Environmental Research Section A **85,** 145–158 (2001)
doi:10.1006/enrs.2000.4107, available online at http://www.idealibrary.com on IDEAL®

Measurement of Urinary Methoxyphenols and Their Use for Biological Monitoring of Wood Smoke Exposure

Russell L. Dills, Xiaoqiang Zhu, and David A. Kalman

Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98195-7234

Received November 3, 1999

A urinary assay for methoxyphenols was developed for the biological monitoring of wood smoke exposure. Methoxyphenols in 10-ml samples of urine were extracted after acid hydrolysis using XAD in a solid-phase extraction cartridge. The methoxyphenols were eluted with ethyl acetate and then analyzed by gas chromatography/mass spectrometry. Specific chemicals quantified were guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-propylguaiacol, syringol, 4-methylsyringol, 4-ethylsyringol, vanillin, eugenol, and syringaldehyde. Recoveries ranged from 60 to 90%, with coefficients of variation of $\leq$20%. Background levels of the compounds were measured in 21 nonsmoking adults. Guaiacol, 4-methylguaiacol, eugenol, and vanillin were detected in all subjects. An experimental feeding of a commercial wood smoke flavoring demonstrated that methoxyphenols were rapidly and efficiently eliminated in urine. Preliminary field studies demonstrated that urinary excretion rates of some methoxyphenols increased after inhalation exposure to wood smoke.

Key Words: **wood smoke; methoxyphenol; biomonitoring; urine; air pollution.**

INTRODUCTION

Particulate air pollution has been implicated in an increase of adverse health effects (Schwartz *et al.*, 1993; Pope *et al.*, 1991) and mortality (Schwartz *et al.*, 1996; Pope *et al.*, 1995). The National Research Council (NRC, 1998) has indicated a need for better definition of the relationship between ambient and personal exposures in particular for sensitive populations. Exposure assessments coupled with health studies are considered an efficacious way to determine what characteristics of particulate matter cause adverse health effects in susceptible subpopulations. Biological monitoring would permit retrospective assessment of exposure after health effects are noted. Other potential advantages of a biomarker approach are more accurate dosimetry, the ability to assess inter- and intraindividual variability of internal exposure, and the integration of exposures to an individual.

Wood burning is one source of particulate air pollution, and a preponderance of data suggested that exposure to wood smoke increased respiratory symptoms and diminished pulmonary function in children (Larson and Koenig, 1994). Potential impacted populations include residents in communities that allow heating by wood stoves (Larson and Koenig, 1994), individuals cooking with wood stoves (Pérez-Padilla *et al.*, 1996), and firefighters at wildfires (Reinhardt *et al.*, 1995) or prescribed burns (Reinhardt *et al.*, 1994). Furthermore, the 1998 wildfires in Mexico, the Southwest, and Florida have shown that millions may be exposed to air pollution in the form of wood smoke.

Wood smoke particulate was found to be almost entirely in the inhalable size range, $<1\,\mu$m (Kamens *et al.*, 1984; Dasch, 1982). Methoxyphenols comprised 20–30% of the carbon particulate weight in smoke from wood stoves and fireplaces (Hawthorne *et al.*, 1989) in the inhalable fraction (Hawthorne *et al.*, 1992).

Wood produces methoxyphenols as volatile emissions (Lustre and Issenberg, 1969; Kornreich and Issenberg, 1972; McKenzie *et al.*, 1994). Pyrolysis of wood lignins (polymeric syringyl or guaiacyl propane derivatives) has been shown to be the source of the methoxyphenols (Simonelt *et al.*, 1993; Edye and Richards, 1991) and because of this specific origin, air monitoring for these compounds has been investigated as a means of tracing emissions from wood burning (Hawthorne *et al.*, 1988, 1989a, 1992; Simonelt *et al.*, 1993). Methoxyphenols found in wood

0013-9351/01 $35.00

smoke include the 4-H-, methyl-, ethyl-, propyl-, propenyl-, formyl-, acetyl-, and acetonyl-substituted compounds of guaiacol and syringol. Proportions of among these compounds differ depending on wood species; hardwood and softwood smoke differ in the ratios of syringol to guaiacol, with syringol derivatives being 2 orders of magnitude higher in hardwood smoke (Hawthorne *et al.*, 1989a). In contrast, grasses (*Graminaceae*) contain lignin that is predominantly composed of *p*-hydroxyphenylpropane units (Sarkanen and Hergert, 1971) and produces cresol derivatives upon pyrolysis (Simonelt *et al.*, 1993). Smoke from grasses is thus not likely to confound source analyses for wood smoke.

Human and animals studies have shown that by the oral route of exposure, guaiacol and syringol did not undergo appreciable phase I metabolism but were rapidly eliminated ($t_{1/2} \approx 2$ h) in urine as glucuronide and sulfate conjugates (Ogata *et al.*, 1995; Miller *et al.*, 1974, 1976). Eugenol (4-allyl-2-methoxyphenol) was more extensively metabolized by phase I processes than guaiacol and syringol, with only 50% of the dose recovered as conjugated eugenol (Fischer, 1990). Vanillin (4-formyl-2-methoxyphenol) was also extensively metabolized; 50% of the dose was recovered as vanillic acid in rats (Strand and Scheline, 1975) and 70% was metabolized to vanillic acid in rabbits (Sammons and Williams, 1941). The extent of absorption via inhalation for guaiacol, syringol, and their derivatives apparently has not been studied. However, phenol was rapidly absorbed after inhalation exposure in humans (Piotrowski, 1971) and it is likely that the methoxyphenols would also be.

Currently, there is no biomarker of exposure to wood smoke; exposure assessment relies on personal or environmental sampling of particulate. We hypothesized that urinary measurement of methoxyphenols would be a biomarker for personal exposure to wood smoke and therefore developed an analytical method for measurement of methoxyphenols in urine.

METHODS AND MATERIALS

Materials

A commercial smoke flavoring (No. 51878-797, Western Flavors and Fragrances, Livermore, CA) was used in the feeding study; it was approximately 10-fold more concentrated than the retail flavorings examined (Wright's Concentrated Hickory Seasoning and Arizona Mesquite Smoke Flavoring). Methoxyphenols in the flavorings were quantified by gas chromatography–mass spectrometry (GC-MS) after extraction with ethyl acetate.

Solid-phase extraction (SPE) cartridges were made from 6-ml polypropylene filtration tubes with polyethylene frits (Supelco, Bellefonte, PA). Amberchrom CG-161C (400 mg, 80–160 μm; Supelco) was dry-packed into the filtration tubes and then compressed with the top frit. The tubes were conditioned with 12 ml ethanol and 24 ml 10 mM HCl (pH 2). Commercially available SPE tubes containing XAD absorbents did not work well, presumably because of the large particle size (≈800 μm).

Chemicals

Chemicals were purchased from Aldrich (Milwaukee, WI) unless otherwise indicated. Chemicals were reagent grade (≥98%) and used without purification. 4-Ethyl-2-methoxyphenol (4-ethylguaiacol) and 2-methoxy-4-*n*-propylphenol (4-propylguaiacol) were bought from Lancaster Synthesis (Windham, NH). 4-Nitrophenol-β-D-glucuronide was from Sigma (St. Louis, MO).

4-Methylsyringol. Syringaldehyde (55 mmol) was reduced with $LiAlH_4$ (165 mmol in 200 ml dry tetrahydrofuran) to form 4-methylsyringol. After 4 h reflux with vigorous stirring, the reaction mixture was externally cooled with ice water. Water-saturated diethyl ether was added slowly until gas evolution ceased. The reaction mixture was then poured into 100 ml of ice water. Following the addition of 25 ml of concentrated HCl and 400 ml of water, the reaction mixture was extracted four times with 200 ml ether. The ether was dried with Na_2SO_4 and then evaporated under reduced pressure at room temperature. The crude product was purified by silica gel chromatography (60 A, 70–230 mesh; 3.75 × 35 cm; methylene chloride). The product eluted between 350 and 600 ml. Evaporation of the eluate under reduced pressure at room temperature gave a clear, colorless viscous liquid that turned brown at room temperature. The product was distilled under vacuum using a short path apparatus (b.p. 107/2 mm). The mass spectrum was consistent with previously published data (Hruza *et al.*, 1973).

