Supplementary Material

Biospecimen Collection Procedures

All Adult Interview respondents were asked to provide urine and blood biospecimens. Full-void urine specimens were self-collected by 21,801 (67.5%) consenting participants in a 500 mL polypropylene container (PN 6542, Globe Scientific), immediately placed in a Crēdo Cube shipper (Series 4-496, Minnesota Thermal Science) certified to hold contents between 2°C and 8°C for at least 72 hours and shipped overnight to the PATH Study biorepository. Each specimen was divided into aliquots and stored in FluidX® polypropylene cryovials at -80°C. All containers, pipet tips, and vials that came in direct contact with the urine sample were pre-screened by the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC) Laboratories and determined not to have amounts of metal contamination that would adversely influence the analytical measurements. For more information on the aliquots created from the urine biospecimens please see the PATH W1 Biospecimen Urine Collection Procedures https://doi.org/10.3886/Series606.

Blood was collected by trained phlebotomists from 14,520 (44.9%) participants in one 2.7 mL blue top citrate, two 10.0 mL red top serum, two 10.0 mL lavender top EDTA, and one 2.5 mL PAXgene. and were immediately placed in a Crēdo Cube shipper (Series 4-248, Minnesota Thermal Science) certified to hold contents between 2°C and 8°C for at least 72 hours and shipped overnight to the PATH Study biorepository. For more information on the processing and aliquots created from the blood biospecimens please see the PATH W1 Biospecimen Blood Collection Procedures https://doi.org/10.3886/Series606.

Biomarker Data

Biospecimens collected from a subset of adult respondents were sent for laboratory analyses. A stratified probability sample of 11,522 adults who completed the Wave 1 Adult Interview and who provided a urine specimen were selected for analyses. The sample was selected to ensure respondents represented diverse tobacco product use patterns, including users of multiple tobacco products, and never users of any tobacco product. Of the 11,522 adults who provided urine samples, 7,159 also provided a blood specimen.

Urine and blood (serum) specimens were shipped overnight on dry ice to the laboratories at the Centers for Disease Control and Prevention (CDC), National Center of Environmental Health, Division of Laboratory Sciences, Atlanta, GA, where they were stored at -80°C until ready for laboratory analyses.

All biomarker results reported by CDC met the rigorous accuracy and precision requirements of the quality control/quality assurance program of the CDC (Caudill, Schleicher, & Pirkle, 2008).

In addition, when subject samples are shipped for analysis, the PATH Study team included blinded QC pool samples randomly embedded with the shipped samples to be analyzed during subject sample analysis runs. Blinded replicate subject samples are also included in certain shipments. These blinded samples are used to monitor within-run and between run analysis variability.

Given that not all respondents agreed to provide biospecimens, the resulting biospecimen assay data represent a subsample of adults, therefore specific urine and blood weights are needed to account for potential differences between the full set of adult interview respondents in the specified tobacco product user groups and the set of adults with analyzed biospecimens. Weighted estimates are representative of never, current, and recent former (within 12 months) users of tobacco products in the U.S. civilian, noninstitutionalized adult population at the time of Wave 1. These weighting procedures are outlined in the Biospecimen Restricted Use Files User Guide https://doi.org/10.3886/Series606.

Laboratory Methods

Detailed laboratory procedure manuals are available in the data and documentation section of the PATH Study Biomarker Restricted Use Files - https://www.icpsr.umich.edu/icpsrweb/NAHDAP/studies/36840/datadocumentation#.

Urine Biomarkers

Inorganic Arsenic Species

Inorganic arsenic species and their metabolites (Arsenate [As(V)], arsenite [As(III)], monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)) were measured by high performance liquid chromatography/inductively coupled plasma dynamic reaction cell mass spectrometry (HPLC-ICP-DRC-MS). The limits of detection ranged from 0.11 to 1.91 ng/mL, depending upon the analyte (Caldwell et al., 2009; Verdon et al., 2009). Total inorganic arsenic was calculated by taking the sum of the arsenous acid, arsenic acid, dimethylarsinic acid, and monomethylarsonic acid levels in each urine sample.

