Evaluation of Tobacco Smoke and Diet as Sources of Exposure to Two Heterocyclic Aromatic Amines for the U.S. Population: NHANES 2013–2014

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ABSTRACT

Background: Heterocyclic aromatic amines (HAA) are a group of hazardous substances produced during combustion of tobacco or high-temperature cooking of meats. 2-Amino-9H-pyrido[2,3-b]indole (AαC) is a major carcinogenic HAA in tobacco smoke.

Methods: Urinary AαC, used as a marker of AαC exposure, was analyzed on spot urine samples from adult participants of the 2013–2014 cycle of the National Health and Nutrition Examination Survey ($N = 1{,}792$). AαC was measured using isotope-dilution liquid chromatography–tandem mass spectrometry. Exclusive combusted tobacco smokers were differentiated from nonusers of tobacco products through both self-report and serum cotinine data.

Results: Among exclusive smokers, sample-weighted median urinary AαC was 40 times higher than nonusers. Sample-weighted regression models showed that urinary AαC increased significantly with serum cotinine among both exclusive tobacco users and nonusers with secondhand smoke exposure. Among nonusers, eating beef cooked at high temperature was associated with a significant increase in urinary AαC, whereas consuming vegetables was associated with decreased AαC. In addition, smoking one-half pack of cigarettes per day was associated with a significant increase of 23.6 pg AαC/mL calculated at geometric mean of AαC, controlling for potential confounders. In comparison, increase in AαC attributable to consuming the 99th percentile of beef cooked at high temperature was 0.99 pg AαC/mL.

Conclusions: Both exclusive smokers and nonusers of tobacco in the general U.S. population are exposed to AαC from tobacco smoke, with additional, lesser contributions from certain dietary components.

Impact: AαC is an important biomarker that is associated with tobacco smoke exposure.

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Introduction

Tobacco use is the single largest preventable cause of disease and death in the United States, and tobacco smoking is responsible for more than 480,000 deaths each year in the United States (1). A significant body of evidence accumulated over several decades suggests that tobacco smoke exposure is related to development of lung cancer and other cancers (1–4). Various carcinogens have been identified in tobacco smoke (4–6).

Heterocyclic aromatic amines (HAA) are a group of hazardous substances produced during burning of tobacco or high-temperature cooking of meats (7–10). HAAs are amines that contain at least one heterocyclic ring and one aromatic ring (Supplementary Fig. S1). HAAs are strongly mutagenic and carcinogenic in various *in vitro* and *in vivo* models. HAAs can induce tumors in various organs, including mammary glands, prostate, lungs, colon, skin, bladder, and liver (11–14). Epidemiologic studies associate frequent HAA exposure with elevated cancer risk (15–17). The International Agency for Research on Cancer (IARC) categorized a number of HAAs as possible (Group 2B) and probable (Group 2A) human carcinogens (18).

Among more than 25 HAAs so far identified (19), 2-amino-9H-pyrido[2,3-b]indole (AαC, Group 2B carcinogen) is one of most abundant carcinogenic HAAs in tobacco smoke (10). AαC levels in tobacco smoke can reach as high as 260 ng per cigarette (7, 10, 20, 21). This approaches the levels of other well-known carcinogens in tobacco smoke, such as N'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and benzo[α]pyrene (5, 22, 23).

Another carcinogenic HAA detected in tobacco smoke is 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC, Group 2B carcinogen; Supplementary Fig. S1). MeAαC is a methyl homolog of AαC. MeAαC levels in tobacco smoke are about 10-fold lower than AαC levels (7, 8, 10). These two HAAs are carcinogenic in animal models (5, 24–28), and IARC classifies AαC and MeAαC as suspected human carcinogens (29). In addition, the FDA lists AαC and MeAαC as harmful and potentially harmful constituents in tobacco products and tobacco smoke (30). Besides tobacco smoke, AαC and MeAαC can form in foods prepared at high temperatures (approximately 200°C–300°C), such as barbecued, fried, or broiled meats, poultry, and fish (8, 31–34).

Metabolic activation has been proposed as a mechanism for the carcinogenicity of AαC and MeAαC (9, 35). Cytochrome P450 enzymes are involved in the oxidation of the exocyclic amine group of AαC and MeAαC (35–37). Sequentially, these N^2-hydroxylated metabolites of AαC and MeAαC can be further catalyzed by acetyltransferases, sulfotransferases, or glucuronosyltransferases to generate reactive O-esters that could bind covalently to DNA and elicit genotoxicity (38–43).

Although the potential for AαC and MeAαC to harm human health is well documented, few studies have evaluated human exposure to these two carcinogens. A small-scale study (170 subjects) conducted in

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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China documented that AαC was consistently detected in smokers' urine. The number of cigarettes smoked per day (CPD) was positively associated with urinary levels of AαC in study participants (44). A U.S. study (30 subjects) detected AαC in most of the urine samples of smokers, but those levels had dropped 87% after 6 weeks of not smoking (45). Even fewer studies have measured MeAαC in human biospecimens. The aim of this report is to characterize human exposure to AαC and MeAαC in the general population. We measured AαC and MeAαC as urinary biomarkers of exposure to these carcinogenic HAAs as part of the National Health and Nutrition Examination Survey (NHANES), which conducts biomonitoring of the U.S. civilian, noninstitutionalized population.

Materials and Methods

Study design

NHANES is a national survey that assesses population health and nutritional status based on data collected from a cross-sectional, multistage probability sample that is representative of the noninstitutionalized U.S. civilian population. As part of Centers for Disease Control and Prevention (CDC), the National Center for Health Statistics (NCHS) is responsible for conducting NHANES and collecting questionnaire data, physical examination data, and biospecimens from participants. The NCHS Research Ethics Review Board reviewed and approved the study, and informed written consent was obtained from all participants before they took part in the study.

Spot urine samples were collected during physical examinations carried out in a mobile examination center (MEC) from participants aged ≥18 years in the NHANES 2013–2014 survey cycle. We subsequently measured urinary AαC and MeAαC in a special one third sample of MEC participants (NHANES dataset HCAAS_H; $N =$ 2,605) that included all adult smokers in the 2013–2014 cycle. Results reported here, however, are from a subset of these participants who met eligibility criteria and for whom all required data were present (this attrition is detailed in *Identifying Users of Combusted Tobacco and Non-Users of Tobacco* below).

Analytical method

Spot urine samples were stored at −70°C before assay. The total urinary concentrations of AαC and MeAαC (free and conjugated forms) were analyzed using isotope-dilution LC/MS/MS and robotic sample preparation (46). Briefly, an aliquot of 0.5 mL of urine samples was spiked with internal standards that are stable isotope analogs of targeted analytes. The conjugated forms of AαC and MeAαC in the samples were hydrolyzed at base condition for 5 hours. After hydrolysis, the total urinary AαC and MeAαC were extracted from urine matrix by using diatomaceous earth and mixed mode of cation exchange solid phase extraction plates, respectively. The throughput and precision of sample preparation was improved through automated solid phase extraction using an integrated robotic system (46). Analytes were chromatographically resolved from urinary interferences using a reversed phase column (Agilent Zorbax Eclipse Plus C18, 2.1 × 100 mm 3.5 μm). AαC and MeAαC in the samples were detected by AB Sciex API 5500 QTRAP system. The limits of detection of AαC and MeAαC were 0.62 and 0.33 pg/mL, respectively. Serum cotinine was measured by using LC/MS/MS (47).