4-Ethylsyringol. 3,5-Dimethoxy-4-hydroxyacetophenone (Pfaltz and Bauer, Waterbury, MA; 16 mmol) was reduced with $LiAlH_4$ (67 mmol in 150 ml dry tetrahydrofuran) in the same manner as in the synthesis of 4-methylsyringol to form 4-ethylsyringol. The eluate (methylene chloride) between 200 and 350 ml was collected from a 2.5 × 30 cm silica gel

TABLE 1
Purity and Deuteration Conditions of Synthesized Chemicals

Chemical	Deuteration reaction temperature (°C)	Deuteration reaction time (h)	Chemical purity (%)	Isotopic purity (%)
4-Methylsyringol			97	
4-Ethylsyringol			97	
D_4-Guaiacol	150	16	100	82
D_3-4-Methylguaiacol	150	16	94	100
D_3-4-Ethylguaiacol	150	36	99	88
D_3-4-Propylguaiacol	150	36	99	92
D_3-Syringol	150	10	100	89
D_2-Syringaldehyde	150	10	99	97
D_2-Vanillin	150	33	100	82
D_3-Eugenol	125	6	94	63

column. The mass spectrum was consistent with previously published data (Hruza *et al.*, 1973).

Deuteration of methoxyphenols. The ring hydrogens were exchanged with deuterium under acid conditions (Hawthorne *et al.*, 1989b). The synthesis of D_2-vanillin is given as an example; time and reaction temperatures listed for other compounds are listed in Table 1. All attempts to make D_3-*trans*-isoeugenol in this manner gave hydration products of the alkene. The conditions for the deuteration of eugenol were chosen to minimize its hydration side-product. Isotopic purity was determined by the isotope ratios of the molecular ion. Chemical purity was assessed by gas chromatography with flame ionization detection. A summary of isotopic and chemical purity for the synthesized chemicals is presented in Table 1.

D_2-Vanillin. Vanillin (7 mmol) and approximately 30 ml 4% DCl (99.5% D; Cambridge Isotope Laboratories, Andover, MA) in D_2O (99% D; Cambridge Isotope Laboratories) containing 10 mg/ml $Cr(II)Cl_2$ (anhydrous) were placed in a Teflon microwave digestion vessel. Glass beads were used to reduce the volume of solution needed to completely fill and eliminate all air from the vessel. The vessel was heated to 150°C in a pressure–temperature programmable microwave oven (Floyd, Lake Wylie, SC) for 33 h. The resulting dark solution was rinsed with acetone into a beaker through glass wool and extracted three times with 50 ml of ether. The extract was washed three times with 0.5 M HCl and then dried with Na_2SO_4. The ether was removed under reduced pressure at room temperature. The residue was applied to a silica gel column (60 A, 70–230 mesh; 2.5 × 30 cm; methylene chloride). The product eluted between 150 and 300 ml. Evaporation of the eluate under reduced pressure gave a pink residue, which was recrystallized from petroleum ether-toluene to give tan needles (m.p. 83°).

Potassium α,α,α-trifuoro-m-cresol sulfate. This compound was synthesized from α,α,α-trifuoro-*m*-cresol and sulfur trioxide-pyridine by the method described in Burkhardt and Lapworth (1926). Recrystallization from boiling water gave yellow plates. The sulfate was found to be free of the starting material by reversed-phased HPLC.

Urine Assay

A solution of hydrolysis efficiency standards (potassium α,α,α-trifuoro-*m*-cresol sulfate and 4-nitrophenylglucuronide) in methanol (20 μl, 100 μg/ml) and 0.6 ml of concentrated H_2SO_4 was added to 10 ml of urine. Methoxyphenols were deconjugated by heating the acidified urine in closed screw cap test tubes for 15 min in a boiling water bath. The urine sample was allowed to cool to room temperature and 20 μl of a mixture of deuterated methoxyphenols (100 μg/ml) in ethyl acetate was added. Table 1 lists the deuterated chemicals utilized in this assay. The urine samples were applied over a period of 5 min to preconditioned SPE columns. Multiple samples were processed with a vacuum manifold (Supelco). The SPE columns were then rinsed with 12 ml of 10 mM HCl (pH 2) and then air dried on the vacuum manifold for approximately 2 h. The methoxyphenols were eluted with 4 ml ethyl acetate into a test tube containing 2 μg of 2-chloro-4-methoxyphenol (internal standard). A portion of the eluate was then analyzed by GC-MS.

Gas Chromatography–Mass Spectrometry

Gas chromatography was performed on a 5% diphenyl–95% dimethylpolysiloxane fused-silica capillary column (Rtx-5; 30 m, 0.25 mm i.d., 0.25 μm film thickness; Restek, Bellefonte, PA) utilizing helium carrier gas with a mean linear velocity of 30 cm/s at 200°C. Splitless injections (1 μl) were made with an autosampler (7673, Hewlett–Packard, Avondale, PA). The injection port of the gas chromatograph (5890, Hewlett–Packard,) was maintained at 250°C and contained a deactivated glass, 4-mm-i.d. split liner packed with fused-silica wool (Restek). The oven program started with an isothermal hold of 1 min at 50°C and then was ramped 20°C/min to 275°C. The final temperature was held for 1 min. The mass selective detector (5971, Hewlett–Packard) was utilized in the selected-ion mode. Quantitation ions and retention times are listed in Table 2.

Method Validation

Hydrolysis efficiency of the procedure was determined for potassium α,α,α-trifuoro-*m*-cresol sulfate (0.2 μg/ml) and 4-nitrophenylglucuronide (0.2 μg/ml) in a pooled urine composite. These samples ($N = 6$) were compared to a set ($N = 6$) that had been spiked with the parent phenols and then processed in the same manner.

Recoveries of the methoxyphenols were determined in a urine selected for low background levels of the methoxyphenols. Aliquots of urine (10 ml) were fortified with the methoxyphenols and their deuterated analogs at two levels (0.05 and 0.16 μg/ml) above that of unfortified urine. Background levels of the methoxyphenols were subtracted. Eight replicate analyses were performed at each concentration.

TABLE 2
Quantitation Ions and Retention Times of the Analytes

Peak number[a]	Chemical	Retention time (min)	Quantitation ion (*m/z*)
1	α,α,α-Trifluoro-*m*-cresol	6.24	162
2	D_4-Guaiacol	6.52	113
2	Guaiacol	6.54	109
3	D_3-Methyguaiacol	7.37	126
3	4-Methylquaiacol	7.39	123
4	2-Chloro-4-methoxyphenol	7.75	143
5	4-Ethylquaiacol	8.00	137
5	D_3-Ethylquaiacol	8.02	140
6	D_3-Syringol	8.49	157
6	Syringol	8.51	154
7	D_3-Eugenol	8.54	167
7	Eugenol	8.56	164
8	D_3-Propylquaiacol	8.61	140
8	4-Propylquaiacol	8.63	137
9	D_2-Vanillin	8.87	154
9	Vanillin	8.89	151
10	4-Methylsyringol	9.17	168
11	*trans*-Isoeugenol	9.22	164
12	4-Ethylsyringol	9.66	167
13	4-Nitrophenol	9.76	139
14	D_2-Syringaldehyde	10.46	184
14	Syringaldehyde	10.49	182

[a] See Fig. 1.

Background Levels of Methoxyphenols

Urine samples from 21 adults were collected and analyzed for the methoxyphenols. The volunteers reported no exposure to wood smoke. No restrictions were placed on diet or activities. All subjects lived or worked in metropolitan Seattle, Washington. After initial analysis following the standard method, the samples from these volunteers were concentrated and reanalyzed under scan conditions to verify the identity of the methoxyphenol peaks.

Feeding Experiment

A feeding study was undertaken to demonstrate that administered methoxyphenols were eliminated via urine, to assess the background levels of urinary methoxyphenols, and to determine approximate residence times in humans. Urine from three male adults was collected for 7–10 days as separate voids and analyzed for methoxyphenols. On the 6th day, 0.5 g of wood smoke flavoring was ingested. This amount of flavoring contained approximately 7 mg total methoxyphenol. The dose of each methoxyphenol and its concentration in the wood smoke flavoring are given in Table 3. A diary of food ingestion was kept during the study. Urinary creatinine concentrations were measured by a commercial laboratory. Methoxyphenol concentrations corrected to a standard urinary volume rate of 1 ml/min were also calculated (Araki, 1980).

