolysis procedures likely deconjugated mutagens that were then detected in the assay. None of the samples that underwent acid or alkaline extraction were toxic below 12 mL-eq/plate, whereas samples without this extraction presented toxicity at 3 mL-eq/plate (data not shown). Thus, acid as well as basic cytotoxins were present in the urine.

A pooled sample from exposed smokers was more mutagenic than samples from nonsmokers or nonexposed

Table 2. Geometric mean and geometric standard deviation values for urinary 2-NAP and 1-OHP levels (μmol/mol creatinine) stratified by exposure to wood smoke and smoking

Exposure category	2-NAP		1-OHP	
	N	GM (GSD) (μmol/mol creatinine)	*N*	GM (GSD) (μmol/mol creatinine)
All	253	4.01 (1.55)	250	0.07 (1.14)
Wood smoke				
Nonexposed	67	1.35 (1.33)*	66	0.03 (1.32)†
Low	86	4.51 (1.23)	84	0.06 (1.19)
High	100	7.17 (1.24)	100	0.13 (1.15)
Cigarette				
Nonsmokers	126	2.80 (1.24)*	124	0.04 (1.28)*
Smokers	116	5.17 (1.22)	115	0.08 (1.23)
Nonexposed and nonsmokers	43	0.91 (1.31)	43	0.01 (1.34)

*$P < 0.05$ for difference among means.
†$P = 0.07$ for difference among means.

Table 3. POR (95% CI) for having high urinary 2-NAP and 1-OHP levels for exposure to wood smoke and *GSTM1* genotype

Variable	2-NAP		1-OHP	
	All (N = 209)	Nonsmokers (n = 126)	All (N = 209)	Nonsmokers (n = 126)
No exposure to wood smoke	1.00	1.00	1.00	1.00
Low exposure to wood smoke	4.60 (1.80-11.77)	10.17 (3.00-34.50)	3.67 (31.74-7.73)	6.79 (2.16-21.38)
High exposure to wood smoke	17.13 (6.91-42.44)	17.35 (5.43-55.45)	11.55 (5.32-25.08)	12.36 (4.58-33.35)
GSTM1 positive	1.00	1.00	1.00	1.00
GSTM1 null	2.59 (1.24-.41)	1.57 (0.56-4.37)	2.84 (1.60-5.02)	2.30 (1.08-4.90)

workers either hydrolyzed or nonhydrolyzed (data not shown). From the smoker/exposed pool, samples that did not undergo solvent-solvent extraction and the acid-extracted samples had mutagenicity levels almost 2 times greater than those that were alkaline-extracted (data not shown). Thus, there were acidic mutagens present in the smoker/exposed pooled sample.

Before firm conclusions could be drawn from these preliminary tests with pooled urine samples, additional studies would be required using more samples. Nonetheless, these studies indicated that the most sensitive extraction/strain combination was the C18/methnol extraction of enzymatically hydrolyzed urine combined with strain YG1041 in the presence of S9. Thus, all the samples were processed and tested in this manner.

Using this method, crude urinary mutagenicity levels ranged from 0 to 276.2 rev/mL-eq. The adjusted values varied from 0 to 30.6 rev/μmol creatinine, with the overall geometric mean (geometric SD) of 4.5 (2.8) rev/μmol creatinine (Table 4). Differences in mutagenicity mean values within wood smoke exposure strata as well as between smoking categories were statistically different. The average urine mutagenicity values differed significantly between dichotomized smoking categories, but the difference between wood smoke exposure categories was significant only for the nonsmoker group (Fig. 2). Urinary mutagenicity levels as well as the PORs for having higher mutagenicity levels increased with exposure to wood smoke (Table 4).

Only seven samples showed no mutagenicity, and these belonged to nonsmokers. No effect of the *GST* or *CYP1A1* polymorphisms, determined previously (5), on urinary mutagenicity was observed. The odds of having urine that was toxic to *Salmonella* were associated with exposure to wood smoke; the POR was 1.50, and the 95% CI was 0.75 to 3.15 for the low-exposure group and was 3.95, 95% CI = 1.73-9.02 for the high-exposure group in relation to nonexposed (results not shown). The toxicity was not associated with factors evaluated previously (5), such as smoking or alcohol consumption, genetic polymorphisms, or meat intake on the day before sampling (results not shown).

