Opiate and Tobacco Use and Exposure to Carcinogens and Toxicants in the Golestan Cohort Study

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ABSTRACT

Background: There is little information on human exposure to carcinogens and other toxicants related to opiate use, alone or in combination with tobacco.

Methods: Among male participants of the Golestan Cohort Study in Northeast Iran, we studied 28 never users of either opiates or tobacco, 33 exclusive cigarette smokers, 23 exclusive users of smoked opiates, and 30 opiate users who also smoked cigarettes (dual users; 21 smoked opiates and 9 ingested them). We quantified urinary concentrations of 39 exposure biomarkers, including tobacco alkaloids, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAH), and volatile organic compounds (VOC), and used decomposition to parse out the share of the biomarker concentrations explained by opiate use and nicotine dose.

Results: Dual users had the highest concentrations of all biomarkers, but exclusive cigarette smokers and exclusive opiate users had substantially higher concentrations of PAH and VOC biomarkers than never users of either product. Decomposition analysis showed that opiate use contributed a larger part of the PAH concentrations than nicotine dose, and the sum of 2- and 3-hydroxyphenanthrene ($\sum_{2,3}$-phe) resulted almost completely from opiate use. Concentrations of most VOC biomarkers were explained by both nicotine dose and opiate use. Two acrylamide metabolites, a 1,3-butadiene metabolite and a dimethylformamide metabolite, were more strongly explained by opiate use. Acrylamide metabolites and $\sum_{2,3}$-phe were significantly higher in opiate smokers than opiate eaters; other biomarkers did not vary by the route of opiate intake.

Conclusions: Both cigarette smokers and opiate users (by smoking or ingestion) were exposed to many toxicants and carcinogens.

Impact: This high exposure, particularly among dual opiate and cigarette users, can have a substantial global public health impact.

Introduction

Tobacco control initiatives have led to a decrease in smoking prevalence in most countries (1), but this decrease has not been uniform across all populations and subgroups. People with substance use disorders are among the subgroups with high prevalence of cigarette smoking and do not seem to benefit from these control efforts as much as the general population (2). Lifetime drug abuse and dependence have strong associations with nicotine dependence, even after adjusting for other psychiatric disorders (3). Among patients treated for opioid dependence, heavy smoking and increased nicotine dependence are common (4, 5), and people enrolled in methadone maintenance programs because of opioid dependence have difficulty quitting cigarette smoking (6). Cigarette smoking has been associated with higher rates of self-reported disability in illicit substance users (7), and dual users of opiates and cigarettes are at increased risk of cancer compared with single users of either product (8).

In 2017, over 53 million people worldwide had abused opioids in the previous year (56% higher than 2016 estimates; ref. 9). Among them, an estimated 29 million people (50% higher than 2016) abused nonprescription opiates, including opium-derived products common in Central Asia and the Middle East. Opiates are structurally related to compounds found in the resin of the opium poppy, *Papaver somniferum*. Opiate products (opium, morphine, and heroin) are derived from this resin, and an opioid is any agent (synthetic or natural) with the functional and pharmacologic properties of an opiate (10). Chronic opiate use can have long-lasting effects on health, and there is accumulating evidence about the potential carcinogenicity of opiate use (11), with associations reported between chronic opiate use and the risk of esophageal (12), gastric (13), pancreatic (14), and bladder cancers (8). Chronic opiate use has also been associated with increased risk of cardiovascular disease (15) and all-cause premature mortality (16). Many of these diseases are also associated with tobacco use, and because of the high prevalence of dual use, potential

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synergistic effects of opiates and tobacco on health are important. An objective method to investigate these effects is to study biomarkers of exposure to toxicants and carcinogens, such as those included in the FDA's list of "Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke" (17), among exclusive and dual users of opiates and tobacco. While we know that cigarette smoke contains more than 70 carcinogens (18), no study has investigated the toxicant and carcinogen exposure among opiate users, particularly dual users of opiates and tobacco.

Population-based studies of exposure biomarkers are complex because of challenges in obtaining detailed and reliable exposure data. Besides, most of these biomarkers are only measured in urine, which is not available in most large-scale population-based studies (19). The Golestan Cohort Study (GCS) in Iran provides a unique opportunity for such studies. In 2018, 82% of the total estimated global opium production originated from Afghanistan. Most of the seizures of opiates are made close to the production, and neighboring countries in the Near and Middle East/Southwest Asia, particularly Iran, constitute important targets for global illicit drug markets (9). In the GCS population, 17% of the participants are current opiate users, about 11% are current cigarette smokers, and 6% are dual users of cigarettes and opiates (20). Detailed and validated self-reported tobacco and opiate use information (21), along with concurrent urine samples were collected from all participants at the time of recruitment. We have previously shown that several chemical biomarkers can be successfully measured at the Centers for Disease Control and Prevention (CDC) National Center for Environmental Health (NCEH) Laboratory (22, 23) in the urine samples from this cohort to evaluate toxicant and carcinogen exposures (24). In this study, we evaluated such exposure biomarkers among never tobacco/opiate users, exclusive cigarette smokers, exclusive opiate users, and dual opiate and cigarette users.