Inhalation Exposures

We did not have facilities for controlled exposure to wood smoke and therefore, to demonstrate that methoxyphenols from inhaled wood smoke could be detected in urine, we opportunistically obtained urine from an individual on two recreational camping trips with wood smoke exposure from campfires. Urine from this male adult was collected as separate

TABLE 3

Concentrations of Methoxyphenols in the Wood Smoke Flavoring Used in the Feeding Experiment, the Ingested Dose in the Feeding Experiment, and the Inhaled Dose in the Second Inhalation Study

Chemical	Concentration (mg/g)	Ingested dose (mg)	Inhaled dose (mg)
Guaiacol	2.44	1.23	0.008
4-Methylguaiacol	1.71	0.86	0.005
4-Ethylguaiacol	0.77	0.39	0.002
Syringol	4.59	2.31	0.003
Eugenol	0.21	0.11	0.012
4-Propylguaiacol	0.08	0.04	0.001
Vanillin	3.10	1.57	0.028
4-Methylsyringol	0.21	0.11	0.001
trans-Isoeugenol	0.26	0.13	0.002
4-Ethylsyringol	1.36	0.69	0.001
Syringaldehyde	0.34	0.17	0.008

voids over a period encompassing the wood smoke exposure. Urine samples were kept on ice. Both camping areas were remote from urban areas. The first site was in moderately dense Douglas fir. Wind was stagnant to light. A noticeable smoke haze developed from campfires in the late afternoon to evening. A light rain fell on the second night. Only red alder (*Alnus rubra*) was burned on the first trip. On the second camping trip the same volunteer wore a 6-stage Marple personal cascade impactor (Andersen Samplers, Atlanta, GA) backed up by a low-volume polyurethane foam (PUF) tube (19 mm in diameter by 76 mm in length; SKC, Fullerton, CA). The sampling method was adapted from Hawthorne *et al.* (1992). The second site was in narrow valley amidst Ponderosa pine (*Pinus pondersa*). Wind was very light, and a short (5 min) and light rain shower occurred during the sampling. Mostly pine with a small amount of western red cedar (*Thuja plicata*) and big leaf maple (*Acer macrophyllum*) was burned on the second trip.

The mylar films and final filter (glass fiber) from the cascade impactor were extracted for 24 h with 50 ml ethyl acetate that had been spiked with the deuterated methoxyphenols. The extract was evaporated by a nitrogen stream to 1 ml and then quantified by GC-MS. The PUF was soxhlet extracted with 250 ml ethyl acetate for 16 h after spiking with the deuterated methoxyphenols. The extract was concentrated to 1 ml and then analyzed by GC-MS.

Measurement of methoxyphenol concentrations in the personal air space during the second trip allowed us to estimate the dose assuming a standard ventilation rate of a male doing light activity (USEPA, 1989) and 100% bioavailability. The total exposure dose was estimated by this calculation to be 70 μg. The dose for each compound is given in Table 3.

RESULTS

Method Validation

The methoxyphenols and α,α,α-trifuoro-*m*-cresol chromatographed well (Fig. 1), and the ion chromatograms were adequately resolved for the individual compounds. Poor chromatography and diminished response was observed for 4-nitrophenol after approximately 40 urine samples had been run (Fig. 1C,

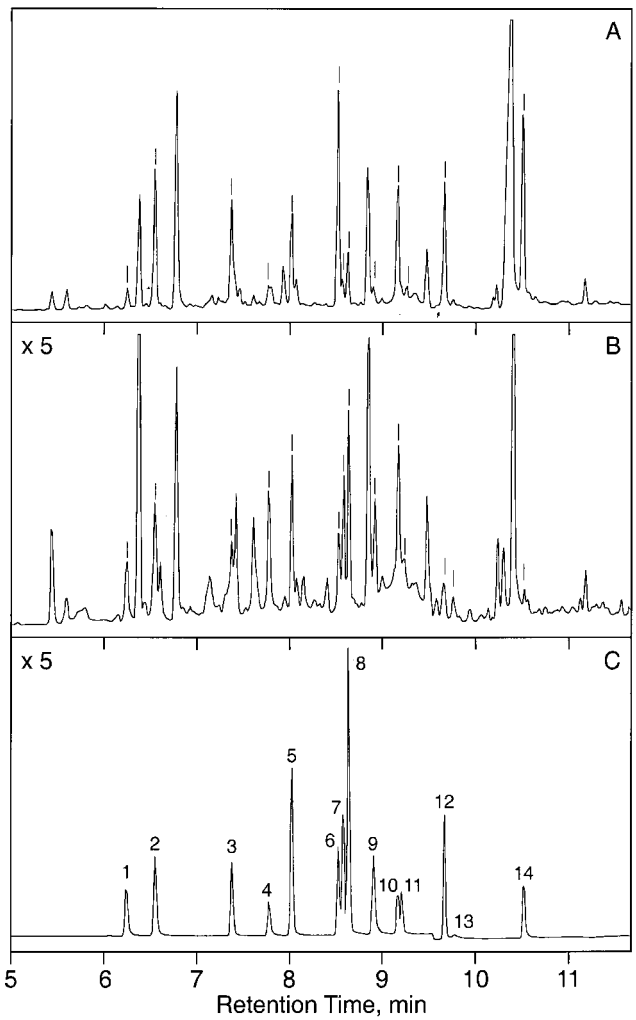

FIG. 1. Total ion chromatograms of methoxyphenol samples from an individual before (B) and after (A) incidental ingestion of wood-smoked food. The vertical scales in B and C are magnified fivefold. Chromatogram B was from the sample taken on January 26, 1998, at 8:56 PM in Fig. 2 (point B). Chromatogram A was from the sample taken on January 27, 1998, at 6:18 PM in Fig. 2 (point E). The concentration of the methoxyphenols in the standard (C) was 0.5 μg/ml. Identities of the peaks are listed in Table 2.

TABLE 4

Recoveries [Mean (%CV)] of the Methoxyphenols at Two Spiking Levels and Background Levels [Mean (%CV)] of Methoxyphenols in Urine

	Recovery Spiking level[a] (μg/ml)		Background level[b] (μg/ml)
	0.05	0.16	
Guaiacol	89(20)	78(15)	0.75(3.7)
4-Methylguaiacol	72(12)	71(8)	0.21(3.2)
4-Ethylguaiacol	68(6)	71(5)	ND
Syringol	75(6)	74(6)	ND
Eugenol	66(24)	64(13)	0.46(2.8)
4-Propylguaiacol	67(5)	70(5)	ND
Vanillin	63(12)	59(7)	0.15(5.7)
4-Methylsyringol	72(6)	70(5)	ND
trans-Isoeugenol	9(9)	11(5)	ND
4-Ethylsyringol	74(7)	73(6)	0.06(3.7)
Syringaldehyde	79(7)	63(9)	0.21(4.1)

Note. ND, not detected. Detection limits were approximately 0.004 μg/ml urine.

[a] Represents the fortification above the background level. $N = 8$.

[b] $N = 4$.

No. 13). This could be rectified by column trimming and inlet liner replacement but the diminishing response made 4-nitrophenylglucuronide a marginal hydrolysis indicator.

Experiments on hydrolysis efficiency showed that α,α,α-trifuoro-*m*-cresol sulfate was nearly completely hydrolyzed (90 ± 4%; mean ± SD) and that 4-nitrophenylglucuronide was completely hydrolyzed (113 ± 35%). The high standard deviation for 4-nitrophenol was do to chromatographic peak tailing.

Recoveries of the methoxyphenols were typically 70% and exhibited no trend with concentration (Table 4). The coefficient of variation (CV) of the recoveries were typically <10%, with the exceptions, such as guaiacol, being chemicals with higher background levels. The low recoveries of *trans*-isoeugenol (Table 4) were caused by its conversion to diisoeugenol, 5-hydroxy-6-methoxy-2-methyl-1-ethyl-3-(4-hydroxy-3-methoxyphenyl)-indan, under hot acidic conditions during the conjugate hydrolysis step of the method.

Background Levels of Methoxyphenols

The background levels of the methoxyphenols were determined in spot (untimed collections) urine samples from 21 adults. Guaiacol, 4-methylguaiacol, eugenol, and vanillin were detected in each sample (Table 5) and had the highest average concentrations. The range of concentrations for guaiacol, 4-methylguaiacol, and eugenol spanned 2 orders of magnitude. 4-Propylguaiacol and *trans*-isoeugenol were detected less than 20% of the time. The ratio of guaiacols to syringols was approximately 8. Background concentrations of methoxyphenols in the exposure subjects were within the range seen in the spot samples (Table 5). The ratio of guaiacols to syringols ranged from 2.5 to 5 in the background samples of the exposure subjects. Subject 1 had a least a 10-fold higher background concentration of 4-methylguaiacol, 4-ethylguaiacol, eugenol, syringol, and 4-propylguaiacol than the other two subjects.