Low-concentration and high-concentration quality control materials and blank urines were run together with NHANES 2013–2014 samples to evaluate method performance on the day of analysis. The reported data satisfy the accuracy and precision requirements of the quality control/quality assurance program of the CDC National Center for Environmental Health, Division of Laboratory Sciences (48). Measurements below the limit of detection were substituted with the limit of detection divided by the square-root of two (49, 50).

Identifying users of combusted tobacco and nonusers of tobacco

Exclusive combusted tobacco smokers were differentiated from nonusers of tobacco products through both self-report and serum cotinine data (51, 52), which is detailed in Supplementary Information. Attrition of participants for statistical analysis is as follows: missing serum cotinine data (146 participants), use of smokeless tobacco and nicotine replacement therapy (135 participants), or missing data for other variables involved in regression models (532 participants). This attrition resulted in 1,792 study participants eligible for statistical analysis for AαC and 1,793 study participants for MeAαC. For the CPD regression model described below, 32 additional participants were excluded for missing CPD data leaving 1,760 study participants.

Statistical analysis

NHANES recruits participants by using a multistage, probability sampling design. This complex design must be accounted for in order to estimate variances correctly and to achieve unbiased, nationally representative statistics. Robust estimation can be made by implementing survey sample weights (NHANES Special Sample weight; WTFSM) on each participant's data and performing Taylor series linearization. This estimation approach was conducted in the statistical software applications SUDAAN, Version 11.0.0 (Research Triangle Institute, Research Triangle Park, NC) and SAS 9.4 (SAS Institute Inc.). Data from the NHANES 2013–2014 sampling cycle were analyzed with sample-weighted linear regression models that were stratified by tobacco use status (exclusive smokers vs. nonusers). Parameters, including absolute change in biomarker concentration (ΔY) relative to the jth predictor ΔX_j, from these models were estimated as described in detail in Supplementary Information.

Sample-weighted regression models were stratified by smoking status, and a collection of demographic variables as follows were included as predictors: sex, age, race/Hispanic origin, body mass index (BMI), impoverishment (poverty income ratio < 1.00, indicating self-reported family income below the U.S. Census poverty threshold), and fasting time (between specimen collection and last consumption of anything other than water). Except for BMI, information for these potential confounders was self-reported. The reference group was male for sex and non-Hispanic white for race/Hispanic origin. Age in years was divided into ranges: 18–39, 40–59, and ≥60, with 40–59 years as the reference group. For adults ≥20 years, BMI was defined using standardized cut-points: underweight (BMI < 18.5), healthy weight (18.5 ≤ BMI < 25), and overweight/obese (BMI ≥ 25). Participants < 20 years were identified as underweight, healthy weight, and overweight/obese if they were below the 5th percentile, between the 5th and 85th percentile, and above the 85th percentile, respectively, for their sex and age (www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html).

Cotinine is a metabolite of nicotine, the primary addictive ingredient in tobacco products. Cotinine in human serum is a highly specific biomarker of tobacco smoke exposure. With a half-life of 16 to 18 hours, cotinine is suitable for biomarker studies concerning recent tobacco use (52). Serum cotinine was included as a continuous predictor to reflect tobacco smoke exposure. Among tobacco nonusers, tobacco smoke exposure is attributable to secondhand tobacco smoke (SHS). SHS exposure can be assessed based on serum cotinine concentrations. In addition, we tested direct association of urinary

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Table 1. Sample-weighted demographic proportions for AαC ($N = 1{,}792$) and MeAαC ($N = 1{,}793$) among NHANES 2013–2014 participants.

	AαC[a]				MeAαC[a]			
	Sample size[b]	Percent (SE[c])	Sample size[b]	Percent (SE[c])	Sample size[b]	Percent (SE[c])	Sample size[b]	Percent (SE[c])
Variables	Tobacco smokers		Nontobacco users		Tobacco smokers		Nontobacco users	
Age (years)								
18–39	276	46.7 (3.08)	419	36.0 (1.43)	276	46.7 (3.01)	419	36.1 (1.41)
40–59	264	40.1 (3.17)	347	34.4 (1.78)	264	40.0 (3.03)	347	34.5 (1.75)
≥60	123	13.3 (1.59)	363	29.6 (1.79)	124	13.3 (1.57)	363	29.4 (1.72)
BMI								
Healthy	222	34.9 (1.79)	337	30.1 (1.86)	223	35.0 (1.81)	337	30.0 (1.88)
Overweight/obese	421	62.4 (2.02)	779	69.2 (1.80)	420	62.3 (2.06)	779	69.3 (1.82)
Under	20	2.70 (0.65)	13	0.77 (0.17)	21	2.73 (0.64)	13	0.77 (0.17)
Poverty income ratio (PIR)								
PIR < 1	260	28.3 (3.61)	213	11.8 (1.55)	261	28.3 (3.42)	213	11.8 (1.54)
PIR ≥ 1	403	71.7 (3.61)	916	88.2 (1.55)	403	71.7 (3.42)	916	88.2 (1.54)
Race								
Mexican American	43	6.21 (2.25)	190	10.4 (2.21)	43	6.21 (2.26)	190	10.4 (2.21)
Non-Hispanic Black	172	17.0 (2.49)	185	9.13 (1.39)	172	17.1 (2.45)	185	9.15 (1.39)
Non-Hispanic White	349	67.4 (3.85)	478	66.9 (3.70)	351	67.4 (3.80)	478	66.9 (3.67)
Other Hispanic	38	3.52 (1.32)	113	5.52 (0.96)	37	3.46 (1.26)	113	5.54 (0.96)
Other/multiracial	61	5.84 (1.11)	163	8.03 (0.85)	61	5.85 (1.11)	163	8.05 (0.85)
Sex								
Female	301	45.6 (2.31)	606	52.8 (1.78)	303	45.9 (2.42)	605	52.7 (1.79)
Male	362	54.4 (2.31)	523	47.2 (1.78)	361	54.1 (2.42)	524	47.3 (1.79)

[a]Same data as in stratified serum cotinine regression models.
[b]Sample size, unweighted.
[c]Standard error.

Table 2. Sample-weighted median (25th, 75th percentile) of urinary AαC ($N = 1{,}792$) and MeAαC ($N = 1{,}793$) concentrations (creatinine adjusted) by age, sex, BMI, and race/Hispanic origin, categorized by smoking status among U.S. population.