Table 4. Geometric mean and POR with 95% CI for urinary mutagenicity (rev/μmol creatinine in *Salmonella* YG1041 +S9) stratified by exposure to wood smoke and smoking

Variable	N	GM (95% CI)	POR (95% CI)
All	132	2.82 (2.37-3.50)	—
Wood smoke*			
No exposure	34	1.79 (1.26-2.54)	1.00
Low exposure	49	2.65 (2.01-3.66)	2.33 (0.83-6.57)
High exposure	49	4.22 (3.27-5.45)	5.31 (1.85-15.27)
Cigarette*			
Nonsmokers	62	1.73 (1.37-2.18)	1.00
Smokers	66	4.23 (3.27-5.25)	4.36 (1.95-9.73)

Abbreviation: GM, geometric mean.
*Statistically significant difference among means.

Using continuous variables in generalized linear models, 2-NAP and 1-OHP levels were both statistically significant predictors for mutagenicity when introduced separately in a model adjusted for the number of cigarettes per day (Table 5). When the levels of both metabolites were present in the model, the levels of 1-OHP did not explain the changes in mutagenicity. When wood smoke exposure was introduced with both metabolite levels in the model, wood smoke exposure was the only explanatory variable that remained in the model, and it was modified negatively by smoking behavior. Therefore, wood smoke exposure was the best explanatory variable for the continuous mutagenicity levels, adjusted for smoking.

Discussion

Urinary Metabolites. Because pyrene is one of the semi-volatile compounds present in high proportion in most PAH mixtures, one of its metabolites, 1-OHP, has been used frequently as a biomarker of exposure to PAH

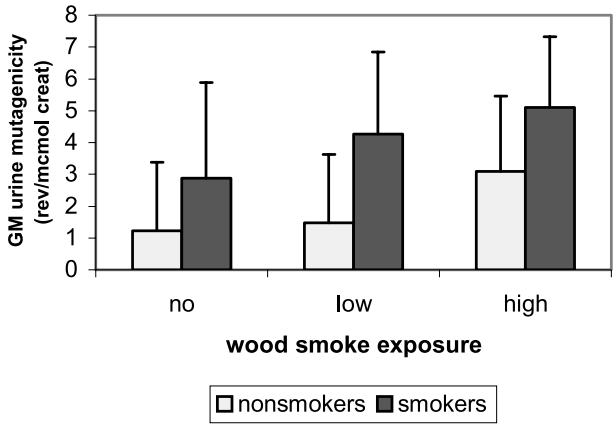

Figure 2. Geometric means of urinary mutagenicity levels in *Salmonella* YG1041 +S9 according to wood smoke exposure categories, stratified by smoking behavior. Difference in wood smoke exposure among nonsmokers was significant ($P < 0.05$); this difference was not significant among smokers ($P = 0.27$).

Table 5. Linear modeling results for the association between urinary mutagenicity levels in *Salmonella* YG1041 +S9 and urinary metabolites (2-NAP, 1-OHP) or wood smoke exposure categories adjusted for smoking (cigarettes/day)

Model	Variable	β (SE)*	95% CI
2-NAP and smoking	2-NAP	0.1417 (0.0479)	0.0470-0.2356
	Smoking	0.0637 (0.0145)	0.0353-0.0922
1-OHP and smoking	1-OHP	0.0856 (0.0383)	0.0105-0.1607
	Smoking	0.0639 (0.0147)	0.0350-0.0928
2-NAP, 1-OHP, and smoking	2-NAP	0.1163 (0.0425)	0.0106-0.2221
	1-OHP	0.0428 (0.0540)	−0.0406-0.1262
	Smoking	0.0632 (0.0145)	0.0348-0.0916
Wood smoke and smoking	Wood smoke (WS) low	0.5415 (0.2531)	0.0455-1.0376
	WS high	1.1912 (0.2505)	0.7002-1.6822
	Smoking	0.1437 (0.0393)	0.0667-0.2206
	WS low* smoking[c]	−0.0705 (0.0452)	−0.1591-0.0180
	WS high* smoking[c]	−0.1144 (0.0440)	−0.2005-−0.0282

*Regression coefficient β and SE with 95% CI; n = 121.
[c]Interaction factor between wood smoke and smoking.

in occupational and environmental settings (17, 18, 28). However, naphthalene is present at much higher levels than pyrene. Larson and Koenig (6) reported that 0.24 to 1.6 g of naphthalene was released per kilogram of wood, compared with 8 (10^4) to 3.1 (10^2) g pyrene/kg wood. Our analysis of air samples indicated that workers were exposed to ~15 times higher levels of naphthalene than pyrene in the volatile fraction, which is why we evaluated the urinary levels of a metabolite of naphthalene in this study.