Feeding Studies

Ingestion of wood smoke flavoring produced a similar time course of urinary methoxyphenol concentration in the three subjects. Two episodes of elevated urinary methoxyphenols were observed during the monitoring period for subjects 2 and 3 (Fig. 2). The first was unexpected and was subsequently determined to have occurred after the ingestion of smoked salmon on January 27 at 3:30 PM. Subject 1 did not ingest the smoked salmon and did not exhibit this unexpected rise in urinary methoxyphenol concentrations. All three subjects exhibited an elevation of urinary methoxyphenols (the second peak in Fig. 2) after the planned ingestion of wood smoke flavoring. Urinary methoxyphenols were elevated within 90 min of ingestion. They reached peak concentrations and excretion rates within 4 h and returned to background levels in approximately 18 h. The peak postexposure concentrations for each compound, eugenol excepted, were similar across subjects (Table 5), but because the background levels differed among the subjects, the ratios of background to the peak concentrations varied between the subjects. The peak concentration of eugenol in subject 1 was approximately 10-fold higher than that in the other subjects. Syringol, ethylsyringol, and methylsyringol concentrations increased by 27–32, 120–300, and 200–1200 times, respectively, for subjects 1–3. Unlike the other syringol compounds, syringaldehyde concentrations did not appreciably change after ingestion of the wood smoke flavoring in any of the subjects. The guaiacols (including vanillin, eugenol, and isoeugenol) did not show as dramatic of an increase in urinary concentration and were elevated 2–12, 2–60, and 5–780 for subjects 1–3, respectively.

TABLE 5

Background Concentrations (A) and Excretion Rates (B) of Methoxyphenols in Spot Urine and Pre-exposure Samples Compared to Peak Concentrations and Excretion Rates After Feeding of Wood Smoke Flavoring or Inhalation of Wood Smoke

Chemical	Spot urine 21 Subjects	Feeding experiment[a]			Inhalation exposure[b]	
		Subject 1	Subject 2	Subject 3	1	2
Background concentrations (A) (μg/ml)						
Guaiacol	0.71 ± 1.01[c]	0.97 ± 0.74	0.51 ± 0.32	0.26 ± 0.16	0.99 ± 0.05	1.06 ± 0.59
4-Methylguaiacol	0.10 ± 0.17	0.28 ± 0.90	0.09 ± 0.07	0.04 ± 0.02	0.13 ± 0.01	0.28 ± 0.07
4-Ethylguaiacol	0.06 ± 0.09 (67%)	0.16 ± 0.19 (93%)	0.00 ± 0.00 (12%)	0.01 ± 0.01	0.08 ± 0.04	0.06 ± 0.06
Syringol	0.05 ± 0.05 (57%)	0.27 ± 0.47	0.01 ± 0.01	0.03 ± 0.03 (27%)	0.06 ± 0.01	0.08 ± 0.06
Eugenol	0.27 ± 0.41	1.04 ± 1.71	0.13 ± 0.17	0.20 ± 0.21	0.09 ± 0.04	0.10 ± 0.06
4-Propylguaiacol	0.02 ± 0.02 (19%)	0.10 ± 0.12 (43%)	0.00 ± 0.00 (0%)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Vanillin	0.07 ± 0.07	0.21 ± 0.30 (79%)	0.03 ± 0.01 (94%)	0.04 ± 0.05 (60%)	0.05 ± 0.02	0.06 ± 0.05
4-Methylsyringol	0.02 ± 0.01 (33%)	0.10 ± 0.04 (96%)	0.11 ± 0.05	0.09 ± 0.03	0.41 ± 0.29	0.31 ± 0.08
trans-Isoeugenol	0.14 ± 0.20 (14%)	0.05 ± 0.08 (86%)	0.03 ± 0.01	0.04 ± 0.04 (93%)	0.04 ± 0.01	0.07 ± 0.02 (83%)
4-Ethylsyringol	0.02 ± 0.01 (67%)	0.09 ± 0.12 (75%)	0.02 ± 0.01 (12%)	0.01 ± 0.01	0.11 ± 0.05	0.07 ± 0.05
Syringaldehyde	0.09 ± 0.06 (95%)	0.31 ± 0.38	0.12 ± 0.03	0.19 ± 0.09	0.20 ± 0.09	0.21 ± 0.08
Peak postexposure concentrations (μg/ml)						
Guaiacol		5.71	4.68	10.74	1.69 ± 0.01[d]	1.29
4-Methylguaiacol		3.33	2.50	6.44	0.48 ± 0.21	0.50
4-Ethylguaiacol		1.32	0.81	2.39	0.11 ± 0.01	0.05
Syringol		8.51	8.54	14.53	0.92 ± 0.15	0.08
Eugenol		4.96	0.43	0.65	1.30 ± 0.44	1.47
4-Propylguaiacol		0.11	0.08	0.15	0.010 ± 0.001	0.01
Vanillin		5.62	5.21	9.06	0.38 ± 0.07	0.15
4-Methylsyringol		0.24	0.32	0.88	0.41 ± 0.38	0.32
trans-Isoeugenol		0.27	0.13	0.32	0.09 ± 0.03	0.12
4-Ethylsyringol		2.50	2.26	3.75	0.20 ± 0.02	0.12
Syringaldehyde		0.43	0.34	0.20	0.150 ± 0.000	0.28
Background excretion rates (B), (μg/h)						
Guaiacol		43.7 ± 35.4	33.8 ± 21.3	14.4 ± 13.2	34.6 ± 3.6	36.9 ± 14.8
4-Methylguaiacol		12.5 ± 40.4	5.3 ± 2.3	2.7 ± 2.7	4.7 ± 0.9	11.0 ± 3.7
4-Ethylguaiacol		7.5 ± 9.1 (93%)	0.1 ± 0.1 (12%)	0.9 ± 0.9	2.7 ± 1.4	2.3 ± 1.6
Syringol		12.5 ± 22.7	0.8 ± 0.7	1.3 ± 0.8 (27%)	2.0 ± 0.7	3.2 ± 3.1
Eugenol		39.5 ± 59.3	7.0 ± 6.3	17.3 ± 23.8	3.2 ± 1.7	4.1 ± 3.2
4-Propylguaiacol		4.4 ± 5.1 (43%)	0.0 ± 0.0 (0%)	0.4 ± 0.5	0.5 ± 0.4	0.4 ± 0.3
Vanillin		9.4 ± 13.5 (79%)	2.3 ± 1.6 (94%)	1.6 ± 2.0 (60%)	1.8 ± 0.7	2.6 ± 2.5
4-Methylsyringol		4.5 ± 2.6 (96%)	8.1 ± 4.5	5.5 ± 4.0	15.0 ± 12.1	12.0 ± 3.9
trans-Isoeugenol		2.1 ± 3.0 (86%)	2.7 ± 1.8	1.9 ± 1.2 (93%)	1.2 ± 0.5	2.4 ± 1.2 (83%)
4-Ethylsyringol		4.2 ± 5.4 (75%)	0.7 ± 0.4 (12%)	0.9 ± 1.3	4.0 ± 2.0	2.7 ± 2.2
Syringaldehyde		13.0 ± 16.0	11.4 ± 10.8	9.7 ± 5.6	7.3 ± 3.6	8.1 ± 3.5
Peak postexposure excretion rates (μg/h)						
Guaiacol		210.2	169.1	326.5	76.4 ± 1.3[d]	125.7
4-Methylguaiacol		122.5	90.4	195.9	26.8 ± 3.9	58.7
4-Ethylguaiacol		48.7	29.5	72.8	6.3 ± 2.0	3.6
Syringol		313.2	308.9	441.8	44.5 ± 2.2	9.5
Eugenol		128.0	19.1	19.6	58.1 ± 20.3	172.2
4-Propylguaiacol		4.1	2.9	4.2	0.46 ± 0.04	0.4
Vanillin		206.9	188.5	275.4	18.3 ± 1.2	17.5
4-Methylsyringol		4.0	11.6	26.8	30.1 ± 18.3	30.9
trans-Isoeugenol		9.9	2.8	9.8	4.1 ± 1.5	9.4
4-Ethylsyringol		92.1	81.5	114.1	10.6 ± 1.1	13.9
Syringaldehyde		9.9	11.3	5.0	11.8 ± 0.3	33.3

[a] Subjects 1 and 3 consumed the flavoring dissolved in a soft drink. Subject 2 consumed the flavoring mixed with mashed potatoes. $N = 28$, 17, and 15 for the baseline urine samples for subjects 1, 2, and 3 respectively.

[b] Inhalation exposure 1 was to hardwood smoke; exposure 2 was to softwood smoke. $N = 3$ and 6 for the baseline urine samples for the first and second exposures respectively.

[c] Mean ± SD, with incidences listed as % when less than 100%. Nondetects were not included in the mean.

[d] Average of two peak events. See Fig. 3.

