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Harmful and Potentially Harmful Constituents in the Filler and Smoke of Tobacco-Containing Tobacco Products

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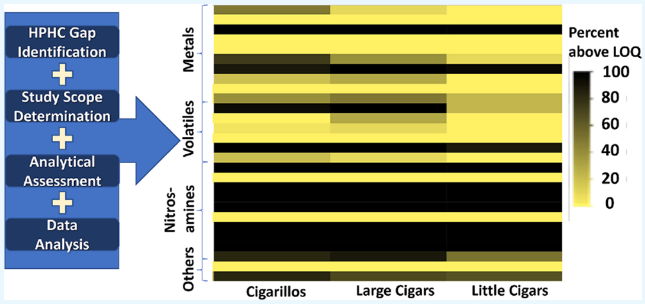
ABSTRACT: The U.S. Food and Drug Administration established a list of 93 harmful and potentially harmful constituents (HPHCs) in tobacco products. While HPHCs are required to be submitted for tobacco products, knowledge gaps exist regarding which tobacco-containing tobacco product (TCTP, i.e., tobacco products that contain tobacco(s) as a component) types (cigarettes, cigars, roll-your-own tobaccos [RYOs], pipe tobaccos [pipes], smokeless tobacco products [STPs], waterpipe tobaccos [waterpipes]) and matrices (filler, smoke) contain which HPHCs. This study identified and addressed such gaps by conducting literature searches and measuring the amount of HPHCs in TCTP types and matrices. First, literature searches, performed for cigarettes, RYOs, and STPs for publications up to 2014 and for cigars, pipes, and waterpipes for publications up to 2016, identified knowledge gaps for the 93 HPHCs (or 119 HPHCs if cresols [*o*-, *m*-, *p*-cresol] are counted as 3 and chlorinated dioxins/furans as 25) across TCTP types and matrices. Then, three ISO 17025 accredited laboratories including two subcontracted laboratories performed the HPHC quantifications. Inclusion of the HPHCs, TCTP types, and matrices in the study scope was also determined by the availability of validated analytical methods in each laboratory. Eleven (9%) HPHCs are quantifiable in all brands for all TCTP types and matrices, 33 (28%) HPHCs are not quantifiable in any brands of any TCTP type and matrix, and 74 (63%) HPHCs are quantifiable only in some brands across TCTP types and matrices examined. Understanding the quantifiability of HPHCs in each TCTP type and matrix can inform the scientific basis for manufacturers regarding the regulatory requirements for reporting HPHCs. The quantity of HPHCs observed can also inform the evaluation of the public health impact of HPHCs and public communications regarding the health risks of tobacco products.



■ INTRODUCTION

The U.S. Federal Food, Drug, and Cosmetic Act (FD&C Act) grants the U.S. Food and Drug Administration (FDA) the authority to regulate the manufacture, marketing, and distribution of cigarettes, cigarette tobaccos, roll-your-own tobaccos (RYOs), and smokeless tobacco products (STPs, e.g., chewing tobacco, moist snuff, dry snuff, and snus)[1] to protect the public health and reduce tobacco use by minors under Section IX. In 2016, under this jurisdiction, FDA issued a final rule to deem products meeting the statutory definition of "tobacco product" to be subject to the FD&C Act.[2] This deeming rule extends the FDA authority to regulate electronic nicotine delivery systems (ENDS), cigars (cigarillos, little cigars, large cigars), pipe tobaccos (pipes), waterpipe tobaccos (waterpipes), heated tobacco products (HTPs), and any other tobacco products meeting the statutory definition of a tobacco product.[2]

The FD&C Act required FDA to establish a list of harmful and potentially harmful constituents (HPHCs) to health in each tobacco product brand and sub-brand by quantity. HPHCs are chemicals or chemical compounds in tobacco products or tobacco product smoke/aerosol that (a) are or potentially are inhaled, ingested, or absorbed into the body and (b) cause or have the potential to cause harm to users or nonusers of tobacco products.[3] The FD&C Act requires tobacco manufacturers or importers to report the HPHC quantities in their tobacco products including the yields in smoke/aerosol. In total, the 2012 established list contained 93 HPHCs (119 if cresols [*o*-, *m*-, *p*-cresol] are counted as 3 and chlorinated dioxins/furans as 25 [see Tables 2 and S1]) when this study started.[4] In 2019, FDA proposed to add 19 additional HPHCs relating to ENDS and other deemed tobacco products to the established list.[5]

Knowledge gaps exist regarding whether an HPHC is quantifiable or not quantifiable in a tobacco product or in tobacco product smoke/aerosol, especially for the newly deemed tobacco products (cigars, pipes, waterpipes, HTPs, ENDS). For example, cigarette smoke contains acrylamide at quantifiable yields,[6−9] but few publications report acrylamide

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yields in cigar smoke.[10] Further, both cigarette[7] and STP filler[7,11] contain quantifiable amounts of acrylamide. However, no publication reported whether acrylamide is in RYOs. Thus, it is unknown whether RYO fillers and cigar smoke contain quantifiable acrylamide. Similarly, it is unknown whether certain HPHCs of regulatory interest vary across marketed brands within a tobacco product type by matrix (e.g., filler, smoke/aerosol).