	AαC[a] [ng/g creatinine (25th, 75th percentile)]		MeAαC[a] [ng/g creatinine (25th, 75th percentile)]	
Variables	Tobacco smokers[b]	Nontobacco users[c]	Tobacco smokers[d]	Nontobacco users[e]
All	31.9 (10.2–79.4)	0.79 (0.45–1.51)	1.57 (0.35–4.43)	0.26 (0.16–0.49)
Age (years)				
18–39	19.7 (5.64–51.7)	0.79 (0.45–1.64)	0.90 (0.23–2.85)	0.23 (0.15–0.45)
40–59	46.0 (18.5–86.7)	0.83 (0.43–1.52)	2.28 (0.53–5.22)	0.29 (0.17–0.53)
≥60	49.5 (18.6–105.2)	0.77 (0.46–1.29)	2.44 (0.66–6.66)	0.28 (0.18–0.52)
BMI				
Healthy	35.4 (8.87–74.27)	0.93 (0.54–1.69)	1.84 (0.33–4.27)	0.37 (0.20–0.55)
Overweight/obese	30.2 (9.18–73.3)	0.72 (0.42–1.35)	1.40 (0.35–3.96)	0.24 (0.16–0.46)
Under	84.8 (16.6–108)	1.41 (0.49–2.12)	5.60 (1.02–7.78)	0.68 (0.23–1.11)
Poverty income ratio (PIR)				
PIR < 1	40.6 (15.4–88.7)	0.69 (0.40–1.34)	2.17 (0.51–5.26)	0.22 (0.15–0.38)
PIR ≥ 1	29.2 (6.68–71.0)	0.80 (0.46–1.52)	1.47 (0.30–4.21)	0.27 (0.17–0.51)
Race				
Mexican American	5.44 (1.84–15.71)	0.83 (0.48–1.62)	0.29 (0.12–0.53)	0.25 (0.16–0.48)
Non-Hispanic Black	27.4 (9.24–50.0)	0.44 (0.31–0.86)	1.19 (0.32–2.96)	0.18 (0.13–0.24)
Non-Hispanic White	38.1 (14.8–92.4)	0.83 (0.47–1.55)	1.73 (0.44–5.27)	0.28 (0.17–0.52)
Other Hispanic	37.3 (23.3–67.7)	0.75 (0.47–1.52)	2.12 (1.17–3.47)	0.25 (0.15–0.39)
Other/multiracial	33.4 (10.8–51.4)	0.79 (0.55–1.62)	1.38 (0.35–3.80)	0.37 (0.21–0.60)
Sex				
Female	42.5 (15.1–91.6)	0.86 (0.52–1.75)	1.94 (0.41–5.53)	0.34 (0.19–0.59)
Male	26.3 (6.55–58.0)	0.70 (0.39–1.29)	1.47 (0.33–3.54)	0.21 (0.14–0.40)

[a]Same data as in stratified serum cotinine regression models.
[b]98.9% (detection rate).
[c]39.5% (detection rate).
[d]75.8% (detection rate).
[e]4.2% (detection rate).

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biomarker concentrations with frequency of tobacco smoking. To perform this analysis, unstratified, sample-weighted regression models were used where serum cotinine concentration was replaced with a measure of exposure comprising self-reported average number of CPD over the five days preceding the NHANES physical exam. Tobacco smoke exposure in the CPD models was divided into five groups. Group 1 (unexposed to tobacco smoke) included participants with the ranges of ≤0.05 ng/mL serum cotinine; Group 2 (presumptively exposed to SHS) included participants with the range of >0.05 to ≤10 ng/mL serum cotinine; and Groups 3, 4, and 5 included participants who consumed 1 to 10 CPD (0.5 pack), 11 to 20 (1 pack), and >20 (>1 pack), respectively. The group of unexposed participants was assigned as the reference category. The unexposed category was designated as ≤0.05 ng/mL serum cotinine, which was its limit of detection in the 1999–2000 NHANES cycle. To allow historic comparison of serum cotinine results, we kept using the limit of detection at 0.05 ng/mL for definition of unexposed participants, though this was improved in 2001 to 0.015 ng/mL.

Exposure through diet is another potential source of AαC, especially when food is prepared at high temperature (31, 33, 34). Dietary exposure was assessed based on the amount participants consumed within each U.S. Department of Agriculture (USDA) food group for the 24-hour period (midnight to midnight; ref. 51), which is described in detail in Supplementary Information. For this assessment, two additional food subgroups were distinguished because of their potential for high AαC exposure arising from high temperature (i.e., broiling, baking, or frying) during preparation (34): high-temperature cooked beef and high-temperature cooked fish. Double counting was avoided by subtracting the amount consumed in each subgroup from the amount consumed in their respective food group. Supplementary Table S1 details the USDA food codes and logic for apportioning dietary intake.

Quantitative analysis of urinary biomarker concentration data must account for normal physiologic variations in urine dilution, which can vary markedly for an individual between voids and thereby confound statistical inference (53). One approach to minimize this problem is to normalize the urinary concentrations of the biomarker to creatinine, which is formed endogenously by lean muscle tissue and cleared by the kidneys into urine at a relatively constant rate. Summary statistics of urinary concentrations are reported as the ratio of AαC and MeAαC to creatinine (ng/g creatinine), and urinary creatinine concentration is included as a continuous predictor in regression models to adjust for potential confounding from urine dilution.

In order to compare the exposure through diet and smoking, we estimated the absolute change in urinary HAA (ΔY) predicted for these two routes under conservative assumptions of exposure. For diet, we estimated the change in urinary HAA in pg/mL associated with consuming the sample-weighted 99th percentile daily amount of specific food groups in the United States. This dietary exposure is then readily compared with the change in urinary HAA, also in units of pg/mL, associated with smoking one-half pack of cigarettes per day from the CPD model.

Results

Sample-weighted demographic distributions of NHANES 2013–2014 participants (AαC: N = 1,792, MeAαC: N = 1,793) were summarized in **Table 1**. Among these participants, AαC was detected in 61.5% of participants, but only in 30.6% for MeAαC, so MeAαC was

