Urinary Methoxyphenol Biomarkers and Woodsmoke Exposure: Comparisons in Rural Guatemala with Personal CO and Kitchen CO, Levoglucosan, and PM$_{2.5}$


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Urinary methoxyphenols have been proposed as biomarkers for woodsmoke exposure, but few field studies have been undertaken. We evaluated these biomarkers for assessing the exposure to woodsmoke of householders in rural Guatemala. The study population was a subset (10 female cooks, 2 female non-cooks, and 8 male non-cooks ranging in age from 7 to 60) drawn from those participating in a long-term randomized intervention trial (RESPIRE) in the highlands. All households rely solely on woodburning for cooking and heating. Approximately half of the homes in the trial used open woodfires in the home, while the intervention group used cookstoves, called “planchas,” that vent most of the woodsmoke outdoors through a chimney. Corrected for creatinine levels, 16 of the 19 methoxyphenols measured were lower in the urine of cooks using the plancha; and 11 of the 19 compounds were lower in the urine of non-cooks from homes using the plancha. Furthermore, the 4 low-molecular-weight syringyl methoxyphenols (syringol, methylsyringol, ethylsyringol, propylsyringol) were each moderately correlated (r$^2 = 0.71, 0.64, 0.68, 0.53$, respectively, with all $p < 0.05$) with personal exposure measurements determined by carbon monoxide (CO) passive diffusion tubes, but not with CO in exhaled breath. 48-Hour kitchen area measurements of PM$_{2.5}$ mass, PM$_{2.5}$ levoglucosan, and CO were highly correlated ($>0.89$) with each other and moderately correlated ($0.54$–$0.78$) with personal CO measurements. Although based on relatively few measurements, this study demonstrates that the urinary concentrations of specific methoxyphenols may be effective biomarkers of short-term exposures to inhaled woodsmoke in field conditions.

Introduction

Wood and other biomass smoke exposures impact a large proportion of the world’s population. As many as three billion people rely on biomass fuels for their daily energy needs (1, 2). As a percentage of total energy use, biomass varies greatly by region with developing countries using wood for a greater proportion of their total energy needs. In addition, smoke from vegetation and forest fires has affected large regions and numbers of people. The 1997–1998 wild fires in Indonesia exposed an estimated 70 million Southeast Asians to woodsmoke (3); smoke was implicated in 527 deaths and 15,800 hospitalizations in eight provinces (4). Smoke from large wildfires can impair air quality at distant urban centers (5, 6).

Indoor levels of particulate matter (PM) have been found to be quite high ($200–5000$ μg/m$^3$), when biomass is burned without venting for cooking and heating in developing countries (7). Reviews of many epidemiological studies of indoor air pollution from household solid fuel use in developing countries find significant health effects (2, 8, 9), including child acute respiratory infections (ARI) and adult chronic obstructive pulmonary disease (COPD), with growing evidence of low birth-weight, cataracts, lung cancer, otitis media, and tuberculosis. Based on the WHO Comparative Risk Assessment, indoor smoke from the burning of solid fuels (predominantly biomass, but also including coal) is the second highest environmental risk factor globally, accounting for 3.6% of the global burden of disease in developing countries and about 1.6 million premature deaths annually around the world (10).

With few exceptions (e.g., (11)) most past epidemiological studies have not attempted to directly measure pollution exposure or dose, but relied on indirect indicators such as type of fuel used. Although such indicators have correlated well with certain health endpoints in these studies, they are inherently imprecise and lead to exposure misclassification and lowered ability to discern effects or to measure the impact of interventions. Obtaining accurate measures of personal exposure and, more importantly, inhaled dose, for an air pollutant is inherently difficult, however. This is due to the substantial spatial and temporal variation in pollutant levels, coupled with the fact that people constantly move among different microenvironments. Thus, traditional fixed-site monitors fail to capture the full variability in exposures experienced by individuals. Although personal monitors are effective in accurately measuring personal exposures, it is impractical and cost prohibitive to implement active personal monitoring on a large scale. Furthermore, external personal monitors are burdensome for participants, particularly young children. Monitors also fail to account for differences in breathing rate, and hence inhaled dose, due to physical exertion. An alternative approach to exposure assessment, which addresses many of the limitations noted above, is biomonitoring.