When examining HPHCs in tobacco products, the exposure and exposure route (smoke inhalation or oral uptake) to users and nonusers need to be considered. An HPHC may be quantifiable in the finished tobacco product, but if it does not reach users and nonusers, the potential harm associated with this HPHC in this tobacco product may be minimal. For STPs, the user's exposure to the HPHC(s) directly links to its amount in the finished tobacco product.[12] For combustible tobacco products, smoke HPHCs transferred from fillers or generated during the combustion process are more important. A well-known example is the formation of cigarette smoke carbonyls from the incomplete combustion of carbohydrates (e.g., sugars).[13,14] For combustible tobacco products, it is important to examine both the tobacco fillers comprising the products and the smoke produced by the products.

This study analyzes only tobacco-containing tobacco products (TCTPs, i.e., tobacco products that contain tobacco(s) as a component) but not other tobacco products (e.g., ENDS containing nicotine extracted from tobacco or non-tobacco nicotine). The study goal is to determine which HPHCs are quantifiable or not quantifiable in which tobacco product types and matrices. Specifically, this study (1) identifies the knowledge gaps regarding if HPHCs are quantifiable/not quantifiable in tobacco product filler and smoke by examining the available literature to find HPHCs (conflicting, limited, or no data available) in TCTP types (cigarettes, cigars, pipes, RYOs, STPs, and waterpipes; see the Methods section for details) and matrices (filler and smoke; see Table S1); and (2) addresses these gaps by quantitatively measuring the HPHCs in the filler of cigarettes, cigars, pipes, RYOs, STPs, and waterpipes as well as HPHCs in the smoke from cigarettes and cigars. Thus, this publication reveals which HPHCs are quantifiable (at and above the limit of quantification [LOQ] of the analytical test method used) and which are not quantifiable (below LOQ of the analytical test method used) across various TCTP brands, types, and matrices.

■ METHODS

Knowledge Gap Identification. This study conducted literature searches for cigarettes, RYOs, and STPs between 2013 and 2014 for publications up to 2014 and for cigars, pipes, and waterpipes from 2014 to 2016 for publications up to 2016 to identify the knowledge gaps regarding the quantifiability/lack of quantifiability of the 93 HPHCs in TCTP fillers and smoke. The literature databases searched include SciFinder, PubMed, ScienceDirect, and Tobacco Industry Documents (owned by the University of California, San Francisco). Keywords used include "constituent" OR "chemical (specific HPHC name)" OR "chemistry" AND "tobacco" OR "tobacco smoke" OR "cigarette smoke" OR "cigar smoke" OR "smokeless tobacco" OR "roll-your-own" OR "pipe tobacco" OR "waterpipe" OR "hookah". The search resulted in 637 articles. After excluding patents, conference presentations, and the articles not in English, 114 articles were considered to contain gap identification information (see Table S1 and references therein). For each TCTP type and relevant matrix, each HPHC is in one of three categories: "established data", "limited data", and "no data". An HPHC is categorized as having established data if (1) the literature searches had relevant results and the HPHC did not fall into the limited data category or (2) other ongoing research projects[15,16] or subsequent FDA publications from these studies[17,18] covered the analysis of this HPHC in a specific tobacco product type and matrix. An HPHC is categorized as having limited data if (1) the publications available did not include domestically available (i.e., United States) commercial TCTP, (2) the publication lacked test method information, (3) the publication methods are outdated (i.e., high detection limit compared to the recent literature), or (4) the publications report conflicting results. Lastly, an HPHC is categorized as having no data if the literature searches had no relevant results for the HPHC for the tobacco product and matrix of interest. Table S1 shows the results of the literature searches. This study examines the HPHCs in the limited data and no data literature search categories.

HPHC Groupings. This study splits the 93 HPHCs on the 2012 established list[4] into 12 groups: alkaloids, amides, aromatic amines, carbonyls, chlorinated dioxins and furans, heterocyclic aromatic amines, metals, nitrosamines, phenols, polycyclic aromatic hydrocarbons, volatiles and semivolatiles, and others. Each group consists of compounds that have similar chemical properties and typically are analyzed using the same or similar testing methods (even though the test methods used may differ between labs), except for the "others" group, which encompasses all chemicals that do not fit into any other groups. Among all 93 HPHCs, cresols and chlorinated dioxins/furans are classes of compounds, not specific chemicals. This study examines all three cresols (*o*-cresol, *m*-cresol, *p*-cresol). For chlorinated dioxins/furans, this study chooses the specific 25 chemicals listed in Table S2 to represent the class of compounds from the testing abilities of the laboratories that conduct tests for pharmaceuticals and environmental industries. This results in the inclusion of 119 HPHCs in the study scope.

Analytical Test Methods. In addition to the literature searches, the availability of the validated analytical methods in the testing laboratories at the time when the study was initiated influences which HPHC gap to address for each TCTP type and matrix of interest. Fully validated analytical methods or analytical methods within the scope of the laboratory quality system were considered available for the study. Table S2 lists the laboratories chosen for analyzing the HPHCs in each TCTP type and matrix. Three separate International Organization for Standardization (ISO) 17025 accredited laboratories were contracted to perform the quantification of the HPHCs between 2014 and 2020. The laboratories are Labstat International Inc. (Lab 1, Maxxam Analytics tested radioactive metals and chlorinated dioxin/furans as a subcontractor), Centers for Disease Control and Prevention (CDC) Tobacco Laboratory (Lab 2), and Enthalpy Analytical (Lab 3, three lab locations, GEL Laboratories tested radioactive metals as a subcontractor). All quantitative methods at each laboratory were fully validated (contained all needed validation parameters: accuracy, precision, limit of detection [LOD], LOQ, specificity, range, linearity, robustness), for the intended purposes, and either in the laboratory's accreditation scope, under a quality control system, or subcontracted to a third-party laboratory that specialized in the methodology. Table S3 provides the instrumentation used at each lab for each HPHC in each

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TCTP type and matrix. In addition, all methods in Table S3 are quantitative and validated unless otherwise noted.