Materials and Methods

The GCS (25) includes 50,045 individuals ages >40 years who live in Golestan Province, in the northeast of Iran. A baseline questionnaire collected information on self-reported use of tobacco (i.e., cigarettes, nass, and waterpipe) and nonmedical opiates, the ages of starting and stopping each product, the route and frequency of use, and daily consumption amount, along with other demographic and lifestyle information. All GCS participants gave a spot urine sample between 2004 and 2008, at the same time as they were interviewed and enrolled in the Cohort. These samples were stored at −20°C until 2015 when they were transferred on dry ice to the U.S. NCI Biorepository and stored at −80°C. The GCS was approved by appropriate ethics committees at Tehran University of Medical Sciences, NCI, and the International Agency for Research on Cancer (IARC). The involvement of the CDC laboratory did not constitute engagement in human subjects research.

From GCS participants who were alive and cancer-free in December 2016, we used self-reported opiate and tobacco use at enrollment to randomly select 60 never users of any tobacco or opiate product during their life (30 male and 30 female), 35 exclusive current cigarette smokers, 30 exclusive opiate users who never used tobacco (all smoked opiates), and 30 current opiate users who currently smoked cigarettes (dual users; 21 smoked opiates and 9 took them by mouth). The type of opiates used were raw opium and opium-derived juice (Shireh). Users of noncigarette tobacco products were excluded from this study. We restricted the current analysis to men as cigarette smoking in GCS is almost exclusive to men.

Validity of self-reported cigarette and opiate use

Self-reported opiate use has been previously tested against urinary codeine and morphine in a random sample of 150 participants of the GCS and proven to be a valid indicator of ever use (k statistics >0.9) with 93% sensitivity and 89% specificity (21).

To validate self-reported cigarette smoking, in this study, we defined active cigarette smoking as urinary cotinine concentrations of 50 ng/mL or greater (26). There was excellent agreement between urinary cotinine and self-reported cigarette smoking (Supplementary Table S1). The only group with a relatively high number of discordant results (7 of 30) were the exclusive opiate users. To reduce any potential error from incorrect reporting of cigarette smoking, we excluded individuals with discordant questionnaire data and urine specimens (self-reported never tobacco users with high cotinine concentrations, and self-reported current cigarette smokers with cotinine values below 50 ng/mL), because self-report was deemed unreliable for these participants. After excluding individuals with discordant self-reports and restricting to male participants, the following individuals were entered into the analyses: 28 never tobacco/opiate users, 33 exclusive cigarette smokers, 23 exclusive opiate users, and 30 dual opiate and cigarette users.

Laboratory measurements

The analytic measurements were conducted at the Division of Laboratory Sciences of NCEH at CDC (Atlanta, GA). We have previously described the panel of 39 exposure biomarkers used in this study (**Table 1**), which were the same used in the National Health and Nutrition Examination Survey (NHANES) and The Population Assessment of Tobacco and Health (PATH) studies (22). These included nine metabolites of tobacco alkaloids (nicotine and its six metabolites and two minor tobacco alkaloids), four tobacco-specific nitrosamines (TSNA), seven metabolites of polycyclic aromatic hydrocarbons (PAH), and 19 metabolites of volatile organic compounds (VOC). Nicotine metabolites were tested in all urine samples, regardless of opiate or cigarette use, to check whether study participants were active cigarette smokers or not. Tobacco-specific metabolites (tobacco alkaloids and TSNAs) would fall below the limits of detection (LOD) in samples with very low or undetectable concentrations of urinary cotinine; these biomarkers were only tested in samples with a cotinine concentration above 20 ng/mL (27), regardless of self-reported tobacco use. However, if the cotinine concentration was below 20 ng/mL, we used a more sensitive cotinine and hydroxycotinine assay to evaluate the participants' secondhand tobacco smoke exposure. PAHs and VOCs are not tobacco specific and were measured in all samples.

Urinary tobacco alkaloids were measured by an isotope dilution high-performance LC/MS-MS method (28). The LODs ranged from 0.39 to 10.5 ng/mL, depending on the analyte. The highly sensitive assays for cotinine and trans-3′-hydroxycotinine used a modified version of isotope dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry as described by Bernert and colleagues (29). The LOD was considerably lower than the usual assay (0.030 ng/mL) for both analytes in this sensitive assay. TSNAs were measured by isotope dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry using a modified version of the method described by Xia and colleagues (30). The LOD for urinary TSNAs ranged from 0.0006 to 0.0042 ng/mL. The seven PAH metabolites were quantified by online solid-phase extraction coupled with high-performance liquid chromatography-isotope dilution tandem mass spectrometry, as described previously (31). The LODs for PAHs ranged from 0.008 to 0.09 ng/mL. Urinary VOC

Table 1. Metabolites used in the biomarker panel developed by CDC National Center for Environmental Health.