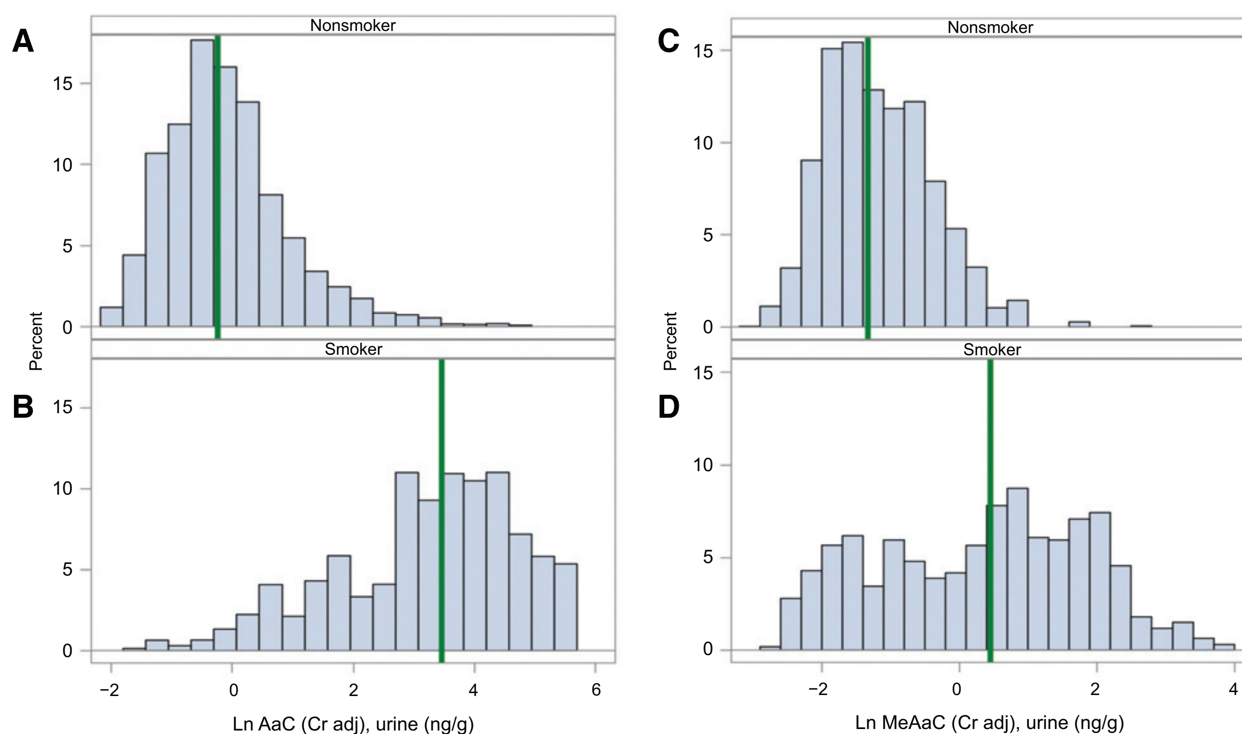

Figure 1.
Histograms of urinary AαC and MeAαC (ng analyte/g creatinine) among exclusive tobacco smokers and nonusers in the NHANES 2013–2014. Green reference line represents median. **A,** The percent-distribution of urinary AαC among nonusers. **B,** The percent-distribution of urinary AαC among exclusive tobacco smokers. **C,** The percent-distribution of urinary MeAαC among nonusers. **D,** The percent-distribution of urinary MeAαC among exclusive tobacco smokers.

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Table 3. Sample-weighted multiple regression results for urinary AαC concentrations (pg/mL) among exclusive smokers from NHANES 2013–2014 participants.

Variables	Exclusive smokers ($N = 663$)		
	Slope (95% CI)[a]	ΔY (95% CI)[b]	P value
Cotinine, serum (ng/mL)	0.0050 (0.0039–0.0061)	0.113 (0.0900–0.136)	<0.0001
Creatinine, urine (g/mL)	293 (43.0–543)	4.37E + 128 (5.78E + 28–3.31E + 228)	0.03
Fasting time (hours)	−0.0227 (−0.0510–0.0056)	−0.507 (−1.07–0.0747)	0.11
Age (years)			
18–39	−0.149 (−0.497–0.199)	−3.13 (−8.45–4.19)	0.37
40–59	Ref.		—
≥60	0.250 (−0.187–0.687)	6.40 (−3.18–20.7)	0.24
Food group			
High-temperature cooked beef (kg)	0.836 (−1.29–2.96)	29.5 (−15.2–345)	0.42
High-temperature cooked fish (kg)	0.162 (−0.580–0.905)	3.98 (−9.15–30.0)	0.65
Meat, poultry, fish, and mixtures (kg)	0.0157 (−0.565–0.597)	0.358 (−9.13–16.5)	0.96
Milk and milk products (kg)	−0.152 (−0.586–0.283)	−3.18 (−9.56–6.35)	0.47
Eggs (kg)	0.893 (−1.23–3.01)	32.5 (−14.7–365)	0.38
Legumes, nuts, seeds (kg)	−0.922 (−3.28–1.44)	−13.6 (−21.5–56.0)	0.42
Grain products (kg)	−0.180 (−0.471–0.112)	−3.71 (−8.14–2.09)	0.21
Fruits (kg)	−0.0643 (−0.449–0.321)	−1.40 (−7.71–7.58)	0.73
Vegetables (kg)	−0.146 (−0.987–0.694)	−3.07 (−13.6–19.7)	0.72
Fats, oils, salad dressings (kg)	0.197 (−4.60–4.99)	4.91 (−22.2–2.24E+03)	0.93
Sugars, sweets, beverages (kg)	0.0578 (−0.0206–0.136)	1.34 (−0.321–3.13)	0.14
BMI			
Healthy	Ref.		—
Overweight/obese	0.140 (−0.186–0.467)	3.40 (−3.34–12.5)	0.37
Underweight	−0.116 (−0.930–0.698)	−2.47 (−13.1–19.9)	0.77
Poverty income ratio (PIR)			
PIR ≥ 1	Ref.		—
PIR < 1	0.273 (0.0584–0.487)	7.08 (1.77–13.5)	0.02
Race			
Mexican American	−0.588 (−1.08 to −0.0938)	−10.0 (−14.6 to −2.82)	0.02
Non-Hispanic Black	−0.189 (−0.545–0.167)	−3.88 (−9.09–3.35)	0.28
Non-Hispanic White	Ref.		—
Other Hispanic	0.212 (−0.379–0.803)	5.33 (−6.37–25.5)	0.46
Other/multiracial	−0.173 (−0.563–0.217)	−3.58 (−9.30–4.60)	0.36
Sex			
Female	0.221 (−0.0862–0.530)	5.60 (−1.34–14.8)	0.15
Male	Ref.		—

Note: The geometric mean of urinary AαC among exclusive smokers used for computing ΔY is 22.6 pg/mL.
Abbreviation: Ref., reference group.
[a]The dependent variable, biomarker concentration, was natural log-transformed for the regression model.
[b]ΔY is the expected change in biomarker concentration in pg/mL associated with a unit increase in the predictor, controlling for other predictors in the model and calculated at the overall geometric mean.

excluded from multiple regression analysis. By comparison, AαC and MeAαC were detected at much higher rates (98.9% and 75.8%, respectively) in exclusive tobacco smokers than those in nonusers (39.5% for AαC and 4.2% for MeAαC). Among exclusive smokers, urinary AαC and MeAαC concentrations were significantly correlated, with a sample-weighted Pearson correlation of 0.788. The sample-weighted median of urinary AαC among exclusive smokers was 40 times higher than nonusers (31.90 vs. 0.79 ng/g creatinine, respectively; **Table 2**). Geometric means and selected percentiles of urinary AαC concentrations for U.S. population were summarized in Supplementary Tables S2 and S3. The difference in urinary concentrations of MeAαC in exclusive tobacco smokers and nonusers at selected percentiles was summarized in Supplementary Tables S4 and S5. The percent distribution of urinary AαC and MeAαC depicted these differences among exclusive smokers and nonusers (**Fig. 1**).