For each HPHC, each laboratory conducted at least five replicate measurements for each product brand within each TCTP type for each matrix. For smoke analyses, cigarettes and cigars were smoked under the Canadian Intense smoking regimen (CI: 55 mL puff volume, 30 s puff frequency, 2 s puff duration, 100% vent block) to produce mainstream smoke[19] because CI condition generates a higher amount of smoke and total particulate matter (TPM) compared to the nonintense smoke condition. Therefore, CI is expected to generate a higher amount of target HPHCs, which is more appropriate for this study to determine if an HPHC is quantifiable or not quantifiable. Other smoke conditions for cigarettes, such as conditioning, TPM collection, butt length, and insertion depth, followed those defined in ISO 3402,[20] ISO 4387,[21] and ISO 3308.[22] Other smoke conditions for cigars, such as conditioning (e.g., minimum 72 h), TPM collection, butt length (e.g., 33 mm, filter +8 mm, or artificial mouthpiece + 17 mm), and insertion depth (i.e., 28.0 ± 1.0 mm) followed Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Recommended Method (CRM) numbers 46,[23] 65,[24] and 64.[25] This study defined the maximum conditioning time as 10 days for both cigarettes and cigars.

Product Types, Brands, and Sample Matrix Selection. This study selected products (except waterpipe) that reflected both high and low market shares (i.e., large and small manufacturers) based on the 2012 sales data published by Euromonitor International. Market available off-brands with low market shares were included to provide a better representation of the entire marketplace. As shown in Tables S4 and S5, selected products include both flavored and nonflavored and STPs contain different subcategories (chewing tobacco, moist snuff, dry snuff, snus). Waterpipe brands were solely based on market availability at the time when the study began (2014−2018). The study was designed to select 30 cigarette, 30 little cigar, 20 cigarillo, 10 large cigar, 30 pipe, 30 RYO, 30 STP, and 25 waterpipe brands. However, because the marketplace availability changed at the time of each laboratory study, the number of brands examined differed from the number initially planned (Table 1). The number of brands was reduced when no proper replacement was available. When available, a replacement brand was selected by matching the original brand in flavor, product design, manufacturer, or/and market size. CDC located in Atlanta, Georgia, acquired the TCTPs analyzed in lab 2 in 2014, while NorthStar Technology Corp located in Irvine, California, and Bizzell Group LLC located in Bowie, Maryland, acquired the TCTPs for lab 1 and lab 3 in 2017−2018 and 2019, respectively. This study required that all products be from the U.S. market but did not specify the sampling procedure for either product acquisition (e.g., specific geographical locations within the United States) or sampling at the time of testing. The actual product acquisition procedure at the product purchasing stage was similar to that of "at point of sale at one time" specified in ISO 8243.[26] Sufficient amounts of products for each brand within each TCTP type were acquired. The sampling procedure at the time of testing followed the established sampling protocols of each testing laboratory. Samples were stored at −20 °C in the testing laboratory upon receiving before conditioning for HPHC analysis.

This study analyzed smoke HPHCs for cigarettes and cigars and filler HPHCs for cigarettes, cigars, pipes, RYOs, STPs, and waterpipes. Although RYOs and pipes (when used with paper tubes as an RYO alternative) are also combustible tobacco products, this study does not include smoke HPHC analysis for these types because the smoke HPHCs from both are expected to be qualitatively similar to those in cigarette smoke. Further analysis of HPHCs in smoke from RYOs and pipes (when used with both paper tube and pipe) will be considered if new evidence proves otherwise. Testing of HPHCs in waterpipe smoke may also be considered in the future when validated test methods based on the application of a consensus smoke collection standard become available in testing laboratories, considering the recent (i.e., 2019) publications of the consensus standard for smoking conditions and technical specifications for determining the total collected matter from waterpipe use.[27−30]

Data Quality, Processing, and Statistics. The data generation quality control of this study is at the testing level by the ISO 17025 accredited laboratory quality systems. Data quality checks were performed to ensure all data across the laboratories are accurate, reliable, processed the same way, and error-free. These data quality checks include one researcher examining the contracted results to compare the raw data to the summarized data to (1) ensure no transcription errors, (2) determine that the values labeled as "below the LOD" (BDL) or below the LOQ (BLOQ) were correctly labeled, and (3) ensure that the tobacco product name is correctly labeled. The researcher also examined the test information, including method validation parameters, to ensure that the information supports the claimed method validation status and reported data. The data summary is in Table S4 for filler and Table S5 for smoke. After the data quality check, the mean HPHC quantity or yield for each brand set was categorized as quantifiable or not quantifiable. For this study, quantifiable is defined as at least three (out of five) replicates of a brand having a quantity or yield at or above LOQ, while not quantifiable is defined for any data that does not meet the above criteria. Once quantifiable and not quantifiable were coded, the percent quantifiable for each HPHC for each TCTP type in each matrix was calculated based on the total number of brands tested within that product type. For example, aflatoxin B1 in cigarette filler is quantifiable in 24 out of the 27 brands examined; thus, the percent quantifiable is 89%. Another researcher involved in this study verified the first researcher's data quality checks and the HPHC categorizations. This study visualized the calculated quantifiable percentage for

Table 1. Number of Brands for Each TCTP Type and Matrix Examined at Each Laboratory

	Lab 1 (Labstat International Inc.)		Lab 2 (CDC)		Lab 3 (Enthalpy Analytical)	
	smoke	filler	smoke	filler	smoke	filler
cigarette	27	27	27	27	27	27
large cigar	10	10			10	10
little cigar	29	29			29	29
cigarillo	20	20			19	19
pipe		28		2[a]		28 + 2[a]
RYO		24		27		22[b]
STP		27		30		27
waterpipe						21 or 19[c]

[a]Two brands were purchased as RYO but were pipe tobaccos. [b]Two brands have nearly identical descriptions (currently listed as 05a and 05b). [c]The number of brands purchased differed by the year the experiments were performed.