Biomarker class	Full compound name	Parent compound	Abbreviation	CV (%)
Nicotine and its metabolites	Cotinine	Nicotine	COTT[a]	4.6
	Trans-3′-hydroxycotinine	Nicotine	HCTT[a]	4.3
	Cotinine N-oxide	Nicotine	COXT[b]	7.3
	Norcotinine	Nicotine	NCTT[b]	6.7
	Nicotine	Nicotine	NICT[b]	2.5
	Nicotine 1′-oxide	Nicotine	NOXT[b]	5.0
	Nornicotine	Nicotine	NNCT[b]	3.7
Other tobacco alkaloids	Anabasine	Anabasine	ANBT[b]	3.1
	Anatabine	Anatabine	ANTT[b]	3.9
TSNAs	*N*′-nitrosoanabasine	NAB	NABT[b]	4.1
	N′-nitrosoanatabine	NAT	NATT[b]	4.6
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol	NNK	NNAL[b]	1.4
	N′-nitrosonornicotine	NNN	NNNT[b]	13.7
Metabolites of PAHs	1-Hydroxynaphthalene	Naphthalene/carbaryl[c]	1-nap[a]	2.2
	2-Hydroxynaphthalene	Naphthalene	2-nap[a]	2.9
	1-Hydroxyphenanthrene	Phenanthrene	1-phe[a]	7.5
	Sum of 2- and 3-hydroxyphenanthrene	Phenanthrene	$\sum_{2,3}$-phe[a]	6.9
	2-Hydroxyfluorene	Fluorene	2-flu[a]	3.1
	3-Hydroxyfluorene	Fluorene	3-flu[a]	5.7
	1-Hydroxypyrene	Pyrene	1-pyr[a]	20.1
Metabolites of VOCs	2-Methylhippuric acid	Xylene	2MHA[a]	7.4
	3-Methylhippuric acid + 4-Methylhippuric acid	Xylene	34MH[a]	10.8
	N-Acetyl-S-(2-carbamoylethyl)-L-cysteine	Acrylamide	AAMA[a]	13.5
	N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	Acrylamide	GAMA[a]	11.0
	N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	Acrylonitrile	CYHA[a]	16.5
	N-Acetyl-S-(2-cyanoethyl)-L-cysteine	Acrylonitrile	CYMA[a]	12.3
	N-Acetyl-S-(2-carboxyethyl)-L-cysteine	Acrolein	CEMA[a]	12.8
	N-Acetyl-S-(3-hydroxypropyl)-L-cysteine	Acrolein	HPMA[a]	15.1
	N-Acetyl-S-(benzyl)-L-cysteine	Toluene[c]	BMA[a]	12.2
	Mandelic acid	Styrene	MADA[a]	21.4
	Phenylglyoxylic acid	Ethylbenzene/styrene	PHGA[a]	13.6
	N-Acetyl-S-(phenyl)-L-cysteine	Benzene	PMA[a]	17.3
	N-Acetyl-S- (2-hydroxypropyl)-L-cysteine	Propylene oxide	HPM2[a]	10.1
	N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	Dimethylformamide[c]	AMCA[a]	11.8
	N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	1,3-Butadiene	DHBM[a]	11.5
	N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	1,3-Butadiene	MHB3[a]	15.3
	N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	Crotonaldehyde	HPMM[a]	11.3
	N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	Isoprene	IPM3[a]	17.1
	2-Thioxothiazolidine-4-carboxylic acid	Carbon disulfide	TTCA[a]	10.1

[a]Measured in all individuals.
[b]Measured only among those with cotinine above 20 ng/mL.
[c]Multiple other parent chemicals can also be metabolized to these compounds.

metabolite concentrations were measured using ultra high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry according to a published procedure (32). LODs for VOC metabolites ranged from 0.500 to 15.0 ng/mL. Finally, creatinine was measured by a commercial automated, colorimetric enzymatic (creatinase) method implemented on a Roche/Hitachi Cobas 6000 Analyzer. **Table 1** shows the coefficients of variation (CV) of the tests calculated on blind pooled samples from GCS; all but two were below 20%, and 19 were below 10%, showing excellent assay performance.

Statistical analysis

For most biomarkers, fewer than 10% of the values were below the LOD, and none of the biomarkers had 20% or more below-LOD values. Concentrations below the LOD were replaced by the LOD divided by the square root of 2. All biomarker concentrations were adjusted for urinary dilution by dividing by urinary creatinine and were log-transformed. Because the distribution of biomarker concentrations is skewed, we calculated geometric means (GM) and 95% confidence intervals (95% CI) of these creatinine-corrected values. The total nicotine equivalent (TNE) is a standard method of estimating nicotine exposure and was calculated as the molar sum of nicotine metabolites (19). Depending on the number of metabolites measured in each person, we calculated TNE2 (the molar sum of cotinine and hydroxycotinine) for everyone, and TNE7 (the molar sum of all seven nicotine metabolites) for cigarette smokers.

To establish the proportion of each biomarker concentration explained by opiate use and nicotine dose, we used a decomposition method called the Oaxaca–Blinder method (33, 34). Oaxaca–Blinder decomposition uses stratified linear regression to segregate the observed differences among study groups into the differences in observed characteristics of individual participants (termed "endowments") and unexplained differences due to their group membership (coefficients). We stratified the study participants to

Table 2. Baseline and demographic characteristics of the study population selected from the Golestan Cohort male participants.