The sample-weighted multiple regression model among exclusive smokers (**Table 3**) found that urinary AαC was positively associated with serum cotinine (0.113 pg AαC/mL per ng cotinine/mL), controlling for sex, age, race/Hispanic origin, BMI, fasting time, urinary creatinine, diet, and impoverishment. Mexican American exclusive smokers had significantly lower AαC in comparison with non-Hispanic White exclusive smokers. Urinary AαC level among impoverished exclusive smokers was significantly higher than among nonimpoverished participants.

Among nonusers of tobacco products (**Table 4**), urinary AαC was positively associated with serum cotinine (0.140 pg AαC/mL per ng cotinine/mL), presumptively attributable to secondhand smoke exposure, controlling for sex, age, race/Hispanic origin, BMI, fasting time, urinary creatinine, diet, and impoverishment. Non-Hispanic Black and other/multiracial nonusers had lower AαC than non-Hispanic White nonusers. Urinary AαC was also positively associated with the amount consumed (kg) of beef cooked under high temperature (15.5 pg/mL per kg), whereas the association with consumption of fish cooked at high temperature was not significant. Consumption of vegetables (−0.264 pg/mL per kg) was

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Table 4. Sample-weighted multiple regression results for urinary AαC concentrations (pg/mL) among nonusers of tobacco from NHANES 2013–2014 participants.

	Nonusers ($N = 1,129$)		
Variables	**Slope (95% CI)[a]**	**ΔY (95% CI)[b]**	***P* value**
Cotinine, serum (ng/mL)	0.171 (0.0771–0.264)	0.140 (0.0665–0.220)	0.001
Creatinine, urine (g/mL)	304 (193–415)	8.13E + 131 (4.58E + 87–1.45E + 176)	<0.0001
Fasting time (hours)	−0.0161 (−0.0256 to −0.0066)	−0.0120 (−0.0185 to −5.53E-03)	0.003
Age (years)			
18–39	0.0301 (−0.148–0.208)	0.0230 (−0.0940–0.161)	0.72
40–59	Ref.	Ref.	—
≥60	−0.128 (−0.293–0.0375)	−0.0903 (−0.184–0.0184)	0.12
Food group			
High-temperature cooked beef (kg)	3.07 (0.337–5.81)	15.5 (0.561–201)	0.03
High-temperature cooked fish (kg)	−0.501 (−1.07–0.0736)	−0.296 (−0.484–0.0209)	0.08
Meat, poultry, fish, and mixtures (kg)	0.245 (−0.155–0.645)	0.209 (−0.0870–0.637)	0.21
Milk and milk products (kg)	−0.0927 (−0.298–0.112)	−0.0667 (−0.185–0.0757)	0.35
Eggs (kg)	−0.200 (−1.06–0.662)	−0.137 (−0.474–0.609)	0.63
Legumes, nuts, seeds (kg)	−0.0525 (−0.892–0.787)	−0.0385 (−0.423–0.793)	0.90
Grain products (kg)	−0.0978 (−0.417–0.222)	−0.0702 (−0.244–0.163)	0.52
Fruits (kg)	−0.0182 (−0.271–0.235)	−0.0136 (−0.167–0.180)	0.88
Vegetables (kg)	−0.431 (−0.775 to −0.0863)	−0.264 (−0.396 to −0.0812)	0.02
Fats, oils, salad dressings (kg)	1.198 (−0.838–3.23)	1.74 (−0.369–15.5)	0.23
Sugars, sweets, beverages (kg)	0.0236 (−0.0170–0.0642)	0.0180 (−0.0102–0.0473)	0.23
BMI			
Healthy	Ref.		—
Overweight/obese	−0.0304 (−0.204–0.144)	−0.0225 (−0.131–0.104)	0.72
Underweight	−0.0487 (−0.500–0.403)	−0.0358 (−0.280–0.333)	0.82
Poverty income ratio (PIR)			
PIR ≥ 1	Ref.		—
PIR < 1	−0.0502 (−0.206–0.105)	−0.0368 (−0.132–0.0731)	0.50
Race			
Mexican American	0.0523 (−0.189–0.294)	0.0404 (−0.118–0.238)	0.65
Non-Hispanic Black	−0.325 (−0.520 to −0.130)	−0.209 (−0.298 to −0.102)	0.003
Non-Hispanic White	Ref.		—
Other Hispanic	−0.0235 (−0.253–0.206)	−0.0175 (−0.157–0.155)	0.83
Other/multiracial	−0.147 (−0.280 to −0.0141)	−0.103 (−0.178 to −0.0184)	0.03
Sex			
Female	−0.0271 (−0.106−0.0513)	−0.0202 (−0.0712–0.0347)	0.47
Male	Ref.		—

Note: The geometric mean of urinary AαC among nonusers used for computing ΔY is 0.75 pg/mL.
Abbreviation: Ref., reference group.
[a]The dependent variable, biomarker concentration, was natural log-transformed for the regression model.
[b]ΔY is the expected change in biomarker concentration in pg/mL associated with a unit increase in the predictor, controlling for other predictors in the model and calculated at the overall geometric mean.

a significant negative predictor of urinary AαC. Fasting time also was negatively associated with AαC.

In the unstratified, sample-weighted CPD regression model for nonusers and exclusive smokers (Supplementary Table S6), each CPD exposure group had significantly higher urinary AαC levels compared with those of nonusers whose serum cotinine levels were <0.05 ng/mL, controlling for sex, age, race/Hispanic origin, BMI, fasting time, urinary creatinine, diet, and impoverishment. **Figure 2** displays the sample-weighted least-square means of urinary AαC from the CPD model by exposure category, where it can be seen that urinary AαC increased in a dose-dependent manner across exposure categories.

Discussion

From a national, population-based study, we show that tobacco smoke and diet are significantly associated with AαC exposure. Sample-weighted linear regression models showed that serum cotinine was a significant positive predictor of urinary AαC among both exclusive smokers and nonusers. Moreover, we found a significant dose-dependent association between exclusive smokers' urinary AαC levels and CPD smoked. Our finding for tobacco smoke exposure among both exclusive smokers and nonusers is consistent with the fact that AαC is the most abundant carcinogenic HAA in tobacco smoke (7, 10, 20, 21), and indicates that tobacco smoke is a major source of AαC exposure in the U.S. population.