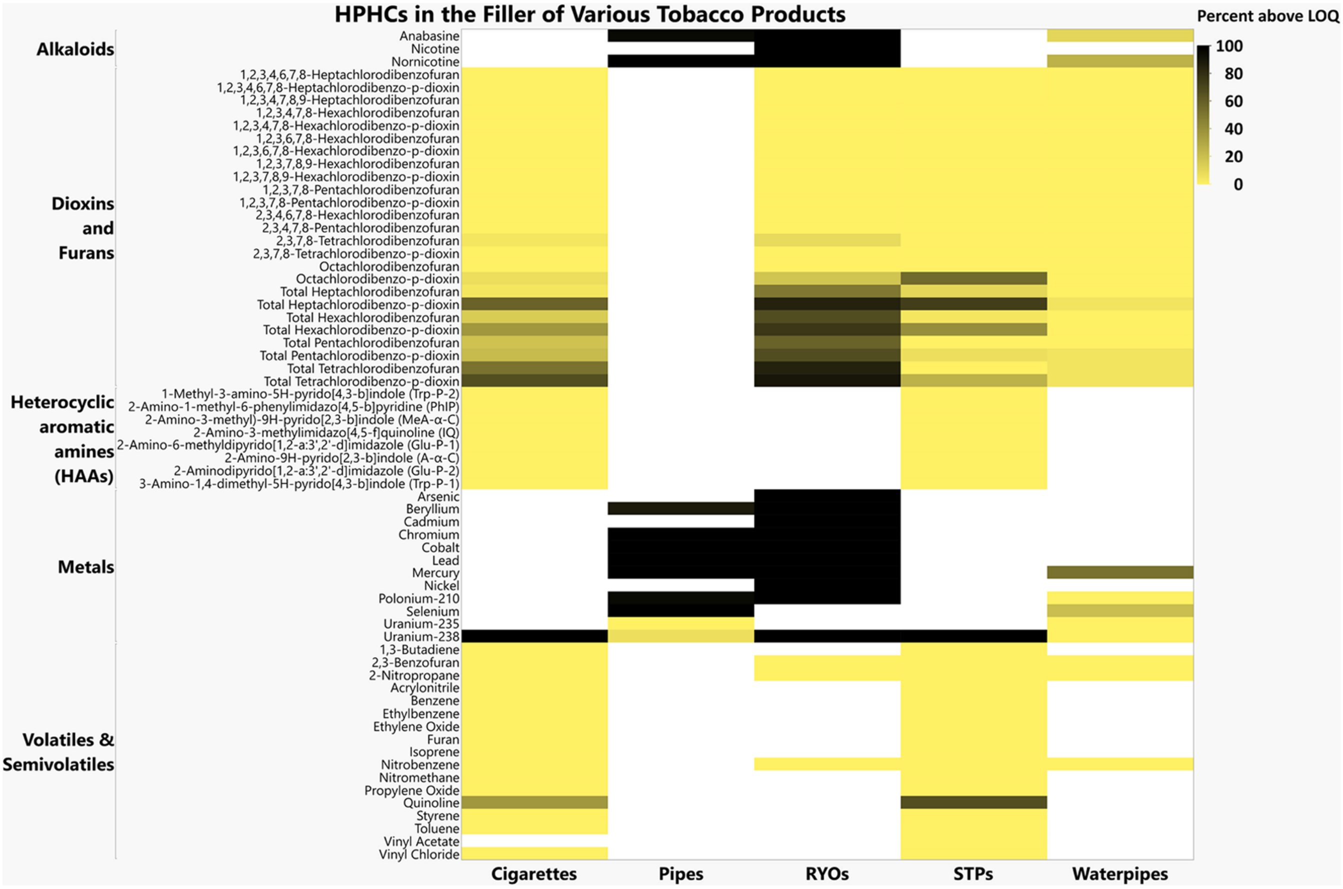

Figure 1. Heatmap of HPHCs in the filler of cigarettes, pipes, RYOs, STPs, and waterpipes examined.

each HPHC by brand in each TCTP type and matrix examined by heatmaps generated using JMP 14 (Figures 1−4).[31]