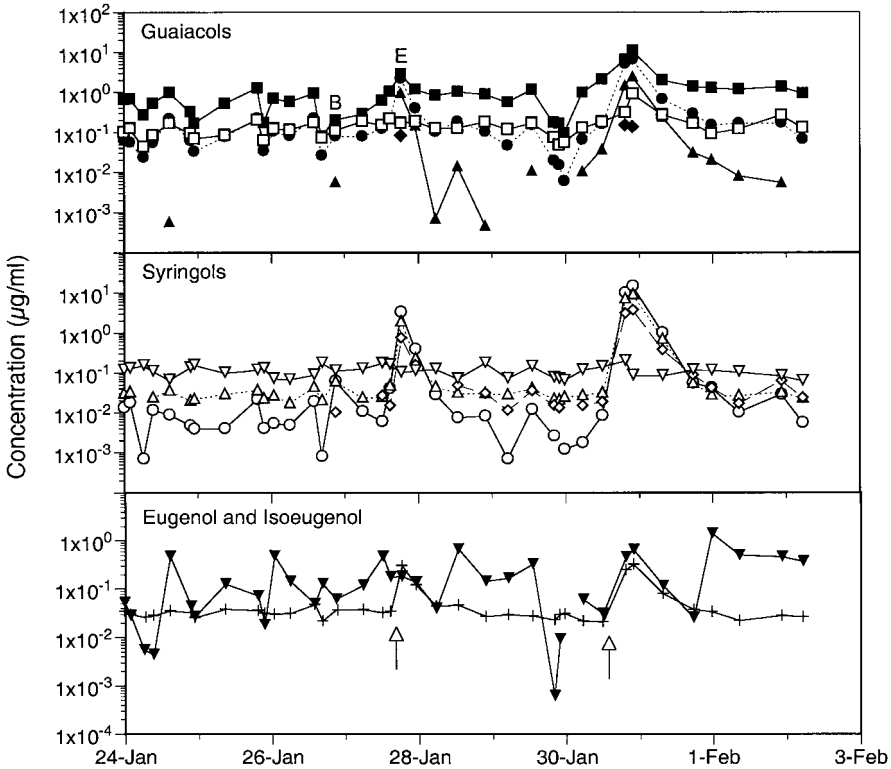

FIG. 2. Urinary concentrations of methoxyphenols in one subject over a period of 9 days. Arrows indicate the time at which wood smoke chemicals were ingested. The first was an unplanned ingestion of a smoked food and the second was the experimental ingestion of wood smoke flavoring. Chromatograms of the sample from points B and E are shown in Fig. 1. Symbols: (■) guaiacol; (●) 4-methylguaiacol; (▲) 4-ethylguaiacol; (◆) 4-propylguaiacol; (□) vanillin; (○) syringol; (△) 4-methylsyringol; (◇) 4-ethylsyringol; (▽) syringaldehyde; (▼) eugenol; (+) *trans*-isoeugenol.

The peak, postexposure excretion rate for each compound, eugenol and vanillin excepted, were similar across subjects and compounds (Table 5), with the ratio of maximum to minimum values ranging from 1.4 to 2.5 (1.6–2.9 for concentration); this ratio for eugenol and vanillin was 6.7 (3.7 and 11.5 for concentrations of vanillin and eugenol). As was seen in the comparison of background concentrations, the ratios of background to peak excretion rates varied between the subjects, because the background excretion rates differed among the subjects. The peak excretion rate of eugenol in subject 1 was approximately 10-fold higher than that in the other subjects. Syringol, ethylsyringol, and methylsyringol excretion rates increased by 22–25, 90–240, and 120–590 times, respectively, for subjects 1–3. Unlike the other syringol compounds, syringaldehyde excretion rates actually diminished slightly (0.4–0.8 of the background average) for subjects 1 and 3 and did not appreciably change for subject 2 (1.2 of the background average). The guaiacols again did not show as dramatic an increase in urinary excretion rate and were elevated 4–10, 11–33, and 9–590 times for subjects 1–3, respectively. Vanillin and 4-propyl guaiacol excretion rates did not change for subject 1.

Concentrations of the methoxyphenols were measured for 6 days prior to ingestion of wood smoke flavoring and gave an indication of the daily fluctuation of these chemicals in urine (Fig. 2; Table 5, background data). The CV for concentration during the time preceding smoked-food ingestion was high for eugenol (150%) and guaiacol (30%), moderate for methylguaiacol (12%) and vanillin (6%), and low (< 1%) for the other chemicals that were detected. The fluctuations in the concentration of eugenol appeared to exhibit periodicity (Fig. 2).

Inhalation Exposures

On the first trip, changes in urinary concentration and excretion rates of methoxyphenols generally followed the pattern of exposure—increasing during the two periods of exposure and decreasing during the periods without exposure (Fig. 3). The urinary

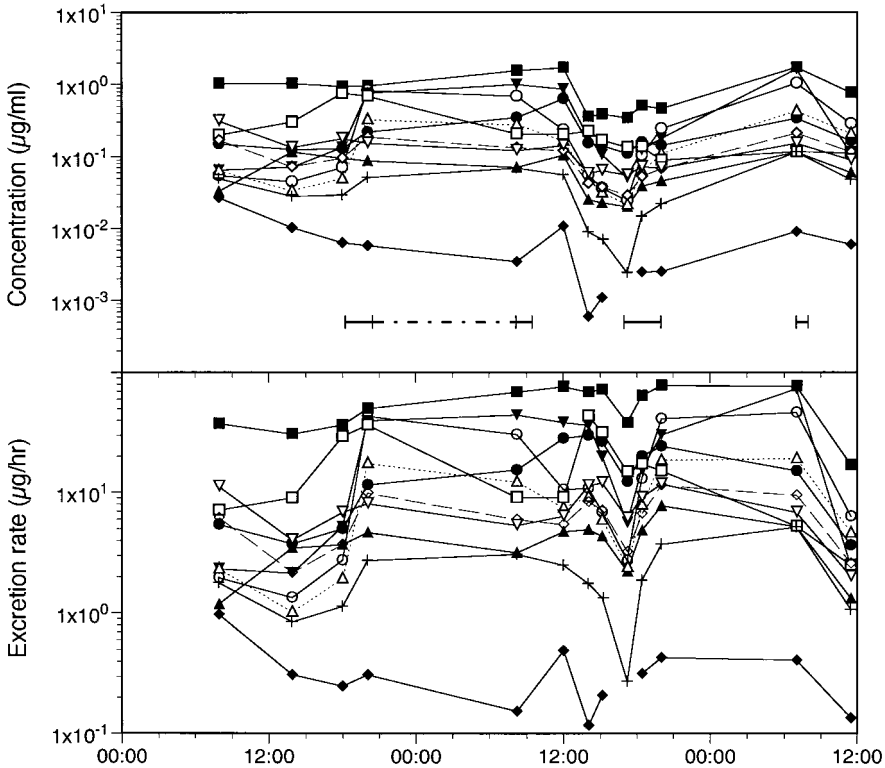

FIG. 3. Urinary concentrations and excretion rates of methoxyphenol before and after inhalation of wood smoke. The bars on the bottom of the top panel mark periods of exposure to wood smoke from a proximal wood fire. During the period marked by the dashed bar, exposure occurred from ambient wood smoke of more distant fires. Symbols are the same as those in Fig. 2.

concentration of guaiacols increased by 1.7–4.7 times the average of the three urinary measurements prior to exposure. The syringols showed a larger increase in concentration (1.8- to 18-fold). A similar pattern but with higher magnitudes was seen with the excretion rates; guaiacols increased 2.2- to 6.3-fold and syringols increased 2.8- to 23-fold. 4-Propylguaiacol, vanillin, and syringaldehyde did not track exposure as well as the other compounds. Vanillin concentration peaked before exposure started and then declined over the next 2 days. Syringaldehyde concentrations did not change appreciably during the experiment, and the highest concentration also occurred prior to exposure, as was the case for 4-propylguaiacol. Normalization of urinary concentrations by urinary creatinine concentration did not change appearance of the time profiles.

On the second trip, the air concentrations of methoxyphenols in the personal air space of the subject were measured (Fig. 4). Vanillin was predominant in concentration (16 $\mu g/m^3$). Eugenol, guaiacol, syringaldehyde, and 4-methylguaiacol were the next highest in concentration (2.7–6.2 $\mu g/m^3$).