Only three small-scale studies previously reported human AαC exposure levels. A study in China (78 subjects) found that the mean of total urinary concentrations of AαC (free and conjugated forms) for smokers (20 ng/g creatinine) was significantly higher than that for nonsmokers (7 ng/g creatinine; ref. 54). Compared with above study, median urinary AαC was slightly higher for exclusive smokers (31.90 ng/g creatinine) and much lower for nonusers (0.79 ng/g creatinine) in the NHANES 2013–2014 samples. The lower median urinary AαC of nonuser in our study could be related

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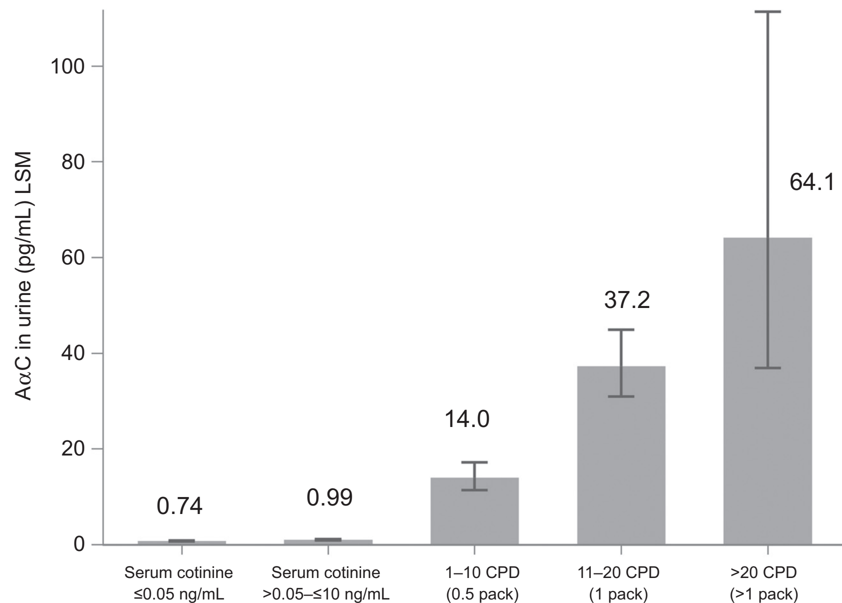

Figure 2.
Least-square means of urinary AαC concentrations (pg/mL), adjusted for sex, age, race/Hispanic origin, BMI, fasting time, urinary creatinine, diet, and impoverishment ($N = 1,760$; error bars denote 95% CIs). Group ≤0.05 ng/mL serum cotinine: participants unexposed to tobacco smoke; Group >0.05 to ≤10 ng/mL serum cotinine: participants presumptively exposed to SHS; Exclusive tobacco smokers were further classified based on their CPD consumed.

to lower levels of secondhand smoke exposure in the United States than in China. Another study in China measured free urinary AαC in 170 volunteers (44). The mean urinary AαC among light (1–19 CPD) smokers was 7.50 ng/g creatinine (9.2 pg/mL urine), and among heavy (>20 CPD) smokers, it was 11.92 ng/g creatinine (13.8 pg/mL urine), whereas mean urinary AαC among nonsmokers was 2.54 ng/g creatinine. In contrast to Turesky and colleagues' study, we observed higher exposure levels to AαC at corresponding CPD (least squared means 1–10 CPD: 14.0 pg/mL; 11–20 CPD: 37.2 pg/mL; >20 CPD: 64.1 pg/mL). These differences may be explained, at least in part, to differences in sample preparation. Turesky and colleagues measured free AαC in nonhydrolyzed samples, whereas our measurements were preceded by hydrolysis, which enables measurement of both free and base-sensitive conjugated species of AαC, thereby leading to systematically higher measured concentrations. In addition, there is large variation of AαC in cigarettes between manufacturers that would increase the observed variation in urinary AαC at the same CPD consumed in the two countries (7, 10, 21). Nonetheless, both studies corroborated that urinary AαC followed a dose response pattern with CPD. In one small U.S. study (30 subjects) where urinary AαC measurements comprised both free and base-sensitive conjugated species (45), the measurements had magnitudes closer to ours. Konorev and colleagues observed that mean urinary AαC among smokers decreased from 24.3 ng/g creatinine to 3.2 ng/g creatinine after subjects had stopped smoking tobacco for 6 weeks.

In the NHANES 2013–2014 population, urinary AαC in non-Hispanic White smokers was around 7-fold higher than that in Mexican American smokers (**Table 2**). The effect of race/Hispanic origin cannot be completely explained by differences in tobacco smoke exposure alone because serum cotinine was included in the model. Difference in toxicokinetics may partly contribute to the race/Hispanic origin-related differences. CYP1A2 is one of the major P450 isoforms responsible for AαC metabolism (35, 55–57). N-hydroxylation of the exocyclic amine group results in HONH-AαC, and ring oxidation of AαC at the C-3 and C-6 positions results in AαC-3-OH and AαC-6-OH, respectively (35, 56). Various factors such as sex, race/Hispanic origin, and smoking status can affect CYP1A2 activity (58–60). Unfortunately, standards for these compounds and their corresponding stable isotope–labeled internal standards are not commercially available, so hydroxylated metabolites of AαC were not included in our assay. Therefore, the influence of race/Hispanic origin on AαC metabolism remains unclear and awaits future investigation.

We also found that certain diet components are significantly associated with AαC exposure. Beef and fish cooked at high temperature have been regarded as the most likely sources of AαC from foods eaten in the United States (34). Based on the formation mechanism, AαC is produced from pyrolysis of proteins or amino acids heated at high temperature (8). In the nonuser and CPD regression models, eating beef cooked at high temperature was shown to be significantly associated with increased urinary AαC. In contrast, we found no significant association between urinary AαC and the amount of "meat, poultry, fish, and mixture" eaten without also specifying meat types or cooking temperature, which is consistent with a process requiring high temperature to contribute to dietary AαC exposure.

Eating vegetables was associated with significantly diminished urinary AαC levels in nonsmokers. Either decreased HAA absorption or enhanced hepatic elimination of HAA via induced CYP2A1 activity from eating vegetables has been proposed to explain reduced urinary HAA in persons who ate cooked meat (61, 62). Because the hydroxylated AαC metabolites formed by CYP2A1 were not measured in our assay, we cannot say whether the influence of eating vegetables on urinary HAA levels in previous studies could be directly used to interpret our findings.

Fasting time was a significant negative predictor of urinary AαC levels in nonusers of tobacco products, indicating that urinary excretion of AαC declined with time after the last meal. Because the AαC half-life is around 3.0 hours (63), urinary AαC levels would be expected to decline rapidly after complete AαC absorption from the food matrix.

We compared the potential influence of diet versus smoking on urinary AαC by estimating the 99th percentile of beef consumption cooked at high temperature for the U.S. population, which we found to be 0.22 kg/day. At this level of consumption, we estimated the likely increase in urinary AαC ΔY (95% confidence interval, CI) to be 0.99 (0.16–2.29) pg AαC/mL, compared with the increase associated

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with smoking one-half pack of cigarettes per day of 23.6 (18.7–29.6) pg AαC/mL. Under these assumptions, the likely level of dietary exposure is considerably lower than from exclusive cigarette smoking.