Several classes of chemicals have been proposed as biomarkers to assess wood smoke exposure, of which PAH metabolites (e.g., 1-hydroxypyrene) and lignin pyrolysis products are the most promising (12–15). Kato et al. reported increases in urinary 1-hydroxypyrene concentrations associated with woodsmoke exposure in charcoal production workers from Brazil (13). Woodsmoke exposure was assigned based on job classification and no exposure measurements were made. Fuenkes found urinary 1-hydroxypyrene concentrations in structural firefighters were higher after periods of smoke exposure (12), however no significant correlation was found between urinary 1-hydroxypyrene concentrations and personal exposure to airborne PAHs. Methoxyphenols are derived from the pyrolysis of the wood polymer lignin, and have been used as specific tracers of woodburning.
Once these chemicals enter the body through respiration, they are metabolized and excreted in the urine. We have previously reported the use of urinary methoxyphenols as biomarkers of woodsmoke exposure in a preliminary study (15) and a managed exposure study (16). We extend this preliminary work to examine the applicability of this family of biomarkers to assess indoor exposures to woodsmoke in a group of subjects from a rural community in Guatemala, in which householders rely on wood burning for cooking and home heating.

**Experimental Section**

**Study Subjects.** The study was conducted in communities in the San Marcos region of rural highland Guatemala, located at 2200–3000 m. Subjects were recruited from a larger cohort involved in a randomized intervention trial (RESPIRE; Randomized Exposure Study of Pollution Indoors and Respiratory Effects), which was examining the effects of introducing a chimney stove (the plancha) on indoor woodsmoke exposures and child and mother health (see Supporting Information and ref 20). Twenty subjects from ten households (2 subjects/household) were recruited to participate in the biomarker evaluation study. These subjects were recruited from a random subset comprising 10 of the 534 houses in the RESPIRE study. One hundred percent of potential subjects who were interviewed agreed to take part in the study, and informed consent was obtained from all subjects.

For the biomarker evaluation study, the participants from each household included the mother of the index child and one other family member. The index children were under 18 months of age and spot urine collection was deemed not feasible with the children due to foreseeable logistical difficulties, namely the use of nonabsorbent diapers by a population unaccustomed to diaper use. The other family member could have been the index child’s father, grandparent, or older sibling and were selected to capture a range of ages and gender (8 males and 2 females between the ages of 7 and 60 were recruited). Mothers were specifically recruited because previous studies have shown that they typically experience the highest woodsmoke exposure due to the time they spend cooking and tending to the fire (1, 7, 21–26). Of the ten households recruited, six were plancha homes and four were open-fire homes. A balanced selection of 5 open fire and 5 plancha homes was originally proposed but not met because the household members of the planned fifth open fire home were not available at the time of consent and an open-fire replacement could not be recruited within the time constraints of the study. To avoid PM and CO exposures from other sources, the subjects agreed not to use the local wood-fired sauna (temascal) the day preceding the urine collection period. Temascal refers to small huts that serve as saunas and are used extensively among this population for bathing purposes. Temascal exposure was a concern because fires are used in the unventilated huts to heat rocks, which provide the heat for the bath.) Furthermore, none of the participants in the study were smokers or lived with smokers.

**Urine Collection and Analysis.** Each of the 20 participants in the biomarker evaluation study collected their first morning urine voids, defined as the first urination after midnight, for two consecutive days in a 1-L polypropylene container. Researchers visited each home daily to retrieve the urine samples and conduct a brief interview. Subjects reported the time of day at which they provided the urine sample as well as the time of their previous urinary void. Because most of the participants were illiterate and unable to write down the times of their activities, researchers relied on the participants to recall the time of these activities. The researchers provided clocks and wristwatches at the time of consent because many of the subjects did not have them.

For each urine sample, the volume was measured and aliquots (40 mL) of the void were transferred to 50 mL polypropylene tubes and frozen at −20 °C. Most samples were frozen within 2 h of pickup, however since urination times were typically quite early in the morning, as much as 8–12 h passed before certain samples were frozen. Urine samples were kept frozen as they were transported to the University of Washington for analysis. No degradation of methoxyphenols was observed in urine samples stored frozen for 2 years.

Urine samples were analyzed for methoxyphenols by GC/MS using the procedure of ref 14. Additional details are provided in the Supporting Information. Urinary creatinine was measured by a clinical laboratory using a colorimetric assay. Urinary creatinine measurements were used to adjust for diuresis.