	Opiate nonusers			Opiate users		
	Never tobacco users ($n = 28$)[a]	Exclusive cigarette smokers ($n = 33$)[a]	Total ($n = 61$)	Exclusive opiate users ($n = 23$)[a]	Dual users of opiates and cigarettes ($n = 30$)	Total ($n = 53$)
Age: mean (SD)	51.9 (7.5)	51.7 (7.6)	51.8 (7.6)	52.1 (5.5)	49.3 (7.2)	50.5 (6.6)
Sex: M/F	28/0	33/0	61/0	23/0	30/0	53/0
Turkmen ethnicity (%)	67.9	72.7	71.4	100	86.7	92.5
Residence						
Urban (%)	21.0	42.4	32.8	4.3	20	13.2
Rural (%)	79.0	57.6	67.2	95.7	80	86.8
Education						
None (%)	53.6	39.4	45.9	52.2	56.7	54.7
1–8 years (%)	28.6	30.3	29.5	34.8	23.3	28.3
>8 years (%)	17.8	30.3	24.6	13.0	20.0	17.0
BMI						
Underweight (%)	3.6	0	1.7	4.3	16.7	11.3
Normal (%)	35.7	42.4	39.3	26.1	70	51.0
Overweight (%)	39.3	39.4	39.3	60.9	13.3	34.0
Obese (%)	21.4	18.2	19.7	8.7	0	3.7
Age when use started: mean (SD)						
Tobacco	NA	25.2 (7.4)	25.2 (7.4)	NA	27.2 (11.1)	27.2 (11.1)
Opiate	NA	NA	NA	45.2 (7.2)	35.4 (8.0)	39.7 (9.0)
Use intensity: mean (SD)						
Cigarettes per day	NA	10.9 (7.3)	NA	NA	16.4 (8.9)	NA
Opiate nokhods[b] per day	NA	NA	NA	3.5 (2.7)	4.7 (3.4)	4.2 (3.2)

Abbreviations: BMI, body mass index; NA, not applicable.
[a]Numbers exclude individuals who had self-reported tobacco status discordant with measured cotinine concentrations.
[b]Nokhod is a local measurement unit equal to approximately 0.2 g (43).

opiate users and nonusers, and then decomposed the differences in biomarker concentrations between these two groups into the proportion explained by "endowments" (nicotine dose, age, ethnicity, place of residence, education, and BMI) and the unexplained part due to being either an opiate user or nonuser. Because nicotine dose was, by far, the strongest component of those "endowments," we report this endowment part as the share of nicotine dose and (a much smaller share of) all other variables combined. Including cigarettes smoked per day or using nonlogarithmic transformations of TNE did not improve the model predictions. Decomposition was done using the Oaxaca ado file for Stata (StataCorp Inc.).

Comparisons between biomarker concentrations in different groups were made using the 95% CIs of their geometric means, and whether or not these confidence intervals overlapped. Whenever adjusted regression models were used, we defined a P value of 0.05 or lower as a statistically significant finding.

Results

Table 2 shows the baseline characteristics of the four groups (never tobacco/opiate users, exclusive cigarette smokers, exclusive opiate users, and dual opiate and cigarette users). Opiate users who also smoked cigarettes (dual users) were both heavier smokers and heavier opiate users than exclusive users of either cigarettes or opiates. Among dual users, 90% used opiates every day, compared with 74% of exclusive opiate users. All cigarette smokers (dual and exclusive) smoked cigarettes every day.

In **Table 3**, we compare the geometric means and 95% CI of the 39 study biomarkers among four groups (never tobacco/opiate users, exclusive cigarette smokers, exclusive opiate users, and dual opiate and cigarette users). Exclusive opiate users (i.e., those who did not smoke cigarettes) had high concentrations of most biomarkers, relative to nonopiate users who also did not smoke cigarettes. Except for one PAH (sum of 2- and 3-hydroxyphenanthrene or $\sum_{2,3}$-phe) and two VOC (BMA and PHGA) biomarkers, opiate users who also smoked cigarettes had the highest concentrations of all biomarkers. As a sensitivity analysis, we recalculated geometric means after excluding nondaily opiate users (six exclusive and three dual users). The Supplementary Table shows that the geometric means of PAHs and VOCs increased in both groups, but the differences between groups stayed the same.

Opiate users had higher nicotine doses (i.e., TNE2) than nonopiate users with a similar cigarette smoking history (**Table 3**). We used Oaxaca–Blinder decomposition to explore the proportion of the biomarker differences between opiate users and nonusers that could be explained by the amount of exposure to tobacco (their nicotine dose) versus the proportion explained by opiate use. As seen in **Table 4**, differences between opiate users and nonusers in PAH concentrations were explained by both the nicotine dose and opiate use, but opiates seemed to contribute a larger part of the differences in PAHs. The most striking of these was for $\sum_{2,3}$-phe, which was almost completely explained by opiate use. For the VOCs, both nicotine dose and opiate use seemed to explain the observed biomarker differences between opiate users and nonusers. However, a few VOC biomarkers were strongly explained by opiate use, including acrylamide metabolites (AAMA: 96.0%, GAMA: 95.3%), the 1,3-butadiene metabolite (DHBM: 92.0%), and the dimethylformamide metabolite (AMCA: 85.0%). Two other VOCs (BMA and TTCA) had a high percentage of the difference explained by opiate use, but because the difference between opiate users and nonusers was low, these differences were not statistically significant. All other variables in the model (age, ethnicity,

Table 3. Geometric means and 95% CIs of several urinary biomarkers across groups of Golestan Cohort male participants.