RESULTS

Table S4 for filler and Table S5 for smoke list the measured mean, standard deviation, and range for each examined HPHC in each product brand and product type. Table 2 summarizes the number of brands that had quantifiable HPHCs. The parentheses in Table 2 show the percentage of these brands of the total number of brands examined. Figures 1−4 are heatmaps visualizing the percent of brands where the HPHC is quantifiable for each examined HPHC in each TCTP type and matrix. From these results, this study sorts HPHCs into one of three categories: (**1**) quantifiable in all brands for all TCTP types and matrices examined, (**2**) not quantifiable in any brands of any TCTP type and matrix examined, and (**3**) quantifiability varies by tobacco product brands, types, and/or matrices examined. Category 3 is the largest category and has four subcategories: (**3a**) quantifiable in some brands in every TCTP type and matrix examined, (**3b**) quantifiable in some brands in some TCTP types and matrices, and quantifiable in all brands of others examined, (**3c**) quantifiable in some brands in some TCTP types and matrices and not quantifiable in any brand of others examined, and (**3d**) quantifiability varies among TCTP brands, types, and matrices examined. The HPHC categorizations are dynamic, and the categorization of an HPHC may change when new data that fill the knowledge gaps of certain HPHCs appear. Table 3 summarizes the quantifiability categories of HPHCs. This categorization of the quantifiable status of HPHCs does not include the HPHC quantities and yields that were found to have established data by this study during the literature search because the test information (e.g., lab accreditation and method validation status, smoking regimen, number of replicates used, number of product brands tested) provided for the published data is limited. Thus, data quality is highly likely to be at different control levels for the data collected in this study and those in publications.

DISCUSSION

TCTP brands in this study are brands representative of the U.S. market. Each TCTP type and matrix has 10 or more selected brands, as listed in Tables S4 and S5. Thus, the results from this study are expected to be representative of these TCTP types and matrices in the U.S. market.

Category 1: Quantifiable in All Brands for All TCTP Types and Matrices Examined. As shown in Table 3, 11 (9%) examined HPHCs are in category 1 and are quantifiable in all brands of every TCTP type and matrix examined. Category 1 HPHCs include nicotine, acetamide, two aromatic amines (o-anisidine, o-toluidine), two carbonyls (acetaldehyde, formaldehyde), two metals (cadmium, nickel), two nitrosamines [*N*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)], and ammonia. These HPHCs may be of high interest to tobacco product manufacturers, academic researchers, and regulators as these HPHCs could potentially reach any users of relevant tobacco products.

Category 2: Not Quantifiable in Any Brand of Any TCTP Type and Matrix Examined. Thirty-three (28%) examined HPHCs are in category 2 and are not quantifiable regardless of TCTP type and matrix examined. HPHCs in

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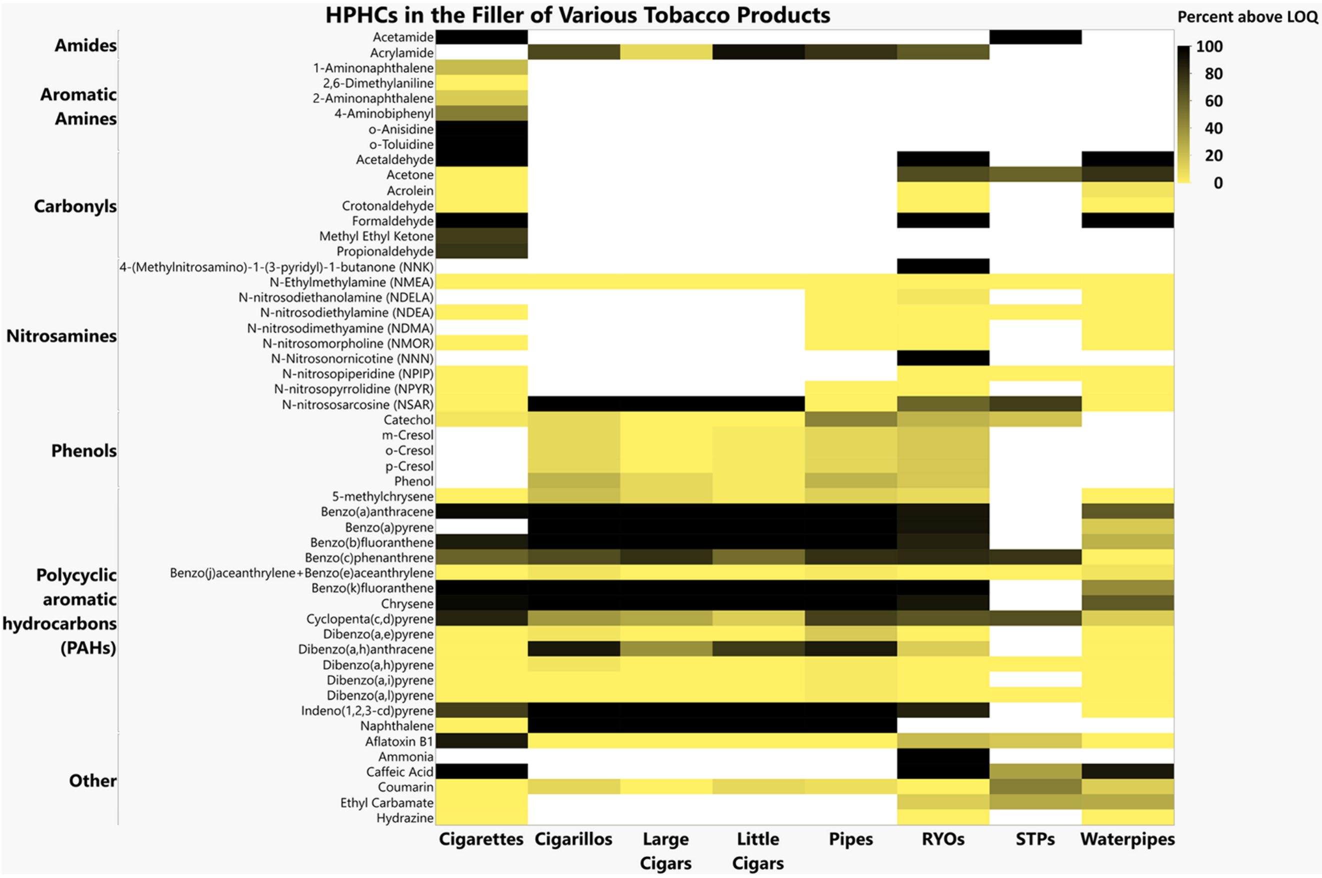

Figure 2. Heatmap of HPHCs in the filler of cigarettes, cigarillos, large cigars, little cigars, pipes, RYOs, STPs, and waterpipes examined.