Even though the duration of exposure was brief (2.5 h), elevations in the excretion rate of these five methoxyphenols were seen (Fig. 5). Similar behavior was seen for the urinary concentrations after adjustment for urinary flow rate. The rank order of maximum excretion rate (eugenol, guaiacol, 4-methylguaiacol, syringaldehyde, and vanillin) was not the same as the order of air concentrations (vanillin, eugenol, guaiacol, syringaldehyde, and 4-methylguaiacol; Fig. 4). When compared to the average preexposure excretion rate, guaiacol methoxyphenols increased 1.4- to 37-fold, while syringol methoxyphenols increased 2.5- to 9-fold after exposure to the softwood smoke. Similar comparisons of pre- and postexposure data failed for methoxyphenol urinary concentrations and creatinine-adjusted concentrations because of the absence of a postexposure peak for compounds other than eugenol (Fig. 5). The highest guaiacol concentration occurred before exposure. Vanillin and syringaldehyde concentrations in urine did not appear to rise above the preexposure levels. Eugenol and 4-methylguaiacol did increase in concentration above preexposure levels. When concentrations were

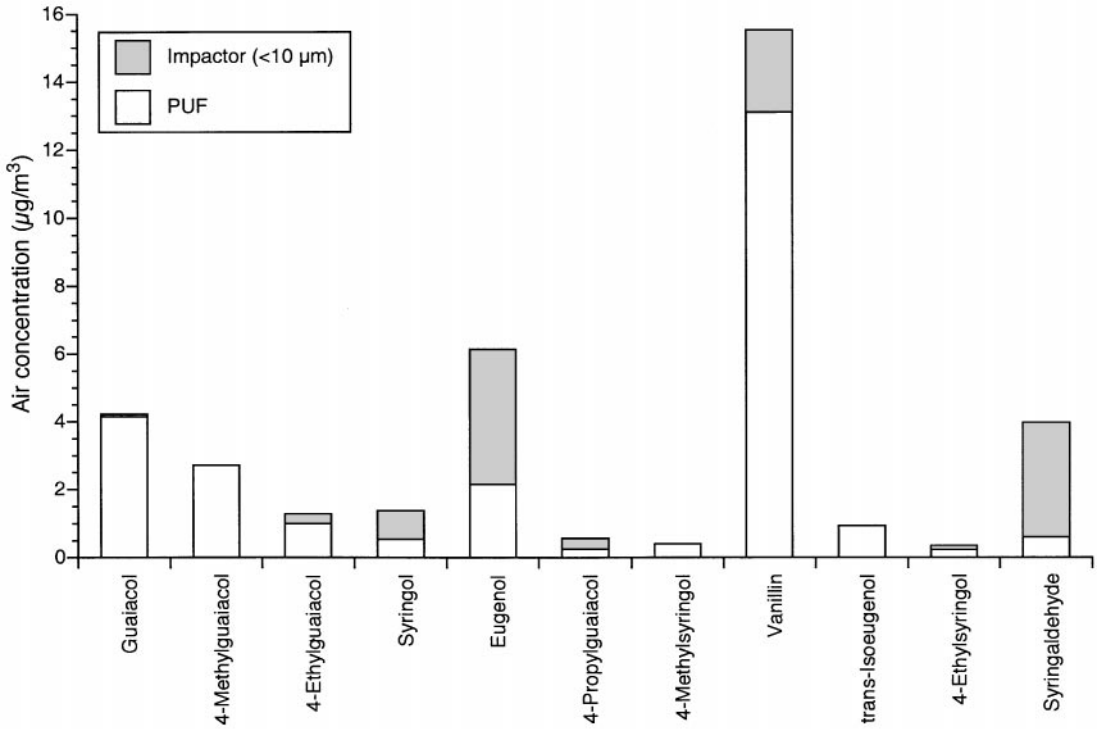

FIG. 4. Composition of wood smoke in the breathing zone of a exposed subject. A personal cascade impactor was backed up by a polyurethane foam (PUF) plug for air sampling during the duration of exposure to wood smoke from a fire.

expressed relative to creatinine, only eugenol had a marked increase after exposure.

DISCUSSION

Method Validation

Parallel treatment of deuterium-labeled standards and analytes would necessitate addition of the labeled standards prior to hydrolysis. However, deuterium in D_3-syringol rapidly exchanges for hydrogen under the hydrolysis conditions but not under subsequent conditions of sample preparation. Therefore, we added the labeled standards after the hydrolysis. Isoeugenol was not adequately quantified by this method because its dimerization produced low recoveries (9–25%). Enzymatic hydrolysis was evaluated and was found to be not as efficient as and much more expensive than acid hydrolysis (but did not dimerize isoeugenol).

Comparison of Exposure Routes

The feeding experiments were an expedient approach to determining whether the methoxyphenols would be eliminated from urine in a timely manner and confirmed that the assay could measure the conjugated methoxyphenols. The inhalation exposures were a test of our hypothesis that airborne methoxyphenols are eliminated in the urine and could be measured. The absorption of the methoxyphenols by either route of exposure had to be rapid to give a rise in urine concentration or elimination rate in the next void (within 1–2 h). The elimination half-life of the methoxyphenols by either route of exposure appeared to be independent of exposure route and short (2–3 h) based on visual inspection.

The oral doses of the methoxyphenols were 100 times the dosage calculated for the second inhalation exposure (Table 3). The peak concentrations of methoxyphenols in urine from the oral dosing exposures were 2- to 120-fold higher than those in the inhalation exposures (Table 5). Excretion rates were 2- to 40-fold higher in the oral dosing exposures. The high values corresponded to syringols, which were less in abundance in the softwood smoke of the inhalation exposure. The higher dose did produce a higher urinary level but the likelihood of obtaining a proportional dose–response in this instance was reduced because the composition of the exposure agents differed and the bioavailability of the two routes may not be equivalent.

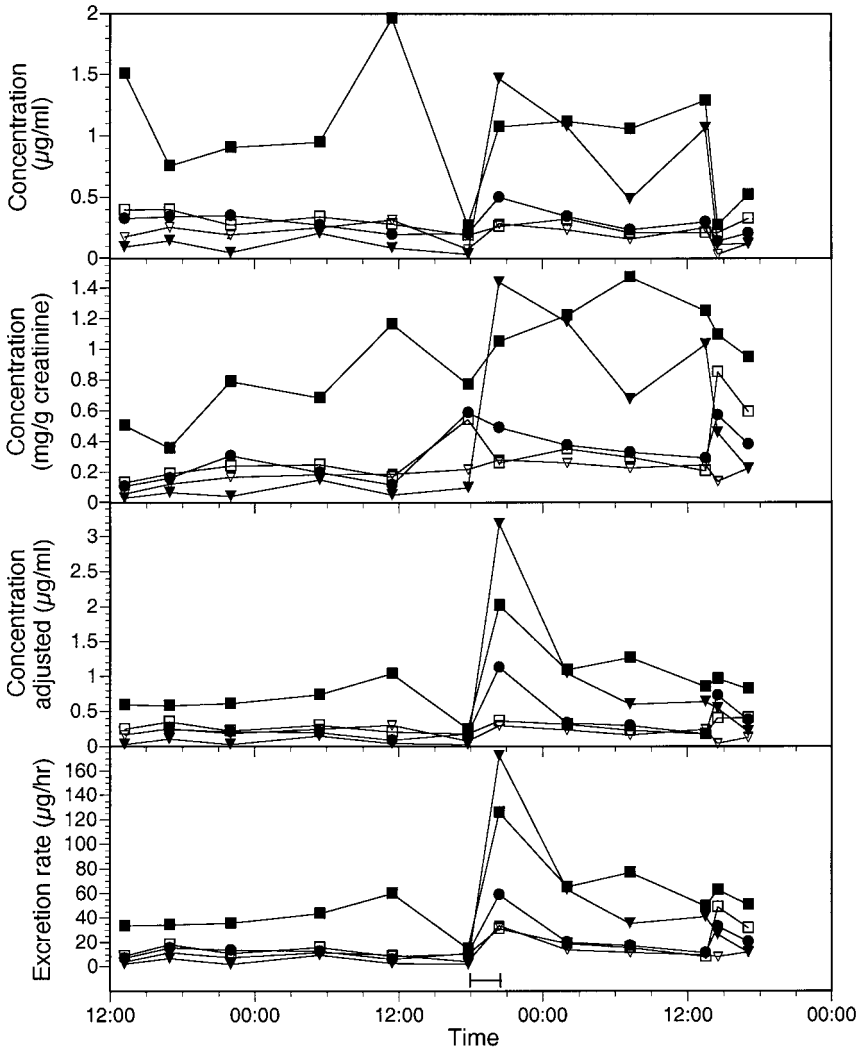

FIG. 5. Three measures of urinary methoxyphenol elimination. The bar marks the period of exposure to wood smoke. The compounds plotted were the five that had the highest air concentrations. Urinary concentration was adjusted to constant urinary flow (1 ml/min). Symbols: (■) guaiacol; (●) 4-methylguaiacol; (□) vanillin; (▽) syringaldehyde; (▼) eugenol.