The urinary levels of the hydroxymetabolites of naphthalene, 1-NAP and 2-NAP, have been suggested as biomarkers of exposure to jet fuel emissions (19) and airborne PAH (29, 30). Kim et al. (24) suggested that 2-NAP was a better marker of exposure for inhaled PAH than 1-NAP, the latter being better associated with smoking. 1-NAP has been proposed as a biomarker of occupational exposure to the insecticide carbaryl (31, 32) and could represent sources of exposure other than wood smoke.

The urinary levels of 2-NAP among charcoal workers (Table 2) were higher than those reported for subjects exposed occupationally to jet fuel (19) or for shipyard workers (30) and lower than the levels found in coke-oven workers (33). The concentrations of 2-NAP found among workers not exposed to wood smoke were similar to the values reported for nonexposed controls (34). The urinary concentrations of 2-NAP found in the group not exposed to wood or cigarette smoke were comparable to those found by Yang et al. (29). The geometric mean of urinary 1-OHP among charcoal workers was lower than that reported for other occupational exposures (17, 19) but was close to the values reported for fire fighters (20). Although both metabolites were able to discriminate between those exposed or not exposed, 2-NAP values were more distinct within categories, indicating that urinary concentrations of 2-NAP were more suitable as a biomarker of exposure to wood smoke than were urinary 1-OHP levels.

Cigarette smoke is one of the nonoccupational sources associated commonly with urinary 1-OHP or 2-NAP (29, 35), but the low smoking rate among charcoal workers obscured this potential association. The same reasoning can be presented for the effect of alcohol intake among charcoal workers.

The positive effect of the *GSTM1* null genotype on the excretion of PAH metabolites observed in our study also has been reported by others (19, 29, 36). However, we found no effect of *GSTT1* here, and we were unable to investigate the joint effect of *GSTM1* and *GSTT1* null genotypes due to the small number of subjects in this category. The effect of *CYP1A1* was also not evident, as also shown by Yang et al. (37) for urinary 1-OHP in a nonexposed population. The sample size actually prevents any firm conclusions about the role of genotype, and further and larger studies are needed to confirm the results.

Urinary Mutagenicity. Pimenta et al. (12) assessed acute toxicity and genotoxicity of *Eycalyptus grandis* wood smoke condensate with *Photobacterium phosphoreum* and *Vibrio fisheri*, respectively. The phenolic fraction showed more acute toxicity, whereas the fraction containing only PAH (total pyrolysis extract) was genotoxic. Charcoal itself, however, was not mutagenic in *Salmonella*.[7] Therefore, urinary mutagenicity observed in the present study was probably related to exposure to wood smoke and not to charcoal dust.

Strain YG1041 used in this study is sensitive to frameshift mutations caused by complex mixtures containing PAH and aromatic amines, especially nitroarenes and arylamines (26), such as those present in cigarette smoke. Cerná et al. (38) have shown that YG1041 with the addition of S9 was more sensitive than the more traditional TA strains in the evaluation of the mutagenicity of urban air pollution and also of urine of subjects exposed occupationally to outdoor pollution. This observation suggests the presence of aromatic amines and nitroarenes in the urine of the exposed workers whose urine was significantly more cytotoxic relative to that of the nonexposed workers. Compounds such as these may have accounted for our finding that exposed workers had increased odds of having toxic urine (i.e., cytotoxic to *Salmonella*) relative to nonexposed workers.

The use of strain YG1041 to assess mutagenicity in urine samples is not common, and the literature offered only a

[7] Umbuzeiro G, Rego MAV, Kato M, Unpublished observations.

few examples to which our results could be compared. Only Cerná et al. (38) used a sample preparation procedure similar to ours. Their values (rev/μmol creatinine) for smokers and nonsmokers exposed occupationally to air pollution were higher than those of charcoal workers. The effect of smoking behavior did not seem to be remarkable in the group with outdoor work exposure to combustion emissions from fossil fuels reported by Cerná et al. (38).