**Exposure Assessment.** Comprehensive exposure monitoring was undertaken for a 48-hour period co-incident with the biological monitoring. Pollutant concentrations (PM2.5, levoglucosan, CO) were measured indoors in the kitchen. In addition, personal exposure to CO was measured on the mothers. Although urine samples were collected from the mother and one other member of each study household, personal monitoring which included CO tubes and real-time CO monitors was only conducted on the mother. Although personal CO monitoring for the other family members in addition to mothers would be preferable for the biomarker validation, it was not part of the testing protocol for the exposure monitoring concurrently employed by the RESPIRE study. As not to overburden study participants who were engaged in ongoing monitoring for the main study, personal monitoring was not extended to other household members.

PM2.5 was collected in the kitchen using a personal sampling pump (model 224-PCXR8, SKC Inc.), aluminum triplex cyclone (Scc1.062, BGI Inc.), and 37 mm PTFE membrane filters (2.0 μm pore size, Pall Life Sciences). The pumps were installed in the kitchen during the first visit; visits occurred at approximately the same time (mid-day) for each home in the study. Researchers returned at the same time on the following day to collect the used filter and install a new filter. Two 24-hour samples were taken in the kitchen of each home. Each pump intermittently sampled for 1 min every 3 min during each 24-hour deployment resulting in 480 min of sampling per day. The pump was set to a flow rate of 1.5 L/min, appropriate to capture PM2.5, yielding a total of 720 L of air sampled. Each pump was calibrated in the field office and again in the field with a rotameter, (E1–4Y601-ES00, Matheson) which itself was calibrated using a wet flow cell calibration system (Gilibator, Gillian Inc.). The flow was then checked and recorded again when the filters were collected.

PM2.5 concentrations were determined gravimetrically (see Supporting Information) from the filter samples. Filters were then extracted by a previously described protocol (14, 27), and levoglucosan concentrations were determined by GC/MS (14) (see Supporting Information). Levoglucosan is formed from the pyrolysis of cellulose; it is a major component of woodsmoke (6) and measurements of levoglucosan in PM2.5 have been used to quantify woodsmoke levels in air samples (28).

CO has been used as a surrogate for exposure to woodsmoke because it is cheaper and easier to monitor than PM concentrations. Previous studies in this population showed that CO was a good surrogate longterm (24 h) marker for PM2.5, with correlations (Spearman rho) of 0.92–0.94 between 24 h CO and 24 h PM2.5 (29–31).

Continuous datalogging electrochemical CO monitors (HOBO CO monitor, Onset Corp.) were placed in the kitchen and on the mother of each study home. These monitors were programmed to log CO readings to 0.2 ppm resolution every
Levels of woodsmoke-associated air pollutants including PM$_{2.5}$, levoglucosan, and CO (as measured by the HOBO datalogger) were dramatically higher (20–50 fold) in homes with open fires compared to homes with the plancha. All differences were statistically significant. Mothers in homes that had a plancha experienced 2–3 fold lower exposures to CO compared to mothers from homes with an open fire.

As shown in Table 2, indoor air pollution metrics were highly correlated with each other. To construct this comparison matrix, 2-day (48 h) measurements were calculated for each monitoring technique. The comparison entailed calculating the 2-day means of PM$_{2.5}$ and levoglucosan concentrations and comparing these measures with the TWA of the CO data as determined by the CO tubes and realtime CO measures during the 2-day deployment. Because the CO tubes placed in open fire kitchens exceeded the upper limit of detection, these were not included in the correlation analysis.

PM$_{2.5}$ concentrations in the study homes were closely correlated with the other pollutants measured in the kitchen (levoglucosan ($r = 0.94$), continuous CO ($r = 0.95$)). The kitchen PM$_{2.5}$ concentrations were less closely associated with personal exposure readings collected on the mothers, (continuous CO ($R = 0.74$), CO tube worn by mother ($r = 0.78$)). The two personal monitoring devices were moderately correlated ($r = 0.83$). The exhaled breath CO measurements were poorly correlated with the area and personal air quality measurements.