Methoxyphenols as Biomarkers

Excretion rates of methoxyphenols tracked the wood smoke exposures in the inhalation study. Furthermore, the relative increases of the guaiacols to syringols depended on what type of wood was being burned. When nonconiferous wood (hardwood) was being burned, urinary syringols were predominant and when coniferous wood (softwood) was being burned, urinary guaiacols were predominant. This conformed with the distributions of guaiacols and syringols observed in hard- or softwood smoke (Hawthorne *et al.*, 1988, 1989; Simonelt *et al.*, 1993). Although vanillin was previously identified in air samples from near a coniferous forest fire (Veltkamp *et al.*, 1996), we cannot ascribe all vanillin to burning wood since pines also emit vanillin (Little, 1992).

Since we lacked data on particulate concentrations during the inhalation exposures, we could not experimentally relate urinary elimination of methoxyphenols to the concentration of inhaled particulate and therefore, sensitivity of the method to atmospheric particulate concentration cannot yet be determined. However, given the brief duration (2.5 h; Fig. 5) of the inhalation exposure to a small source, it is likely that the assay would be at least applicable to exposures such as wildfires and cooking fires. Particulate concentrations from these types of exposures ranged from 30 to 4000 $\mu g/m^3$ (Table 6). We do not know if exposures to typical

TABLE 6
Assesments of Wood Smoke Particulate Concentrations

Assesment	Concentration: Mean ± SD ($\mu g/m^3$)	Concentration: Range ($\mu g/m^3$)	Reference
Outdoor air sampling			
City, residential, winter	1.8[a]	0.6–3.4	Hawthorne *et al.* (1992)
City, nonresidential, winter	0.8[a]	0.3–1.6	
City, nonresidential	1.07 ± 0.24[b,d]		Schauer *et al.* (1996)
City, residential	1.55 ± 0.24[b,d]		
City, residential, day	0.09[c]	0.06–0.11	Scheffield *et al.* (1994)
City, residential, night	0.78[c]	0.04–1.65	
Rural, residential, winter	5.5[a]	1.8–12	Hawthorne *et al.* (1992)
Personal air sampling			
Prescribed burn workers		750–4000	Reinhardt *et al.* (1994)
Wildfire fighters		240–3700	Reinhardt *et al.* (1995)
Indoor air sampling			
Biomass burning, kitchen	555[c]	30–1492	Bauer *et al.* (1996)
Biomass burning, kitchen	768[a]	49–1654	

[a] PM_{10}.
[b] PM_2.
[c] $PM_{2.5}$.
[d] Annual average.

urban air levels of wood smoke particulate (≤ 1 $\mu g/m^3$; Table 6) are detectable by the assay. Also, we do not know if the background levels of methoxyphenols in the 21 subjects were in part do to urban air concentrations of wood smoke particulate.

Excretion rate and concentration adjusted to a standard urinary flow rate were better able to indicate exposures to methoxyphenols than urinary concentration with or without creatinine adjustment. It is generally accepted that concentration and creatinine adjustment do not account for effects on elimination due to variation in diuresis (Boenger *et al.*, 1993). Alteration of methoxyphenol concentration by variation in diuresis adds an unknown bias to concentration-based metrics. Excretion rate (mass eliminated/hour) is independent of the diuresis and therefore will not be influenced by the hydration state of the subject. Concentration adjusted to a standard urinary flow rate also is independent of diuresis but requires prior knowledge of the elimination of each compound for each subject (Araki, 1980), which in practice is not likely. Therefore, if the previous and current void times are obtained, excretion rate is the best metric; otherwise, unadjusted concentration should be used.

Intersubject variability of background levels of the methoxyphenols, most likely caused by diet, must be considered when utilizing these compounds as a biomarker. We suspect that comparison of a preexposure baseline to peak levels (either concentration or excretion rate) would to some extent compensate for the dietary or metabolic components of intersubject variability. The individual would in essence serve as their own control. The sensitivity of the biomarker would also depend upon individual variability in background. As a corollary, high-level exposures (as in our feeding study) may well be discerned with only a single background urine sample, while low-level exposures may require an extensive background survey.

The short half-lives of the methoxyphenols suggest that episodic exposures would be detectable only for 1–2 days after sampling. We do not know if prolonged exposures may cause accumulation of the biomarkers and produce a elevated urinary level with a corresponding longer period for detection.

Confounding Sources of Methoxyphenols

Results from the feeding study showed that consumption of smoked foods or smoke flavoring elevated urinary concentrations of the methoxyphenols. However, ingestion of small quantities of retail food products, such as one serving of "alder-smoked" potato chips, did not elevate urinary levels (data not shown). Vanillin (vanilla) and eugenol (cloves) are

commonly used flavorings, which makes these compounds less suitable biomarkers. Oil of cloves is also a dental analgesic. Guafenesin (glyceryl guaiacolate) is an expectorant. The metabolism of guafenesin has been poorly characterized in humans; a major metabolite was identified as β-(2-methoxyphenoxy) lactic acid but minor metabolites and mass balance were not established (vandenHeuvel *et al.*, 1972). Guaiacol was not a reported metabolite of guafenesin in rats (Naito *et al.*, 1969) or ponies (Davis and Wolff, 1970), although the later study reported catechol as a metabolite, which may have been formed through a guaiacol intermediate. Thus, we cannot predict whether over-the-counter cough medicines could be a confounder. Further study is also needed to determine the effect of smoking on the urinary levels of the methoxyphenols. While guaiacols and syringols do appear in tobacco smoke condensate (Arnarp *et al.*, 1989; Arrendale *et al.*, 1982), the cresols predominate because nonwoody plants have lignins primarily composed of hydroxyphenyl units (Ralph and Hatfield, 1991; Saiz-Jimenez and de Leeuw, 1985).

Conclusion

Urinary methoxyphenol elimination rate and concentration adjusted for urine flow appear to be effective biomonitors of wood smoke exposure. The relative amounts of syringol and guaiacol elimination gave an indication of whether the methoxyphenols came from hard- or softwood. The relatively high urinary concentrations of guaiacol in individuals without known exposure to wood smoke would limit the sensitivity of this specific chemical as an indicator. The widespread use of vanillin and eugenol as flavorings diminishes the value of these two compounds as indicators. The aldehydes, syringaldehyde and vanillin, undergo extensive metabolism and are more susceptible to oxidation in air than the other methoxyphenols, which lessens the amount of these compounds eliminated in urine. We suggest that quantifying the suite of the methoxyphenols as a screen for exposure to wood smoke overcomes the difficulties of interpretation for individual compounds. While it is unclear whether interindividual variability and dietary sources will permit these markers to be used for characterization of background wood smoke exposures, we anticipate that higher acute exposures such as would be expected during regional air quality episodes, particularly during fall/winter seasonal inversions, would be assessable using this approach combined with ambient air quality measurement.

ACKNOWLEDGMENTS

Funding was provided by NIEHS Grant ES 08614-01. This study was performed in accordance with University of Washington guidelines for human subjects under the review of the Human Subjects Division.

REFERENCES

Araki, S. (1980). Effects of urinary volume on urinary concentrations of lead aminolaevulinic acid, coproporphyrin, creatinine, and total solutes. *Br. J. Ind. Med*. **37,** 50–54.

Arnarp, J., Bielawski, J., Dahlin, B.-M., Dahlman, O., Enzell, C. R., and Pettersson, T. (1989). Tobacco smoke chemistry. 2. Alkyl and alkenyl substituted guaiacols found in cigarette smoke condensate. *Acta Chem. Scand.* **43,** 44–40.

Arrendale, R. F., Severson, R. F., Chortyk, O. T., and Snook, M. E. (1982). Analyses of mono- and dihydroxybenzenes in tobacco smoke and pyrolyzates by glass capillary gas chromatography. *J. Chromatog. Sci*. **20,** 136–143.

Boeniger, M. F., Lowry, L. K., and Rosenberg, J. (1993). Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: A review. *Am. Ind. Hyg. Assoc. J*. **54,** 615–627.

Brauer, M., Bartlett, K., Regalado-Pineda, J., and Pérez-Padilla, R. (1996). Assessment of particulate concentrations from domestic biomass combustion in rural Mexico. *Environ. Sci. Technol.* **30,** 104–109.

Burkhardt, G. N., and Lapworth, A. (1926). Arylsulphuric acids. *J. Chem. Soc*. 684–690.

Dasch, J. M. (1982). Particulate and gaseous emissions from wood-burning fireplaces. *Environ. Sci. Technol*. **16,** 639–645.