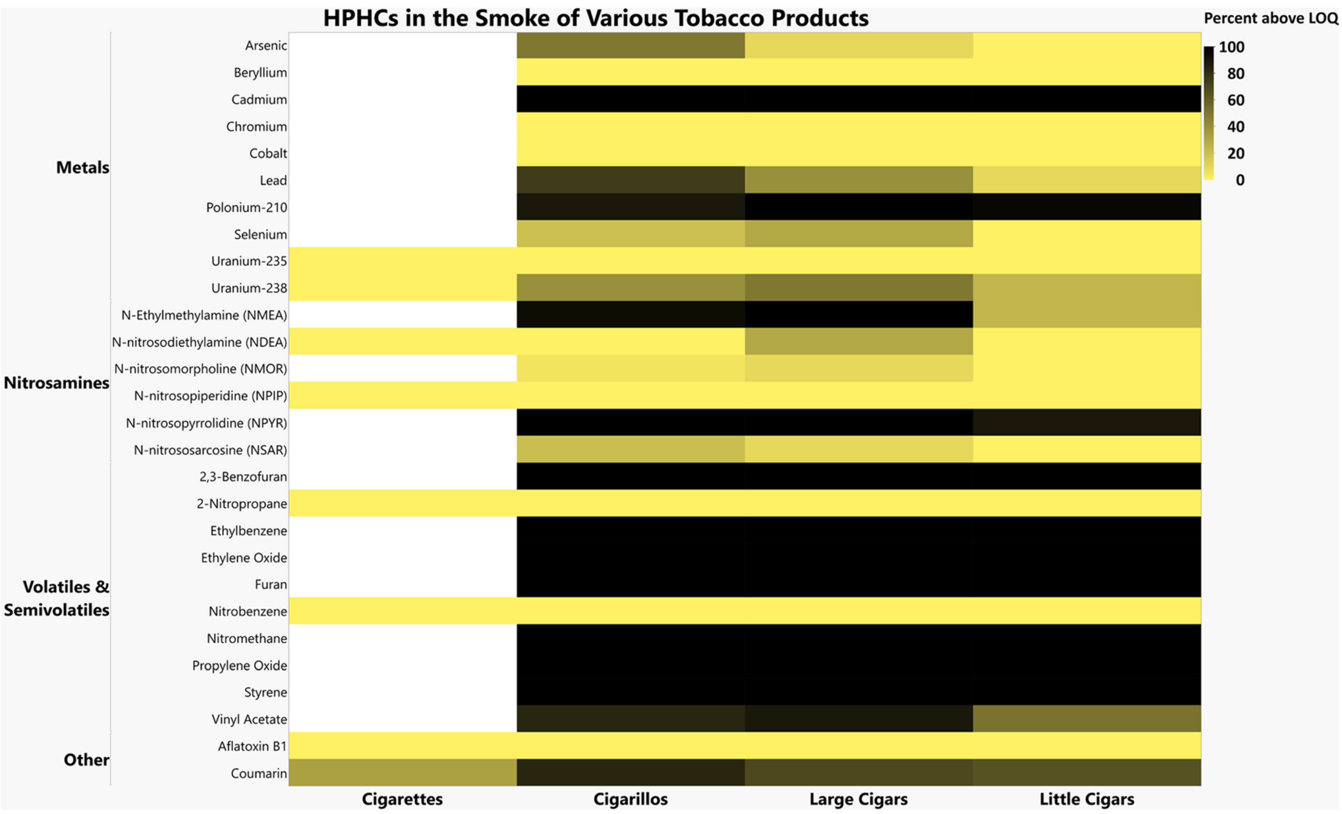

Figure 3. Heatmap of HPHCs in the smoke of cigarettes, cigarillos, large cigars, and little cigars examined.