**Urinary Measures.** First morning void urine samples were obtained from all subjects. These urine samples typically were voided between 4:30 and 7 a.m., (mean time 5:15 a.m.; standard error of the mean (SE), 15 min). All participants reported collection of their first morning void, defined as the first urine void after midnight. Additionally all participants reported one void after the previous night’s dinner. Participants began eating dinner at a mean time of 7:40 p.m. (SE 5 min), and they voided after that meal at 8:52 p.m. (SE 6 15 min). All participants reported collection of their first morning void, defined as the first urine void after midnight. Additionally all participants reported one void after the previous night’s dinner. Participants began eating dinner at a mean time of 7:40 p.m. (SE 5 min), and they voided after that meal at 8:52 p.m. (SE 6

### Results

**Indoor Air Pollution.** Measures of indoor air pollution due to biomass smoke were measured in the kitchens of the 10 households we studied. These data are presented in Table 1. Levoglucosan and PM$_{2.5}$ measurements represent 24 h averages, typically covering a midday-to-midday period. The CO tube measured integrated CO concentrations over the same continuous 48-hr period (also nominally starting at midday), and these data are expressed as a time weighted average in Table 1. Continuous CO data were collected at 30-second intervals, and are expressed in Table 1 as 48-h time-weighted averages. Personal exposures to CO were also measured on the mothers both by the diffusion tubes and the continuous monitors, and these data are included in Table 1.
Unequal variances. P2: p value of 2-tailed t-test comparing methoxyphenol levels in non-mothers from open fire and plancha households, assuming samples are included as an “unexposed” reference group. Plancha refers to study participants using ventilated cook stoves provided in the intervention trial. P1: p value of 2-tailed t-test comparing methoxyphenol levels in mothers from open fire and plancha households, assuming limit of quantitation (LOQ) were replaced with a value equal to one-half of the LOQ. Urine samples were also collected from 2 office workers to creatinine to account for diuresis. Our previous report indicated that normalization of urinary methoxyphenols due to the diurnal nature of activities in this population. Instead the consistency between activity times is with woodsmoke exposure than un-normalized urinary concentrations, and were essentially equivalent in predictive ability. In this study, creatinine measures in the urine samples of the participants ranged from 14 to 226 mg/dL (mean ± SE, 88 ± 7.5 mg/dL). Excretion rate, determined by dividing the measured volume of the sample by the time duration since the previous void, ranged from 5.3 to 133 mL/hr. These two measures of urine dilution correlated poorly (r² = 0.2) even after removing an outlier that resulted from an incomplete sample collection. Since some study participants could not accurately record sample times or collect full urine voids, creatinine was chosen as the preferred normalization tool. Although the WHO recommends an optimal creatinine range of 30–300 mg/dL for analysis of urine samples, the three urine samples outside that range (14, 27, 28 mg/dL) were included in all data analyses (32). Correlations were determined between the creatinine-corrected methoxyphenol concentrations (see Table 1 of the Supporting Information). Compounds in the syringyl and guaiacyl families are closely associated with other chemicals in that family but were not consistently associated with chemicals in the other family. Controlled combustion studies of different biomass fuels have shown that methoxyphenol emissions from combustion of angiosperms show a predominance of syringyl compounds, whereas combustion emissions from gynosperms show a predominance of guaiacyl compounds (19,33). The strong correlations observed among the syringyl compounds (Supporting Information, Table 1), and the associations observed between these compounds and the other metrics of woodsmoke exposure (Table 4) indicate that the biomass fuels burned in this study were largely angiosperms, and the methoxyphenols in the woodsmoke from these fuels were dominated by syringyl compounds.

### Table 3. Comparison of Creatinine Normalized Urinary Methoxyphenol Concentrations between Mothers and Non-Mothers in Open Fire and plancha Homes