	Opiate nonusers		Opiate users	
	Never tobacco users ($n = 28$)[a]	Exclusive cigarette smokers ($n = 33$)[a]	Exclusive opiate users ($n = 23$)[a]	Dual users of opiates and cigarettes ($n = 30$)
Nicotine and other tobacco alkaloids (ng/mg creatinine)				
COTT	1.0 (0.7–1.5)	1,799.9 (1,200.7–2,698.3)	3.5 (1.9–6.4)	4,238.2 (3,420.8–5,250.9)
HCTT	2.3 (1.5–3.6)	2,661.6 (1,742.6–4,065.3)	5.4 (2.9–10.0)	7,201.6 (5,768.9–8,990.0)
TNE2[b]	0.02 (0.02–0.03)	24.9 (16.6–37.4)	0.05 (0.03–0.09)	62.6 (51.0–77.0)
COXT	—	214.9 (142.7–323.8)	—	437.3 (353.8–540.4)
NCTT	—	56 (37.1–84.4)	—	136.5 (109.2–170.7)
NICT	—	676.8 (382.1–1,198.8)	—	1,872.9 (1,329.1–2,639.4)
NOXT	—	182.6 (109.2–305.6)	—	307.6 (234.7–403.1)
NNCT	—	42.5 (27.0–67.0)	—	101 (79.4–128.4)
TNE7[b]	—	33.4 (21.9–50.8)	—	82.1 (66.2–101.8)
ANBT	—	4.6 (3.0–7.1)	—	12 (9.0–16.0)
ANTT	—	6.5 (3.9–10.7)	—	16.6 (12.3–22.5)
TSNAs (pg/mg creatinine)				
NABT	—	9.1 (6.2–13.5)	—	19.5 (15.1–25.1)
NATT	—	53.7 (34.5–83.6)	—	107.9 (83.9–138.6)
NNAL	—	130.9 (92.6–185.1)	—	230 (181.9–290.7)
NNNT	—	10.4 (6.9–15.8)	—	13.3 (8.7–20.1)
PAHs (ng/g creatinine)				
1-nap	9,102.5 (6,058.4–13,676.2)	14,637 (11,511–18,613)	14,443.8 (9,310.7–22,406.9)	28,993.2 (21,459.5–39,171.8)
2-nap	1,925.6 (1,474.6–2,514.5)	9,048.9 (6,864.2–11,929.0)	3,544.7 (2,475.5–5,075.7)	18,857.0 (15,123.1–23,512.8)
1-phe	219 (178.8–268.2)	264.5 (227.4–307.8)	290.6 (228.2–370.1)	491.9 (410.3–589.8)
$\sum_{2,3}$-phe	300.1 (227.8–395.3)	482.9 (371.5–627.6)	2,561 (1,290.9–5,080.7)	2,213.4 (1,326.8–3,692.6)
2-flu	404.8 (312.9–523.7)	1,238.6 (973.2–1,576.5)	538 (371.8–778.4)	2,044.2 (1,656.6–2,522.4)
3-flu	172.1 (126.3–234.6)	755.7 (566.1–1,008.9)	775.8 (452.9–1,328.9)	2,467.5 (1,835.1–3,317.8)
1-pyr	330.3 (258.0–422.7)	636.0 (504.0–802.4)	966.3 (665.2–1,403.7)	1,421.9 (1,111.6–1,819.0)
VOCs (ng/mg creatinine)				
2MHA	63.8 (40.8–100.0)	148.2 (113.5–193.5)	95.1 (53.5–168.9)	190.3 (133.3–271.8)
34MH	311.2 (212.1–456.6)	839.4 (650.3–1,083.5)	450.4 (264.7–766.3)	1,231.0 (875.6–1,730.7)
AAMA	43.1 (35.3–52.5)	124.7 (97.2–159.9)	243.5 (171.4–346.0)	281.0 (214.0–368.8)
GAMA	6.8 (5.8–7.8)	15.2 (12.4–18.7)	28.8 (19.3–42.9)	31.5 (23.8–41.8)
CYHA	0.6 (0.5–0.7)	14.2 (9.1–22.1)	4.7 (2.7–8.4)	35.5 (26.9–46.8)
CYMA	0.9 (0.7–1.3)	86.4 (58.3–127.9)	17.4 (9.7–31.0)	190.6 (148.6–244.4)
CEMA	74 (60.6–90.5)	186.2 (153.9–225.3)	105.4 (81.6–136.1)	244.2 (193.1–308.9)
HPMA	170.7 (125.6–232.1)	881.8 (661.8–1,174.9)	300.5 (211.7–426.5)	1,243.6 (855.6–1,807.7)
BMA	4 (2.9–5.4)	6.4 (4.4–9.2)	6.9 (4.5–10.5)	6.1 (4.5–8.2)
MADA	164 (135.3–198.8)	274.2 (225.7–333.2)	248.4 (201.2–306.8)	464.9 (375.9–575.0)
PHGA	73.1 (48.5–110.1)	101.3 (68.8–149.3)	60 (35.6–101.0)	97.9 (65.1–147.2)
PMA	1.2 (0.9–1.7)	1.5 (1.2–1.8)	1.3 (0.8–2.0)	2.0 (1.6–2.5)
HPM2	22.5 (17.4–29.1)	53.9 (42.9–67.7)	22.4 (17.3–29.0)	70.9 (56.7–88.7)
AMCA	66.9 (48.1–93.0)	296.3 (233.1–376.7)	283.3 (187.7–427.4)	771.1 (631.1–942.3)
DHBM	227.5 (166.8–310.4)	384.5 (337.3–438.4)	326.5 (259.8–410.3)	443.0 (371.5–528.4)
MHB3	3.9 (2.8–5.3)	23.3 (16.5–32.8)	5.3 (4.1–6.8)	32.9 (23.0–46.9)
HPMM	318.9 (243.7–417.4)	1,359.7 (980.2–1,886.0)	422.7 (304.2–587.5)	2,068.9 (1,434.0–2,985.0)
IPM3	1.8 (1.4–2.5)	21.6 (12.9–36.2)	3.4 (2.3–4.9)	39.0 (23.8–64.0)
TTCA	11.3 (7.1–18.1)	12 (9.3–15.4)	13 (8.3–20.4)	19.5 (12.5–30.4)

[a]Numbers exclude individuals who had self-reported tobacco status discordant with measured cotinine levels.
[b]TNE in nmol/mg creatinine. TNE2 was calculated on the basis of cotinine and hydroxycotinine only; TNE7 based on all seven nicotine metabolites.

place of residence, education, and BMI) contributed a much smaller share of biomarker differences than nicotine dose and opiate use.