Dietary exposure was based on 24-hour recall data collected in the MEC, thus reflecting dietary consumption for a single day. Although interviewers elicited recall with structured questions and standardized techniques, retrospective responses are generally susceptible to recall bias.

So far, no population-based study has identified tobacco smoke as a source of MeAαC exposure. A study in China lacked sufficient sensitivity of the urinary MeAαC assay to detect MeAαC in smokers (54). With the improved sensitivity of MeAαC measurement in our assay, our analysis provides the first population-based data on MeAαC. MeAαC was found to co-occur with AαC at about 10-fold lower content in tobacco smoke (7, 8, 10). As with AαC, MeAαC was mostly detected in smokers at around 20 times lower levels than those of AαC. Moreover, urinary AαC and MeAαC concentrations were significantly correlated among exclusive smokers with a sample-weighted Pearson correlation of 0.788. Therefore, MeAαC could serve as a valuable ancillary biomarker to assess AαC exposure.

In the United States, urinary AαC is significantly associated with serum cotinine levels among both exclusive smokers and nonusers of tobacco, and tobacco smoke is a major source of AαC exposure in the general population. Much smaller increases in urinary AαC were associated with secondhand smoke exposure and eating beef cooked at high temperature.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC and FDA. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, CDC, or FDA.

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References

1. U.S. Department of Health and Human Services. The health consequences of involuntary exposure to tobacco smoke: a report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2006.
2. Doll R, Hill AB. Smoking and carcinoma of the lung; preliminary report. Br Med J 1950;2:739–48.
3. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ 2004;328:1519.
4. Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002;3:461–9.
5. Hecht SS. Research opportunities related to establishing standards for tobacco products under the Family Smoking Prevention and Tobacco Control Act. Nicotine Tob Res 2012;14:18–28.
6. Smith CJ, Perfetti TA, Garg R, Hansch C. IARC carcinogens reported in cigarette mainstream smoke and their calculated log P values. Food Chem Toxicol 2003; 41:807–17.
7. Manabe S, Tohyama K, Wada O, Aramaki T. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), in cigarette smoke condensate. Carcinogenesis 1991;12:1945–7.
8. Matsumoto T, Yoshida D, Tomita H. Determination of mutagens, amino-alpha-carbolines in grilled foods and cigarette smoke condensate. Cancer Lett 1981;12: 105–10.
9. Turesky RJ, Le Marchand L. Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. Chem Res Toxicol 2011;24:1169–214.
10. Zhang L, Ashley DL, Watson CH. Quantitative analysis of six heterocyclic aromatic amines in mainstream cigarette smoke condensate using isotope dilution liquid chromatography-electrospray ionization tandem mass spectrometry. Nicotine Tob Res 2011;13:120–6.
11. Sugimura T. Overview of carcinogenic heterocyclic amines. Mutat Res 1997;376: 211–9.
12. Sugimura T. Nutrition and dietary carcinogens. Carcinogenesis 2000;21:387–95.
13. Nagao M. A new approach to risk estimation of food-borne carcinogens–heterocyclic amines–based on molecular information. Mutat Res 1999;431:3–12.
14. Shirai T, Sano M, Tamano S, Takahashi S, Hirose M, Futakuchi M, et al. The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) derived from cooked foods. Cancer Res 1997;57:195–8.
15. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environ Mol Mutagen 2004;44:44–55.
16. Dai Q, Shu XO, Jin F, Gao YT, Ruan ZX, Zheng W. Consumption of animal foods, cooking methods, and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:801–8.
17. Le Marchand L, Hankin JH, Wilkens LR, Pierce LM, Franke A, Kolonel LN, et al. Combined effects of well-done red meat, smoking, and rapid N-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:1259–66.
18. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans: some naturallyoccurring substances - food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monogr Eval Carcinog Risk Chem Hum 1993;56:165–242.
19. Alaejos MS, Afonso AM. Factors that affect the content of heterocyclic aromatic amines in foods. Compr Rev Food Sci Food Saf 2011;10:52–108.
20. Yoshida D, Matsumoto T. Amino-alpha-carbolines as mutagenic agents in cigarette smoke condensate. Cancer Lett 1980;10:141–9.
21. Smith CJ, Qian X, Zha Q, Moldoveanu SC. Analysis of alpha- and beta-carbolines in mainstream smoke of reference cigarettes by gas chromatography-mass spectrometry. J Chromatogr A 2004;1046:211–6.
22. Wu W, Zhang L, Jain RB, Ashley DL, Watson CH. Determination of carcinogenic tobacco-specific nitrosamines in mainstream smoke from U.S.-brand and non-U.S.-brand cigarettes from 14 countries. Nicotine Tob Res 2005;7:443–51.
23. Hecht SS, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis 1988;9:875–84.
24. Ohgaki H, Matsukura N, Morino K, Kawachi T, Sugimura T, Takayama S. Carcinogenicity in mice of mutagenic compounds from glutamic acid and soybean globulin pyrolysates. Carcinogenesis 1984;5:815–9.