<table>
<thead>
<tr>
<th></th>
<th>mothers</th>
<th>non-mothers</th>
<th>open fire</th>
<th>plancha</th>
<th>unexposed</th>
<th>open fire</th>
<th>plancha</th>
<th>unexposed</th>
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<tr>
<td></td>
<td>mean SE</td>
<td>mean SE</td>
<td>P1</td>
<td>mean SE</td>
<td>mean SE</td>
<td>P2</td>
<td>mean SE</td>
<td>mean SE</td>
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<td>0.209</td>
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<td>0.03</td>
<td>0.548</td>
<td>0.093</td>
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<td>0.107</td>
<td>0.100</td>
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<td>0.208</td>
<td>0.058</td>
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<td>ETSY</td>
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<td>0.085</td>
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<td>0.108</td>
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<td>PRSY</td>
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<td>0.023</td>
<td>0.027</td>
<td>0.008</td>
<td>0.14</td>
<td>0.070</td>
<td>0.038</td>
<td>0.027</td>
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<td>summed syringols</td>
<td>1.769</td>
<td>0.518</td>
<td>0.344</td>
<td>0.069</td>
<td>0.03</td>
<td>0.873</td>
<td>0.133</td>
<td>0.443</td>
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<td>GU</td>
<td>3.109</td>
<td>0.374</td>
<td>2.520</td>
<td>0.385</td>
<td>0.29</td>
<td>4.260</td>
<td>0.854</td>
<td>3.829</td>
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<td>MEGU</td>
<td>0.534</td>
<td>0.149</td>
<td>0.581</td>
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<td>0.27</td>
<td>0.706</td>
<td>0.227</td>
<td>0.829</td>
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<td>ETGU</td>
<td>0.240</td>
<td>0.037</td>
<td>0.198</td>
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<td>0.57</td>
<td>0.283</td>
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<td>EUG</td>
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<td>0.031</td>
<td>0.139</td>
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<td>ACETV</td>
<td>1.600</td>
<td>0.204</td>
<td>1.756</td>
<td>0.444</td>
<td>0.75</td>
<td>3.712</td>
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<td>ALLSY</td>
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<td>0.320</td>
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<td>SYALD</td>
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<td>0.009</td>
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<td>CONIF</td>
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<td>PRSYON</td>
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<td>0.128</td>
<td>0.028</td>
<td>0.70</td>
<td>0.145</td>
<td>0.036</td>
<td>0.122</td>
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### Table 4. Relationship Between Methoxyphenols and Indoor Air Quality (Given as Methoxyphenol Biomarker r² (p-value))

<table>
<thead>
<tr>
<th>n</th>
<th>syringol</th>
<th>methylsyringol</th>
<th>ethylsyringol</th>
<th>propylsyringol</th>
<th>sumed syringols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>personal measures: mothers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO tubes</td>
<td>0.74 (0.002)</td>
<td>0.73 (0.002)</td>
<td>0.72 (0.004)</td>
<td>0.50 (0.024)</td>
<td>0.73 (0.003)</td>
</tr>
<tr>
<td>realtime CO (TWA)</td>
<td>0.25 (0.033)</td>
<td>0.19 (0.052)</td>
<td>0.24 (0.046)</td>
<td>0.16 (0.083)</td>
<td>0.25 (0.040)</td>
</tr>
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<td>realtime CO (15 min peak)</td>
<td>0.31 (0.017)</td>
<td>0.28 (0.018)</td>
<td>0.32 (0.018)</td>
<td>0.16 (0.077)</td>
<td>0.33 (0.017)</td>
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<tr>
<td>exhaled breath CO</td>
<td>0.07 (0.46)</td>
<td>0.12 (0.37)</td>
<td>0.15 (0.30)</td>
<td>0.11 (0.36)</td>
<td>0.11 (0.39)</td>
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<tr>
<td>kitchen measures: mothers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PM₂₅</td>
<td>0.43 (0.003)</td>
<td>0.30 (0.013)</td>
<td>0.33 (0.017)</td>
<td>0.21 (0.041)</td>
<td>0.39 (0.007)</td>
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<td>levoglucosan</td>
<td>0.38 (0.006)</td>
<td>0.30 (0.013)</td>
<td>0.36 (0.011)</td>
<td>0.21 (0.045)</td>
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<td>realtime CO (TWA)</td>
<td>0.41 (0.004)</td>
<td>0.27 (0.020)</td>
<td>0.36 (0.011)</td>
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<td>kitchen measures: other householders</td>
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<tr>
<td>PM₂₅</td>
<td>0.37 (0.006)</td>
<td>0.33 (0.008)</td>
<td>0.12 (0.208)</td>
<td>0.16 (0.084)</td>
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<td>levoglucosan</td>
<td>0.42 (0.003)</td>
<td>0.35 (0.006)</td>
<td>0.24 (0.062)</td>
<td>0.15 (0.097)</td>
<td>0.27 (0.048)</td>
</tr>
<tr>
<td>realtime CO (TWA)</td>
<td>0.12 (0.140)</td>
<td>0.16 (0.083)</td>
<td>0.18 (0.115)</td>
<td>0.11 (0.153)</td>
<td>0.24 (0.063)</td>
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</table>
pounds. Descriptive statistics summarizing the urinary methoxyphenol concentrations are listed in Table 3.