Finally, we examined whether the observed higher concentrations of PAH and VOC biomarkers in opiate users came from the opiates themselves or their combustion. Because only the dual use group included oral opiate users, we compared nine dual users who only took opiates by mouth (i.e., "eaters") versus the larger group of dual users ($n = 21$) who only smoked them (**Table 5**). Concentrations of examined PAH and VOC biomarkers were largely similar between opiate eaters and smokers. Among PAHs, the only significant difference was in $\sum_{2,3}$-phe concentration, which was almost five times higher in opiate smokers (geometric mean = 3,477.4 ng/g creatinine; 95% CI, 1,891.3–6,393.6) than opiate eaters (geometric mean = 771.4; 95% CI, 436.4–1,363.4). Among VOCs, two acrylamide biomarkers (AAMA and GAMA) were substantially higher in opiate smokers than eaters.

Discussion

Opiate users in our study were exposed to toxicants and carcinogens, with biomarker concentrations comparable with those among cigarette smokers. Dual users of opiates and cigarettes had higher concentrations of all biomarkers compared with exclusive users of each

Table 4. Geometric means and 95% CIs of different metabolites in male opiate users and nonusers in Golestan Cohort participants and Oaxaca–Blinder decomposition of the percent explained by different factors.

			% of the Difference[a]		
	All opiate nonusers ($n = 61$)	All opiate users ($n = 53$)	Explained by nicotine dose	Explained by opiate use	Explained by other factors
PAHs (ng/g creatinine)					
1-nap	11,769.8 (9,363.0–14,795.1)	21,427.6 (16,449.8–27,911.7)	15.3[b]	88.1[b]	−3.4
2-nap	4,447.5 (3,382.8–5,847.3)	9,129.8 (6,762.5–12,325.8)	36.6[c]	57.7[b]	5.7
1-phe	242.6 (214.6–274.2)	391.5 (333.9–459.0)	10.4[c]	70.8[c]	18.8
$\sum_{2,3}$-phe	388.2 (319.6–471.4)	2,358 (1,579.3–3,520.7)	1.7	94.4[c]	3.9
2-flu	741.3 (592.9–926.9)	1,218.2 (935.7–1,586.0)	53.1[c]	24.5	22.4
3-flu	383.2 (289.6–506.9)	1,493.4 (1,085.3–2,055.0)	16.9[c]	77.9[c]	5.2
1-pyr	470.8 (391.4–566.3)	1,202.5 (972.1–1,487.4)	7.4[b]	79.8[c]	12.8
VOCs (ng/mg creatinine)					
2MHA	100.7 (77.1–131.4)	140.8 (101.9–194.6)	45.5[c]	54.5	0
34MH	532.3 (413.9–684.6)	795.7 (577.2–1,096.9)	42.5[c]	45.0	12.5
AAMA	76.5 (62.1–94.3)	264 (214.0–325.7)	10.5[c]	96.0[c]	−6.5
GAMA	10.5 (8.9–12.4)	30.3 (24.1–38.0)	9.0[c]	95.3[c]	−4.7
CYHA	3.3 (2.0–5.3)	14.8 (10.0–22.0)	29.1[c]	78.8[c]	−7.9
CYMA	10.8 (5.7–20.4)	67.4 (43.8–103.7)	31.1[c]	75.4[c]	−6.6
CEMA	122 (102.0–145.9)	169.6 (138.3–207.9)	45.5[c]	69.7[b]	−15.2
HPMA	415 (309.5–556.5)	671.4 (487.9–924.0)	52.1[c]	47.9	0
BMA	5.1 (4.0–6.5)	6.4 (5.1–8.2)	13.0	113.0	−26.0
MADA	216.6 (186.6–251.4))	356.6 (300.7–423.0)	24.0[c]	72.0[b]	4.0
PHGA	87.2 (66.1–115.0)	79.1 (57.5–108.9)	NA	NA	NA
PMA	1.3 (1.1–1.6)	1.6 (1.3–2.1)	28.6[b]	71.4	0
HPM2	36.1 (29.5–44.1)	43.0 (34.2–54.0)	83.3[c]	−5.5	22.2
AMCA	149.6 (114.0–196.4)	499.3 (390.6–638.4)	15.8[c]	85.0[c]	−0.8
DHBM	302.2 (255.4–357.6)	388.1 (336.7–447.3)	28.0[c]	92.0[b]	−20.0
MHB3	10.2 (7.4–14.1)	14.9 (10.6–20.8)	78.4[c]	10.8	10.8
HPMM	675.2 (509.1–895.5)	1,038.6 (748.3–1,441.4)	69.8[c]	37.2	−7.0
IPM3	7.0 (4.5–10.8)	13.5 (8.5–21.3)	61.5[c]	18.5	20.0
TTCA	11.7 (9.1–14.9)	16.4 (12.0–22.4)	8.8	123.5	−32.3