On the other hand, Vermeulen et al. (39) described the urinary mutagenicity of smokers working in a tire company. In our study, we were able to identify the effect of wood smoke exposure partly because half of the study population consisted of nonsmokers, and 81% of the workers smoked less than 10 cigarettes a day. The fact that the average mutagenicity levels were significantly different among only nonsmokers was expressed mathematically by the generalized linear model. According to this model, if the number of cigarettes per day exceeded 4, nonexposed workers would present levels of urinary mutagenicity comparable to nonsmokers in the low exposure category. If all workers smoked 10 cigarettes per day, mutagenicity levels would not be associated with wood smoke exposure. The higher the cigarette consumption, the more intense was the negative effect of the interaction between smoking and wood smoke, reducing the difference between categories. Our study did not reveal any association between urinary mutagenicity and genetic polymorphisms, in agreement with Binková et al. (40) and Motykiewcz et al. (41).

The diet of the population in the region we investigated consists traditionally of a small variety and amount of vegetables (5). Because most of the work sites did not have electricity and were located far from vegetable production or urban areas, the consumption of fresh vegetables and fruits was even more restricted. Consumption of fresh vegetables and fried/grilled meat was targeted in the questionnaire (5) because the former may enhance protection against carcinogens (42), and the latter is another source of PAH and other carcinogens, such as heterocyclic amines (43). Meat was present frequently in most of the workers' meals (Table 1), and the consumption of salted beef was high. The meat was usually fried before being added to cooked beans or toasted cassava flour. Although urinary mutagenicity can be a biomarker of cooked-meat consumption (22), we were not able to detect any association between urinary mutagenicity and diet variables.

Urinary Biomarkers. Quantification of chemicals in ambient air is considered to be essential for exposure dosimetry, especially for those compounds that have short half-lives in the human body. The first half-lives of elimination of 2-NAP and 1-OH are estimated to be less than 20 hours (17, 44). However, environmental sampling in this cohort had to be considered carefully. Some activities, such as climbing up a ladder to load trucks carrying a basket or a bag full of charcoal, were dangerous *per se*. The extra weight and the size of a personal sampling device could have increased the risk of a fall, interfering with the workers' equilibrium. The changes in temperature and ventilation during the unloading of the kiln might introduce variables that interfere with the adsorption of the volatile compounds by the resin, limiting proper sampling conditions. Because wood smoke is composed of gaseous and particulate phases, analyses of the chemicals in both phases are needed to evaluate correctly the exposure by environmental monitoring. In addition to that, the use of respiratory protection was not uniform among the workers because equipment was not always available and not always used due to discomfort while executing tasks in hot or dusty areas. Therefore, the use of urinary biomarkers as indicators of external exposure and internal dose offered some advantages over environmental sampling in such circumstances. The use of metabolites instead of the parent compounds as biomarkers represented an additional advantage because urine collected at working sites could also be contaminated during sampling.

Urinary metabolites, as with urinary mutagenicity, however, reflect the total intake, rather than exposure only to wood smoke (29), and the relevance of other determinants depends on the magnitude of these exposures in relation to wood smoke. Several of the potential confounders and effect modifiers were distributed rather homogeneously across the wood smoke exposure categories in our study. No significant effect of diet, alcohol intake, smoking behavior, or age was observed. Although some PAHs are human carcinogens, and certain types of exposure to wood smoke have been associated with risk for some types of cancer (45, 46), there are no data about disease incidence among charcoal workers. Future studies of such a cohort are needed to provide information about cancer risk associated with charcoal production.

In conclusion, we have showed the applicability of three urinary biomarkers for monitoring exposure to wood smoke among charcoal workers. For this population, with low smoking rate, all three biomarkers were informative. Urinary 2-NAP was the most sensitive indicator of wood smoke exposure and was associated with the other variables. This is also the first report of the magnitude of exposure to wood smoke among charcoal workers, expressed by the internal dose of some of its components. Our results show that these charcoal workers receive a systemic exposure to genotoxic compounds. Because charcoal production is still an important activity in many developing countries, this cross-sectional study serves as a basis for future research on the health effects of wood smoke exposure on charcoal workers.

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