Urinary levels of syringol were significantly reduced in subjects from plancha homes, and the reduction in three other syringol compounds (4-methylsyringol, 4-ethylsyringol, and 4-propylsyringol) approached significance. The sum of these four compounds was also calculated and included in the analyses. These four methoxyphenols include the most abundant syringol-type compounds, and are all present predominantly in the vapor phase at typical ambient temperatures (34).

Table 4 shows the associations between urinary concentrations of the selected syringol compounds and individual indoor air pollution (IAP) measurements, determined via least-squares linear regression. As judged by the \( r^2 \) values for the regression models, the strongest associations were observed between personal CO passive diffusion tubes and the creatinine-normalized concentrations of syringyl methoxyphenols in the mothers’ urine. Based on the regression models, IAP measurements in the kitchen showed a significant association with syringyl biomarker levels in the mothers, but the association between IAP measures and biomarker levels was weaker (and not always significant) for non-mothers.

Discussion

Data from the urinary biomarker analysis indicate that the cookstove intervention reduced smoke exposures for mothers and non-mothers, and this evidence is corroborated by reductions in personal CO measures observed for the mothers. However, the reduction was much less than that observed in kitchen air concentrations. This is because the subjects do not spend all of their time in the kitchen, and the outdoor air has much lower levels of PM and CO and smaller differences between households using different stoves (35). For the mothers, both of the metrics (personal CO exposure and urinary biomarker levels) showed an approximately 3-fold decrease in exposure, whereas a decrease of 80 to 95% was observed for kitchen measurements in this study and in previous studies (29, 36, 37). Therefore contaminant levels measured in the kitchen do not accurately reflect the mothers’ exposures, and the association between IAP levels in the kitchen and the urinary biomarker, while still statistically significant, is not strong. The consistency between the biomarkers of woodsmoke dose and CO personal exposure suggests that each are appropriate methods for estimating exposure at the level of the individual.

Due to varying time—activity patterns of different family members, woodsmoke concentrations in the kitchen are an even poorer indicator of exposure in non-mothers than they are for mothers. Saksena et al. conducted a study of biomass smoke exposure in rural India that showed that women were exposed to twice the amount of total suspended particulates (TSP) as men during the summer months, and four times the level in winter months (7). In a woodsmoke exposure study in Kenya, Ezzati et al. (26) found that women aged 16–50 spend 54% of their daily time budget indoors and 38% near the fire, as compared with 24% and 6% for men of the same age. Our data show a trend of higher urinary concentrations for the syringyl compounds in mothers compared to non-mothers for the open fire homes, but not for the plancha homes, however these differences were not statistically significant (see Table 3). Since the greatest exposures occur during brief cooking episodes, time-weighted average kitchen concentrations are not an appropriate measure of personal exposure. Ezzati et al. also estimated that study subjects who perform cooking tasks receive 31–61% of their exposure to woodsmoke during short, high-intensity fire emissions periods (26).

In the current study, urinary methoxyphenol concentrations, normalized by creatinine, proved to be a useful biomarker of exposure to woodsmoke. By far, the highest correlations were found between the mothers’ exposure data obtained from the passive diffusion CO tubes and the methoxyphenols in the syringol family (syringol: \( r^2 = 0.71, p = 0.002 \); 4-methylsyringol: \( r^2 = 0.64, p = 0.002 \); 4-ethylsyringol: \( r^2 = 0.56, p = 0.004 \); 4-propylsyringol: \( r^2 = 0.53, p = 0.024 \)). Our data indicate that 53–71% of the variability in the concentrations of syringyl compounds in spot urine samples was explained by the mothers’ exposure to woodsmoke, as determined by the personal CO monitors.

In the current study the average (across all subjects) syringol and guaiacol concentrations are 0.47 and 3.40 mg/mg creatinine respectively. Dills et al. reported average syringol and guaiacol concentrations of 0.06 and 0.95 mg/mg creatinine for the 12 h period following a 2 h exposure to 1500 mg/m^3 woodsmoke (14). While the biomarker concentrations reported by Dills et al. are lower than those in the present study, differences between these two studies in woodsmoke exposure duration and intensity limit our ability to interpret the observed differences in biomarker concentrations.