Metals

Total urine element concentrations of beryllium, cadmium, cobalt, manganese, lead, strontium, thallium, and uranium were measured using inductively coupled plasma mass spectrometry (ICP-MS).

The limits of detection for these elements in urine ranged from 0.002 to 2.34 ng/mL, depending on the analyte (Caldwell et al., 2005; Jarrett et al., 2008).

Nicotine Metabolites

Total urinary nicotine metabolites, including the free and glucuronide conjugated forms, were measured by two separate isotope dilution high performance liquid chromatography/tandem mass spectrometric (HPLC-MS/MS) methods based on the cotinine cutoff value of 20 ng/mL. For samples with cotinine levels above or equal to 20 ng/mL, the "Nicotine Metabolites and Analogs in Urine" method was used to measure anatabine, anabasine, and nicotine plus its six major metabolites (cotinine-N-oxide, nicotine-N-oxide, trans-3'-hydroxycotinine, norcotinine, cotinine and nornicotine; Wei et al., 2014). For samples with cotinine levels less than 20 ng/mL, the "Cotinine and Hydroxycotinine in Urine" method was applied to sensitively measure cotinine and trans-3'-hydroxycotinine using a modified version of the method of Bernert et al. (2005). The limits of detection ranged from 0.030 to 10.5 ng/mL, depending on the analyte. TNE-2 (Total Nicotine Equivalent including two analytes) was calculated for all samples by taking the molar sum of only the two most abundant metabolites of nicotine: cotinine and trans-3'-hydroxycotinine. Furthermore, TNE-7 (Total Nicotine Equivalents including seven nicotine metabolites) was calculated for all samples if cotinine levels were at least 20 ng/mL by taking the molar sum of nicotine and its six major metabolites.

Tobacco Specific Nitrosamines (TSNAs)

Total NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol), NNN (N'-nitrosonornicotine), NAB (N'-nitrosoanabasine) and NAT (N'-nitrosoanatabine) were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (HPLC-MS/MS) using a modified version of the method of Xia et al. (2014). The limit of detection for urinary TSNAs ranged from 0.0006 to 0.0042 ng/mL, depending on the analyte.

Polycyclic Aromatic Hydrocarbons (PAHs)

Seven monohydroxylated metabolites of PAHs (1-hydroxynaphthalene, 1-hydroxyphenanthrene, 1-hydroxypyrene, 2-hydroxyfluorene, 2-hydroxynaphthalene, 3-hydroxyfluorene, the sum of 2 & 3 hydroxynaphthalene) were measured using enzymatic hydrolysis, on-line solid phase extraction, and isotope dilution liquid chromatography tandem mass spectrometry (Wang et al., 2016).

Volatile Organic Compounds (VOCs)

Twenty biomarkers of exposure to VOCs were measured (See PATH Study VOCM Lab Panel Document for complete list of VOCMs measured) using isotope dilution UPLC-MS/MS as described by Alwis et al. (2012) and modified by Alwis et al. (2016).

Urinary Creatinine

Creatinine in urine was measured by an enzymatic assay on a commercial automated clinical chemistry analyzer. In this method, creatinine was converted to glycine, formaldehyde and hydrogen peroxide with the aid of creatininase, creatinase, and sarcosine oxidase. Catalyzed by peroxidase, the liberated hydrogen peroxide reacts with 4-aminophenazone and hydroxyl(tosyloxy)iodobenzene (HTIB) to produce a quinone imine chromogen. The intensity of the color produced by the formation of the quinone imine chromogen is directly proportional to the creatinine concentration. The limit of detection is 1.1 mg/dL.

Blood Biomarkers

Serum Cotinine and trans-3'-hydroxycotinine

Cotinine and trans-3'-hydroxycotinine were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (HPLC-APCI-MS/MS) using a modified version of the method of Bernert et al. (2006). The limits of detection were 0.015 ng/mL for both analytes.

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