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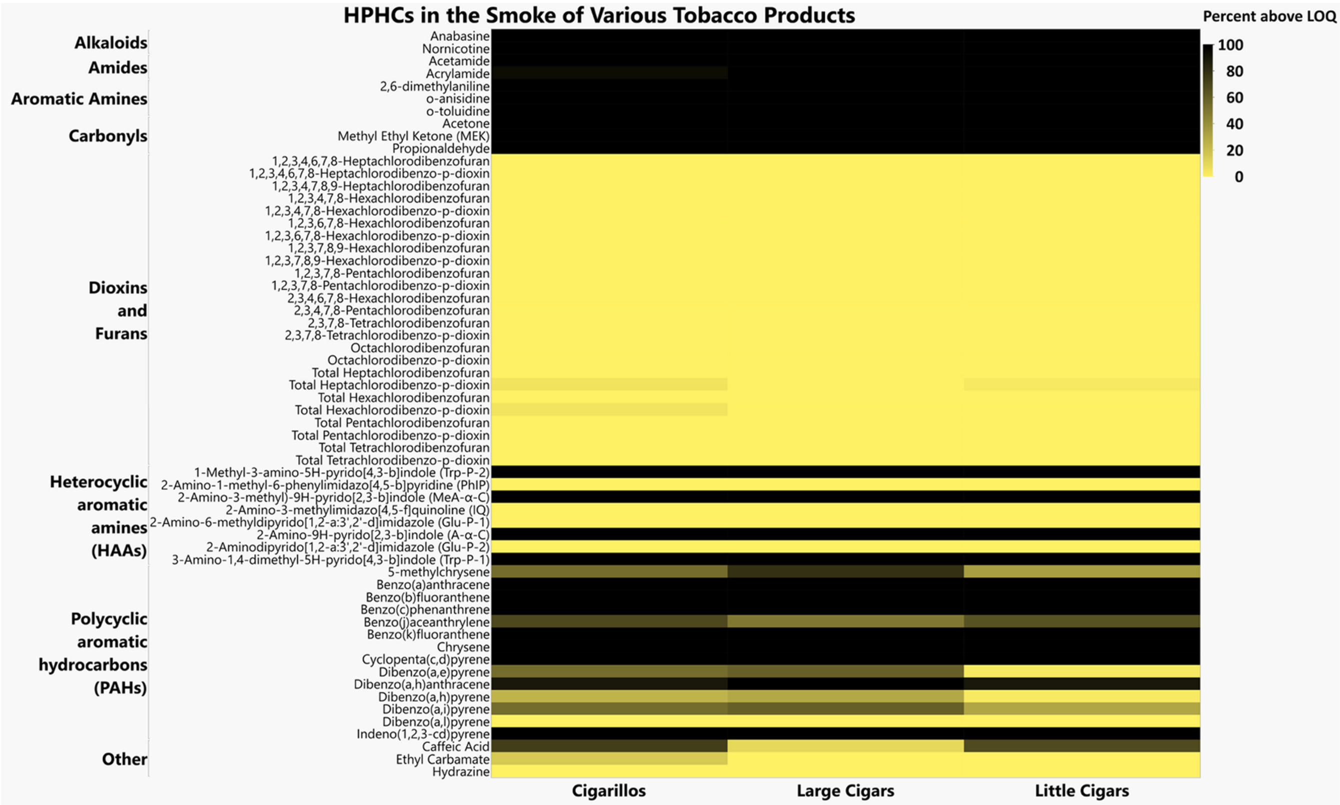

Figure 4. Heatmap of HPHCs in the smoke of cigarillos, large cigars, and little cigars examined.

category 2 include crotonaldehyde, six chlorodibenzo-*p*-dioxins (CDDs), nine chlorodibenzofurans (CDFs), four heterocyclic aromatic amines (2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*]-imidazole [Glu-*P*-1], 2-aminodipyrido[1,2-a:3′,2-*d*]imidazole [Glu-*P*-2], 2-amino-3-methylimidazo[4,5-*f*]quinoline [IQ], 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine [PhIP]), uranium-235, two nitrosamines (*N*-nitrosodimethylamine [NDMA], *N*-nitrosopiperidine [NPIP]), eight volatiles and semivolatiles (acrylonitrile, benzene, 1,3-butadiene, isoprene, nitrobenzene, 2-nitropropane, toluene, vinyl chloride), hydrazine, and hydrogen cyanide. These HPHCs are not quantifiable by the analytical methodologies used in this study. However, this refers only to the TCTP types and matrices examined in this study due to the existence of knowledge gaps, as described above. For example, while benzene is in category 2, this study only examines benzene in tobacco product filler as the literature clearly shows that both cigarette and cigar smoke contain benzene.[6,9,18,32−36] At this time, with the current analytical technology for the examined TCTP types and matrices, category 2 HPHCs may be of lower interest compared to category 1 HPHCs as the users are either not expected to be exposed to these HPHCs or the user exposure to these HPHCs cannot be evaluated due to the lack of quantitative data. These HPHCs will warrant further monitoring when they are found to be quantifiable in other tobacco product types and matrices, when new tobacco product designs appear or when more sensitive analytical methods or techniques become available.

Category 3: Quantifiability Varies by TCTP Brands, Types, and Matrices Examined. The remaining 74 (63%) HPHCs examined are in category 3. The quantifiability of these HPHCs varies across TCTP brands, types, and matrices. These HPHCs fall into one of four subcategories listed in Table 3. In general, more research is indicated for category 3 HPHCs to determine why the quantifiability of these HPHCs varies by brand, TCTP type, and matrix. These HPHCs may be of interest or concern, as users of relevant TCTP types are likely exposed to these HPHCs. The following sections discuss each category 3 HPHC in detail.

Alkaloids. Anabasine and nornicotine are in category 3b. Except for both analytes in waterpipe brands and anabasine in one pipe brand, anabasine and nornicotine are quantifiable in every brand of the TCTP types and matrices examined (i.e., all cigar smoke, pipes, RYOs). Anabasine and nornicotine are minor tobacco alkaloids reported to be quantifiable alongside nicotine in TCTPs.[37,38] As the LOQ for anabasine in pipes for this study is 50 μg/g and the anabasine content in pipes ranges from 54 to 165 μg/g, the one pipe brand that is BLOQ for anabasine is likely just below the LOQ at the double digit ppm level. However, for waterpipes, 84% of brands are below LOD (6.5 μg/g) for anabasine, and 74% of brands are higher than LOD but below the LOQ (76 μg/g) for nornicotine. Thus, it would be helpful to examine how waterpipe differs from other TCTP types for alkaloid quantities.