Previous studies have highlighted the potential for dietary confounding of the relationship between urinary methoxyphenol concentrations and woodsmoke exposure (19). We determined that consumption of food cooked over an open fire did increase urinary methoxyphenols, but the effect was small compared to the effect of smoke exposure on urinary biomarker levels (see Supporting Information). The statistically significant association that we observed between the urinary biomarker and airborne woodsmoke exposure was obtained in spite of some important limitations of this pilot study, including the small number of subjects studied, the collection of spot urine samples rather than 24 h urine samples, and the use of a single 48 h integrated CO measurement to indicate the subject’s exposure to woodsmoke. CO is a reasonable measure of the subjects’ personal exposures to woodsmoke-derived CO for this population, and would be a good surrogate for woodsmoke-derived PM also. CO is a surrogate measure of exposure to woodsmoke-derived methoxyphenols, both in terms of the pollutant (CO vs methoxyphenols), and in terms of the exposure time scale: 48-h integrated CO measurement vs methoxyphenol concentration in first morning urine void.

The correlations between selected methoxyphenols and CO exposure as measured by the continuous CO monitoring device, although statistically significant (except for propylsyringol) was much poorer \( (r^2 = 0.15–0.28) \) than the correlations observed with the CO diffusion tubes. The reason for this is unclear. One explanation is that the instrument lag for the HOBO CO monitor of approximately 10 min may have been too long to accurately monitor very short and intense exposure events. Other CO exposure monitoring devices are available with short response times (on the order of 20 s) that would alleviate this problem. Another explanation may be that the devices were worn in a pouch at approximately waist-level, whereas the tubes were pinned to the mothers’ clothing near the shoulder and closer to the breathing zone. The placement of the tubes nearer to the breathing zone might explain why this measurement is more highly correlated to the biomarker levels than the data from the continuous monitors. Also, some of the monitors may have removed the monitors if they felt uncomfortable wearing them throughout the 48-hr monitoring period. Because of the small sample size in this study, a small amount of noncompliance could have greatly affected monitoring results. In contrast, the tubes are clipped to the shirt of the mothers and are only as large as a standard pencil, so it is
less likely that they would have bothered the study participants.

Interestingly, the 15-minute peak exposure recorded by the CO monitors gave approximately the same results as the TWA during the entire period before urination. Since many commercially produced CO monitoring devices can record the 15-minute peak value even without datalogging capabilities, and they require less programming, downloading and analyzing, the utilization of this parameter may simplify exposure monitoring.

Because it was not feasible in this setting to collect 24- or 48-h urine samples, we were limited to collecting spot samples and trying to correlate this with the exposures that happened previously. An advantage of the continuous monitors was that we could analyze the exposure data to determine the exact times during the day that the most important exposures took place and calculate the expected loss due to evening urinary voids that were not collected. Since cooking events typically took place between 6 and 8 p.m., and the morning void samples occurred at approximately 5 a.m., approximately 10 h elapsed between peak exposures and sample collection. In our previous study we calculated urinary elimination half-lives of 3.1–3.3 h for the syringyl compounds. Therefore ~90% of the syringyl dose from the evening cooking event would have been excreted into the urine by the 5 a.m. first-morning-void sample collection. However, due to the short half-life of these compounds, a significant fraction of the biomarker would have been excreted in any urine voided during the previous evening, and would not have been captured in the first-morning void sample. As noted earlier, all participants reported voiding a urine sample after their evening meal.

Urinary biomarker levels were compared to the exhaled breath CO readings that were taken from the mothers at the time the second urinary void was collected. Although the relationship between the urinary and exhaled breath biomarkers had a positive relationship for each of the four syringols being investigated, none of these trends were significant markers had a positive relationship for each of the four syringols. Therefore the urinary and exhaled breath biomarker approach poses challenges to researchers, such as keeping urine samples frozen during office storage and international shipment. As with any biological sample, researchers must follow stringent data privacy safeguards and successfully communicate these to participants. Urine collection among certain populations requires special cultural sensitivity, for example when female participants may be menstruating. Finally accounting for inter-individual variations in metabolic rates that can affect the excretion half-life of the compounds may further complicate data analysis.

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Supporting Information Available

Additional experimental details regarding subject selection, exposure assessment, and chemical analysis; description of a small study conducted to evaluate the contribution of diet to biomarker levels in this population; and a table showing correlations between the different urinary methoxyphenols. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(11) Ezzati, M.; Kammen, D. M. Quantifying the effects of exposure to indoor air pollution from biomass combustion on acute