Note: NA, Opiate and nicotine contributions could not be calculated because biomarker concentration was lower among opiate users.
[a]Using Oaxaca–Blinder decomposition; negative percentages mean that the factors were in favor of a lower level in opiate users, and percentages above 100 show that the concentrations due to nicotine/opiates could have been higher if not counteracted by other factors acting in the opposite direction.
[b]$P < 0.05$.
[c]$P < 0.001$ for the association between each factor and the biomarker concentration in linear regression models, including TNE2 and opiate use, in addition to age, place of residence, ethnicity, education, and body mass index (BMI).

product. Although some of the biomarker concentrations among opiate users could be explained by their higher nicotine dose, opiate use itself contributed substantially to many biomarkers of exposure, particularly PAHs. Among toxicant and carcinogen biomarkers with high concentrations in opiate users, most had similar concentrations across opiate eaters and smokers, although three biomarkers ($\sum_{2,3}$-phe, AAMA, and GAMA) were higher among opiate smokers.

To the best of our knowledge, this is the first study to specifically compare several tobacco-related biomarkers in the urine from opiate users and nonusers. On the basis of our results, the biomarkers we studied can be broadly categorized into four groups. Group 1 were tobacco-specific biomarkers, which exclusively (or almost exclusively) originated from cigarette smoking. This group included tobacco alkaloids, and TSNAs, which usually have concentrations below LOD in tobacco nonusers. There were also two VOC metabolites (HPM2 and MHB3) in this group. These are normally detectable among tobacco nonusers, but in our study, nontobacco exposures (including opiate use) contributed very little (if at all) to their concentrations. Group 2 included biomarkers moderately ($\leq$75%) associated with opiate use. The contribution of opiate use to these biomarkers ranged between 18.5% (IPM3) and 75.4% (CYMA) and was independent of the route of opiate use. Group 3 biomarkers were strongly associated with opiate use, irrespective of route of use: three PAHs (1-hydroxynaphthalene, 3-hydroxyfluorene, and 1-hydroxypyrene) and three VOCs (CYHA, AMCA, and DHBM) fell into this category. Opiate use contributed almost 75% to 92% of these biomarkers, and their concentrations were comparable between opiate eaters and smokers. Group 4 biomarkers were specifically associated with the combustion of opiates: these included the sum of two phenanthrene metabolites (i.e., $\sum_{2,3}$-phe) and two acrylamide metabolites (AAMA and GAMA). About 95% of these biomarkers came from opiate use, and concentrations were much higher in opiate smokers than eaters. It is noteworthy that many (but not all) of the parent compounds for the biomarkers in our study (such as acrylamide) are on the FDA's list of Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke (17). In addition to these four groups, three VOCs did not show a remarkable contribution from either opiate use or cigarette smoking (BMA, PHGA, TTCA).

Combined use of opiates and tobacco has been more strongly associated with increased risk of bladder (35) and esophageal cancer (22) than with either opiate or tobacco exposure alone. In this study, opiate users who also smoked cigarettes had the highest concentrations of all biomarkers compared with exclusive users of cigarettes and exclusive users of opiates. Two factors can contribute to these

Table 5. Geometric means and 95% CIs of different tobacco-related metabolites among dual opiate and cigarette users by their route of opiate use.

	Opiate smokers ($n = 21$)	Opiate eaters ($n = 9$)
Nicotine and other tobacco alkaloids (ng/mg creatinine)		
COTT	4,448.7 (3,445.8–5,743.3)	3,785 (2,348.1–6,101.3)
HCTT	6,938.9 (5,212.8–9,236.7)	7,853.8 (5,229.4–11,795.2)
COXT	456.3 (352.4–590.8)	396.0 (252.1–621.9)
NCTT	126.5 (97.9–163.4)	163.2 (97.1–274.4)
NICT	1,992.3 (1,296.9–3,060.5)	1,621.5 (814.4–3,228.3)
NOXT	344.3 (252.3–469.8)	236.5 (128.9–434.0)
NNCT	104.5 (77.7–140.3)	93.3 (56.1–155.1)
TNE7[a]	83.1 (63.4–108.9)	79.8 (51.8–122.9)
ANBT	14.0 (10.2–19.2)	8.5 (4.3–16.5)
ANTT	19.3 (14.1–26.3)	11.8 (5.5–25.5)
TSNAs (pg/mg creatinine)		
NABT	21.7 (15.8–29.8)	15.0 (9.4–24.1)
NATT	115.7 (83.7–160.0)	91.6 (59.1–142.1)
NNAL	235.5 (178.3–311.2)	217.5 (127.2–372.0)
NNNT	16.2 (9.6–27.4)	9.1 (4.2–19.5)
PAHs (ng/g creatinine)		
1-nap	27,434.6 (18,469.6–40,751.3)	32,982.9 (19,830.3–54,858.9)
2-nap	18,544.3 (13,979.5–24,599.7)	19,607.2 (12,869.4–29,872.5)
1-phe	486.3 (392.9–601.7)	505.4 (330.2–773.6)
$\sum_{2,3}$-phe	3,477.4 (1,891.3–6,393.6)	771.4 (436.4–1,363.4)
2-flu	1,961.4 (1,510.0–2,547.7)	2,251.2 (1,468.8–3,450.5)
3-flu	2,668.2 (1,827.2–3,896.1)	2,056.0 (1,195.9–3,534.5)
1-pyr	1,488.2 (1,128.1–1,963.2)	1,278.6 (693.9–2,356.0)
VOCs (ng/mg creatinine)		
2MHA	166.9 (107.7–258.6)	258.5 (127.9–522.2)
34MH	1,150.2 (753.8–1,755.1)	1,442.1 (718.1–2,896.0)
AAMA	341.4 (243.7–478.2)	178.3 (124.0–256.5)
GAMA	40.9 (30.2–55.3)	17.2 (10.9–27.2)
CYHA	38.7 (28.0–53.5)	28.9 (15.4–54.5)
CYMA	183.2 (134.7–249.3)	209.0 (124.7–350.3)
CEMA	219.1 (164.7–291.5)	314.5 (199.7–495.4)
HPMA	1,211.3 (743.5–1,973.5)	1,322.4 (677.8–2,579.9)
BMA	5.3 (3.8–7.4)	8.4 (4.4–16.2)
MADA	441.1 (335.9–579.3)	525.6 (356.7–774.5)
PHGA	80.9 (49.3–132.5)	152.8 (69.0–338.2)
PMA	2.0 (1.6–2.5)	2.0 (1.2–3.4)
HPM2	71.4 (53.6–95.1)	69.6 (45.4–106.8)
AMCA	818.9 (627.3–1,069.1)	670.2 (495.0–907.5)
DHBM	415.7 (328.7–525.7)	514 (400.0–660.6)
MHB3	30.7 (19.2–49.2)	38.4 (21.3–69.3)
HPMM	1,945.9 (1,213.2–3,121.2)	2,386.8 (1,217.3–4,680.1)
IPM3	35.9 (18.5–69.8)	47.2 (22.1–100.7)
TTCA	15.3 (10.0–23.4)	34.3 (10.4–113.5)