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25. Okonogi H, Ushijima T, Shimizu H, Sugimura T, Nagao M. Induction of aberrant crypt foci in C57BL/6N mice by 2-amino-9H-pyrido[2,3-b]indole (A alphaC) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). Cancer Lett 1997;111:105–9.
26. Zhang XB, Felton JS, Tucker JD, Urlando C, Heddle JA. Intestinal mutagenicity of two carcinogenic food mutagens in transgenic mice: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and amino(alpha)carboline. Carcinogenesis 1996;17:2259–65.
27. Snyderwine EG, Sadrieh N, King RS, Schut HA. Formation of DNA adducts of the food-derived mutagen 2-amino-9H-pyrido-[2,3-b]indole (A(alpha)C) and bioassay of mammary gland carcinogenicity in Sprague-Dawley rats. Food Chem Toxicol 1998;36:1033–41.
28. Tamano S, Hasegawa R, Hagiwara A, Nagao M, Sugimura T, Ito N. Carcinogenicity of a mutagenic compound from food, 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeA alpha C), in male F344 rats. Carcinogenesis 1994; 15:2009–15.
29. International Agency for Research on Cancer. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon, France: IARC; 1986. p. 37–385.
30. Food and Drug Administration. Harmful and potentially harmful constituents in tobacco products and tobacco smoke: established list. Silver Spring, MD: U.S. Department of Health and Human Services, Food and Drug Administration; 2012.
31. Holland RD, Taylor J, Schoenbachler L, Jones RC, Freeman JP, Miller DW, et al. Rapid biomonitoring of heterocyclic aromatic amines in human urine by tandem solvent solid phase extraction liquid chromatography electrospray ionization mass spectrometry. Chem Res Toxicol 2004;17:1121–36.
32. Yoshida D, Matsumoto T, Yoshimura R, Matsuzaki T. Mutagenicity of amino-alpha-carbolines in pyrolysis products of soybean globulin. Biochem Biophys Res Commun 1978;83:915–20.
33. Gross GA, Gruter A. Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. J Chromatogr 1992;592:271–8.
34. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, Felton JS. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis 1995;16:39–52.
35. Raza H, King RS, Squires RB, Guengerich FP, Miller DW, Freeman JP, et al. Metabolism of 2-amino-alpha-carboline. A food-borne heterocyclic amine mutagen and carcinogen by human and rodent liver microsomes and by human cytochrome P4501A2. Drug Metab Dispos 1996;24:395–400.
36. Frederiksen H, Frandsen H. In vitro metabolism of two heterocyclic amines, 2-amino-9H-pyrido[2,3-b]indole (A(alpha)C) and 2-amino-3-methyl-9H-pyridol2,3-b]indole (MeA(alpha)C) in human and rat hepatic microsomes. Pharmacol Toxicol 2002;90:127–34.
37. Niwa T, Yamazoe Y, Kato R. Metabolic activation of 2-amino-9H-pyrido[2,3-b]indole by rat-liver microsomes. Mutat Res 1982;95:159–70.
38. King RS, Teitel CH, Kadlubar FF. In vitro bioactivation of N-hydroxy-2-amino-alpha-carboline. Carcinogenesis 2000;21:1347–54.
39. Tang Y, LeMaster DM, Nauwelaers G, Gu D, Langouet S, Turesky RJ. UDP-glucuronosyltransferase-mediated metabolic activation of the tobacco carcinogen 2-amino-9H-pyrido[2,3-b]indole. J Biol Chem 2012;287:14960–72.
40. Cai T, Yao L, Turesky RJ. Bioactivation of heterocyclic aromatic amines by UDP glucuronosyltransferases. Chem Res Toxicol 2016;29:879–91.
41. Pfau W, Schulze C, Shirai T, Hasegawa R, Brockstedt U. Identification of the major hepatic DNA adduct formed by the food mutagen 2-amino-9H-pyrido[2,3-b]indole (A alpha C). Chem Res Toxicol 1997;10:1192–7.
42. Frederiksen H, Frandsen H, Pfau W. Syntheses of DNA adducts of two heterocyclic amines, 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAalphaC) and 2-amino-9H-pyrido[2,3-b]indole (AalphaC) and identification of DNA adducts in organs from rats dosed with MeAalphaC. Carcinogenesis 2004;25: 1525–33.
43. Nauwelaers G, Bessette EE, Gu D, Tang Y, Rageul J, Fessard V, et al. DNA adduct formation of 4-aminobiphenyl and heterocyclic aromatic amines in human hepatocytes. Chem Res Toxicol 2011;24:913–25.
44. Turesky RJ, Yuan JM, Wang R, Peterson S, Yu MC. Tobacco smoking and urinary levels of 2-amino-9H-pyrido[2,3-b]indole in men of Shanghai, China. Cancer Epidemiol Biomarkers Prev 2007;16:1554–60.
45. Konorev D, Koopmeiners JS, Tang Y, Franck Thompson EA, Jensen JA, Hatsukami DK, et al. Measurement of the heterocyclic amines 2-amino-9H-pyrido[2,3-b]indole and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in urine: effects of cigarette smoking. Chem Res Toxicol 2015;28:2390–9.
46. Zhang L, Xia Y, Xia B, Nicodemus KJ, McGuffey J, McGahee E, et al. High-throughput and sensitive analysis of urinary heterocyclic aromatic amines using isotope-dilution liquid chromatography-tandem mass spectrometry and robotic sample preparation system. Anal Bioanal Chem 2016;408:8149–61.
47. Pirkle JL, Bernert JT, Caudill SP, Sosnoff CS, Pechacek TF. Trends in the exposure of nonsmokers in the U.S. population to secondhand smoke: 1988–2002. Environ Health Perspect 2006;114:853–8.
48. Caudill SP, Schleicher RL, Pirkle JL. Multi-rule quality control for the age-related eye disease study. Stat Med 2008;27:4094–106.
49. Finkelstein MM, Verma DK. Exposure estimation in the presence of nondetectable values: another look. AIHAJ 2001;62:195–8.
50. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 1990;5:46–51.
51. Espenship MF, Silva LK, Smith MM, Capella KM, Reese CM, Rasio JP, et al. Nitromethane exposure from tobacco smoke and diet in the U.S. population: NHANES, 2007–2012. Environ Sci Technol 2019;53:2134–40.
52. Pirkle JL, Flegal KM, Bernert JT, Brody DJ, Etzel RA, Maurer KR. Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA 1996;275:1233–40.
53. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005;113:192–200.
54. Fu Y, Zhao G, Wang S, Yu J, Xie F, Wang H, et al. Simultaneous determination of fifteen heterocyclic aromatic amines in the urine of smokers and nonsmokers using ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 2014;1333:45–53.
55. Frederiksen H, Frandsen H. Impact of five cytochrome p450 enzymes on the metabolism of two heterocyclic aromatic amines, 2-amino-9H-pyrido[2,3-b]indole (AalphaC) and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAalphaC). Pharmacol Toxicol 2003;92:246–8.
56. Bellamri M, Le Hegarat L, Turesky RJ, Langouet S. Metabolism of the tobacco carcinogen 2-amino-9H-pyrido[2,3-b]indole (AalphaC) in primary human hepatocytes. Chem Res Toxicol 2017;30:657–68.
57. Chevereau M, Glatt H, Zalko D, Cravedi JP, Audebert M. Role of human sulfotransferase 1A1 and N-acetyltransferase 2 in the metabolic activation of 16 heterocyclic amines and related heterocyclics to genotoxicants in recombinant V79 cells. Arch Toxicol 2017;91:3175–84.
58. Carrillo JA, Benitez J. CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine. Br J Clin Pharmacol 1996;41:605–8.
59. Gunes A, Ozbey G, Vural EH, Uluoglu C, Scordo MG, Zengil H, et al. Influence of genetic polymorphisms, smoking, gender and age on CYP1A2 activity in a Turkish population. Pharmacogenomics 2009;10:769–78.
60. Rybak ME, Sternberg MR, Pao CI, Pfeiffer CM. Determination of CYP1A2 activity in the US population by use of caffeine metabolite ratios in spot urine samples: NHANES 2009–2010. FASEB J 2016;30(1 Suppl):271.1.
61. Walters DG, Young PJ, Agus C, Knize MG, Boobis AR, Gooderham NJ, et al. Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans. Carcinogenesis 2004;25:1659–69.
62. Murray S, Lake BG, Gray S, Edwards AJ, Springall C, Bowey EA, et al. Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. Carcinogenesis 2001;22:1413–20.
63. Turesky RJ, Konorev D, Fan X, Tang Y, Yao L, Ding X, et al. Effect of cytochrome P450 reductase deficiency on 2-Amino-9H-pyrido[2,3-b]indole metabolism and DNA adduct formation in liver and extrahepatic tissues of mice. Chem Res Toxicol 2015;28:2400–10.

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