Davis, L. E., and Wolff, W. A. (1970). Pharmacokinetics and metabolism of glyceryl guaiacolate in ponies. *Am. J. Vet. Res*. **31,** 469–473.

Duffy, M. A. (1993). "Physician's Desk Reference for Nonprescription Drugs." 14th ed. Medical Economics Data Production, Montvale, NJ.

Edye, L. A., and Richards, G. N. (1991). Analysis of condensates from wood smoke: Components derived from polysaccharides and lignins. *Environ. Sci. Technol*. **25,** 1133–1137.

Fischer, I. U., von Unruh, G. E., and Dengler, H. J. (1990). The metabolism of eugenol in man. *Xenobiotica* **20,** 209–222.

Hawthorne, S. B., Krieger, M. S., Miller, D. J., and Mathiason, M. B. (1989a). Collection and quantitation of methoxylated phenol tracers for atmospheric pollution from residential wood stoves. *Environ. Sci. Technol*. **23,** 470–475.

Hawthorne, S. B., Miller, D. J., and Aulich, T. R. (1989b). Preparation of deuterated aromatic hydrocarbons, heteroatom-containing aromatics, and polychlorinated biphenyls as internal standards for GC/MS analysis. *Fresenius Z. Anal. Chem*. **334,** 421–426.

Hawthorne, S. B., Miller, D. J., Barkley, R. M., and Krieger, M. S. (1988). Identification of methoxylated phenols as candidate tracers for atmospheric wood smoke pollution. *Environ. Sci. Technol*. **22,** 1191–1196.

Hawthorne, S. B., Miller, D. J., Langenfeld, J. J., and Krieger, M. S. (1992). PM-10 high-volume collection and quantitation of semi- and nonvolatile phenols, methoxylated phenols, alkanes, and polycyclic aromatic hydrocarbons form winter urban air and their relationship to wood smoke emissions. *Environ. Sci. Technol*. **26,** 2251–2262.

Hruza, Sr., D. E., van Praag, M., and Heinsohn, Jr., H. (1974). Isolation and identification of the components of the tar of hickory wood smoke. *J. Agric. Food Chem*. **22,** 123–126.

Kamens, R. M., Rives, G. D., Perry, J. M., Bell, D. A., Paylor, R. F., Jr., Goodman, R. G., and Claxton, L. D. (1984). Mutagenic changes in dilute wood smoke as it ages and reacts with ozone and nitrogen dioxide: An outdoor chamber study. *Environ. Sci. Technol*. **18,** 523–530.

Kornreich, M. R., and Issenberg, P. (1972). Determination of phenolic wood smoke components as trimethylsilyl ethers. *J. Agric. Food Chem*. **20,** 1109–1113.

Larson, T. V., and Koenig, J. Q. (1994). Wood smoke: Emissions and noncancer respiratory effects. *Annu. Rev. Public Health* **15,** 133–156.

Little, E. L. (1992). "The Audubon Society Field Guide to North American Trees." pp. 279–278. Knopf, New York.

Lustre, A. O., and Issenberg, P. (1969). Volatile components of hardwood sawdust smoke. *J. Agric. Food Chem*. **17,** 1387–1393.

McKenzie, L. M., Hao, W. M., Richards, G. N., and Ward, D. E. (1994). Quantification of major components emitted from smoldering combustion of wood. *Atmos. Environ*. **28,** 3285–3292.

Miller, J. J., Powell, G. M., Olavesen, A. H., and Curtis, C. G. (1974). The fate of 2,6-dimethoxy[U-^{14}C]phenol in the rat. *Xenobiotica* **5,** 285–289.

Miller, J. J., Powell, G. M., Olavesen, A. H., and Curtis, C. G. (1976). The toxicity of dimethoxyphenol and related compounds in the cat. *Toxicol. Appl. Pharmacol*. **38,** 47–57.

Naito, S., Mizutani, M., Kitao, K., and Nishimura, S. (1969). Biopharmaceutical studies on guaiacol glycerol ether and related compounds. III. Metabolites of guaiacol glycerol ether and its mononicotinate. *Chem. Pharm. Bull*. **17,** 1794–1798.

NRC (1998). "Research Priorities for Airborne Particulate Matter." Natl. Acad. Press, Washington, DC.

Ogata, N., Matsushima, N., and Shibata, T. (1995). Pharmacokinetics of wood creosote: glucuronic acid and sulfate conjugation of phenolic compounds. *Pharmacology* **51,** 195–204.

Pérez-Padilla, R., Regalado, J., Vedal, S., Paré, P., Chapela, R., Sansores, R., and Selman, M. (1996). Exposure to biomass smoke and chronic airway disease in Mexican women. *Am. J. Respir. Crit. Care Med*. **154,** 701–706.

Piotrowski, J. K. (1971). Evaluation of exposure to phenol: absorption of phenol vapor in the lungs through the skin and excretion of phenol in urine. *Br. J. Ind. Med*. **28,** 172–178.

Pope, C. A., III, Dockery, D. W., Spengler, J. D., and Raizenne, M. E. (1991). Respiratory health and PM_{10} pollution: A daily time series analysis. *Am. Rev. Respir. Dis*. **144,** 668–674.

Pope, C. A., III, Thun, M. J., Namboodiri, M. M., Dockery, D. W., Evans, J. S., Speizer, F. E., and Heath, C. W., Jr. (1995). Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. *Am. J. Respir. Crit. Care Med*. **151,** 669–674.

Ralph, J., and Hatfield, R. D. (1991). Pyrolysis-GC-MS characterization of forage materials. *J. Agric. Food Chem*. **39,** 1426–1437.

Reinhardt, T. E., Black, J., and Ottmar, R. D. (1995). "Smoke Exposure at Pacific Northwest Wildfires." U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station, Seattle.

Reinhardt, T. E., Hanneman, A., and Ottmar, R. D. (1994). "Smoke Exposure at Prescribed Burns." U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station, Seattle.

Sammons, H. G., and Williams, R. T. (1941). Studies in detoxication 12. The metabolism of vanillin and vanillic acid in the rabbit. The identification of glucurovanillin and the structure of glucurovanillic acid. *Biochem. J*. **35,** 1175–1189.

Sarkanen, K. V., and Hergert, H. L. (1971). Classification and distribution of lignins. *In* "Lignins" (K. V. Sarkanen and C. H. Ludwig, Eds.), pp. 74–79. Wiley–Interscience, New York.

Schauer, J. J., Rogge, W. F., Hildemann, L. M., Mazurek, M. A., and Cass, G. R. (1996). Source apportionment of airborne particulate matter using organic compounds as tracers. *Atmos. Environ*. **30,** 3837–3855.

Schwartz, J., Dockery, D. W., and Neas, L. M. (1996). Is daily mortality associated specifically with fine particles? *J. Air Waste Manage. Assoc*. **46,** 927–939.

Schwartz, J., Slater, D., Larson, T. V., Pierson, W. E., and Koenig, J. Q. (1993). Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am. Rev. Respir. Dis*. **147,** 826–831.

Sheffield, A. E., Gordon, G. E., Currie, L. A., and Riederer, G. E. (1994). Organic, elemental, and isotopic tracers of air pollution sources in Albuquerque, NM. *Atmos. Environ*. **28,** 1371–1384.

Saiz-Jimenez, C., and de Leeuw, J. W. (1985). Lignin pyrolysis products: Their structures and their significance as biomarkers. *Adv. Org. Geochem.* **10,** 869–876.

Simonelt, B. R. T., Rogge, W. F., Mazurek, M. A., Standley, L. J., Hildemann, L. M., and Cass, G. R. (1993). Lignin pyrolysis products, lignins, and resin acids as specific tracers of plant classes in emissions from biomass combustion. *Environ. Sci. Technol*. **27,** 2533–2541.

Strand, L. P., and Scheline, R. R. (1975). The metabolism of vanillin and isovanillin in the rat. *Xenobiotica* **5,** 49–63.

USEPA (1989). "Exposure Factors Handbook." EPA/600/8-89/043. Chapt. 3, p. 4. U.S. Govt. Printing Office, Washington, DC.

vandenHeuvel, W. J. A., Smith, J. L., and Silber, R. H. (1972). β-(2-Methoxyphenoxy)lactic acid, the major urinary metabolite of glyceryl guaiacolate in man. *J. Pharm. Sci*. **61,** 1997–1998.

Veltkamp, P. R., Hansen, K. J., Barkley, R. M., and Sievers, R. E. (1996). Chromatographic measurement of molecular markers of sources of atmospheric aerosol particles. *Environ. Geochem. Health* **18,** 77–80 (1996).