[a]TNE7, Total nicotine equivalent in nmol/mg creatinine based on all seven nicotine metabolites.

high concentrations: these individuals were receiving PAHs and VOCs from two sources (cigarettes and opiates), and they were heavier users of both cigarettes and opium than individuals who used only one of these products. Previous studies have also shown that opioid use is associated with greater nicotine dependence (3) and poor smoking cessation outcomes (5).

The concentration of $\sum_{2,3}$-phe among opiate users in our study reached almost 10 times the concentration reported among U.S. cigarette smokers participating in NHANES (22). Opiates contain different alkaloids, and among them morphine, codeine, and thebaine are the most abundant. These alkaloids are usually called phenanthrene-type alkaloids and contain a similar heterocyclic ring (36). Previous studies have shown that compounds resulting from high-temperature treatment of these alkaloids, either by burning of opiate products or the direct pyrolysis of alkaloids (particularly morphine), shared a common 3-hydroxyphenanthrene moiety (37). The pyrolysates of phenanthrene-type alkaloids showed strong mutagenic capacity against *Salmonella typhimurium*, which was strongest when morphine itself was pyrolysated, and correlated with their nitrogen content (38), as described for other PAHs such as benzo[*a*]pyrene (39). Phenanthrene-based species are also produced as a result of pyrolysis of heroin, the most common illicit opiate used worldwide (40). Because smoked drugs bypass first-order metabolism in the liver, many other drugs of abuse, even synthetic opioids, are being increasingly used in this way. A study of smoked fentanyl has shown significant amounts of VOCs such as styrene and benzene in the pyrolysis products of this synthetic opioid (41). However, the information on the toxicity of these opioid products, and biomarkers associated with their use are not available.

Pyrolysis does not seem to be the only factor contributing to toxicant exposure among opiate users in our study. Some relatively high PAH and VOC metabolite concentrations were associated with eating opium, and to the best of our knowledge, these opium products were not processed using high temperature (40). Eating opium has also been associated with the risk of some cancers (12) and death from different causes (16, 42). Together, these findings suggest that VOCs and PAHs are present in the opiate products even without exposure to high temperatures. As there are little prior data, we are currently conducting additional studies to evaluate this hypothesis.

Cigarette smokers in GCS seem to smoke, on average, fewer cigarettes than smokers in the United States and have lower risk of mortality (20). We have previously shown the patterns of exposure biomarkers among users of different tobacco products in GCS and compared them with those among U.S. cigarette smokers (24). The current exploratory study is the first to use the same state-of-the-art analytic methods among opiate and dual tobacco/opiate users. Our study has several limitations; it is limited by a relatively small sample size, but, although multiple comparisons are of concern, we did observe clear associations between use of tobacco and/or opiates and biomarker concentrations. Urinary metabolites are markers of relatively recent exposure, but we have previously shown acceptable correlations between biomarker concentrations in baseline urine and repeated samples taken after several years (24), particularly in the presence of a strong source of exposure (such as cigarette smoking). Residual confounding by tobacco use is also of concern for analyses of opiate users. However, our study leveraged both self-report and biomarkers of tobacco exposure, and we observed high levels of VOCs and PAHS among opiate users in the absence of detectable tobacco use. Finally, it should be noted that study participants used raw opium or opium-derived nonmedical opiates that were smoked or taken by mouth. Furthermore, larger studies should be directed toward identifying biomarkers of toxicant exposures among people who use other types of opiates, and/or use them by other routes (such as injection).

In conclusion, this study provides evidence of opiate users' exposure to toxicants and carcinogens from opiate use, and, in dual users, from concomitant cigarette smoking. Given the large number of people using different forms of opiates across the world, and the high prevalence of comorbid nicotine dependence, these exposures can have substantial global public health impact. Future studies examining the chronic effects of such opiate uses, as well as the underlying physiologic mechanisms of those effects, are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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