Amides. Acrylamide is in category 3b. Acrylamide is quantifiable in the smoke of all little and large cigar brands but only some brands of the remaining six TCTP types (95% cigarillo smoke, 63% RYOs, 79% pipes, 93% little cigars, 10% large cigars, 70% cigarillos fillers). Acrylamide is reported to be a Maillard reaction product from the condensation of asparagine with reducing sugars at temperatures above 120 °C.[11,39] Thus, it is not surprising that acrylamide is in the smoke of almost all brands (95−100%) but differs greatly across brands in the filler (10−93%). It may be helpful to examine what leads to filler acrylamide quantity differences across brands.

Aromatic Amines. 1-Aminonaphthalene, 2-aminonaphthalene, and 4-aminobiphenyl are quantifiable in the filler of some

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Table 2. Number of Brands with Quantifiable Quantities or Yields for Each HPHC in Each TCTP Type and Matrix Examined[a]

HPHC Group	HPHCs	filler								smoke				HPHC category
		cigarettes	cigarillos	large cigars	little cigars	pipes	RYOs	STPs	waterpipes	cigarettes	cigarillos	large cigars	little cigars	
alkaloids	anabasine					27 (96%)	27 (100%)		2 (11%)		19 (100%)	10 (100%)	29 (100%)	3b
	nicotine						27 (100%)							1
	nornicotine					28 (100%)	27 (100%)		5 (26%)		19 (100%)	10 (100%)	29 (100%)	3b
amides	acetamide	27 (100%)						27 (100%)			20 (100%)	10 (100%)	29 (100%)	1
	acrylamide		14 (70%)	1 (10%)	27 (93%)	22 (79%)	15 (63%)				19 (95%)	10 (100%)	29 (100%)	3b
aromatic amines	2,6-dimethylaniline	0 (0%)									20 (100%)	10 (100%)	29 (100%)	3d
	o-anisidine	27 (100%)									20 (100%)	10 (100%)	29 (100%)	1
	o-toluidine	27 (100%)									20 (100%)	10 (100%)	29 (100%)	1
	1-aminonaphthalene	6 (22%)												3a
	2-aminonaphthalene	4 (15%)												3a
	4-aminobiphenyl	13 (48%)												3a
carbonyls	acetaldehyde	27 (100%)					22 (100%)		19 (100%)					1
	acetone	0 (0%)					15 (68%)	16 (59%)	15 (79%)		20 (100%)	10 (100%)	29 (100%)	3d
	acrolein	0 (0%)					0 (0%)		1 (5%)					3c
	crotonaldehyde	0 (0%)					0 (0%)		0 (0%)					2
	formaldehyde	27 (100%)					22 (100%)		19 (100%)					1
	methyl ethyl ketone (MEK)	20 (74%)									20 (100%)	10 (100%)	29 (100%)	3b
	propionaldehyde	21 (78%)									20 (100%)	10 (100%)	29 (100%)	3b
chlorinated dioxins and furans	1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,4,6,7,8-heptachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,4,7,8,9-heptachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,4,7,8-hexachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,6,7,8-hexachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,7,8,9-hexachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2

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Table 2. continued

HPHC Group	HPHCs	filler								smoke				HPHC category
		cigarettes	cigarillos	large cigars	little cigars	pipes	RYOs	STPs	waterpipes	cigarettes	cigarillos	large cigars	little cigars	
	1,2,3,7,8-pentachlorodibenzo-*p*-dioxin	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	1,2,3,7,8-pentachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	2,3,4,6,7,8-hexachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	2,3,4,7,8-pentachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	2,3,7,8-tetrachlorodibenzo-*p*-dioxin	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	2,3,7,8-tetrachlorodibenzofuran	1 (4%)					2 (9%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	3c
	octachlorodibenzo-*p*-dioxin	2 (7%)					4 (18%)	15 (56%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	3c
	octachlorodibenzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	total heptachlorodibenzo-*p*-dioxin	16 (59%)					19 (86%)	20 (74%)	1 (5%)		1 (5%)	0 (0%)	1 (3%)	3c
	total heptachlorodibenzofuran	1 (4%)					11 (50%)	3 (11%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	3c
	total hexachlorodibenzo-*p*-dioxin	10 (37%)					17 (77%)	11 (41%)	0 (0%)		1 (5%)	0 (0%)	0 (0%)	3c
	total hexachlorodibenzofuran	4 (15%)					15 (68%)	1 (4%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	3c
	total pentachlorodibenzo-*p*-dioxin	6 (22%)					15 (68%)	2 (7%)	1 (5%)		0 (0%)	0 (0%)	0 (0%)	3c
	total pentachlorodibenzofuran	5 (19%)					13 (59%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	3c
	total tetrachlorodibenzo-*p*-dioxin	18 (67%)					20 (91%)	7 (26%)	1 (5%)		0 (0%)	0 (0%)	0 (0%)	3c
	total tetrachlorodibenzofuran	14 (52%)					19 (86%)	0 (0%)	1 (5%)		0 (0%)	0 (0%)	0 (0%)	3c
heterocyclic aromatic amines (HAAs)	2-amino-9*H*-pyrido[2,3-*b*]indole (A-α-C)	0 (0%)						0 (0%)			20 (100%)	10 (100%)	29 (100%)	3d
	2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-*P*-1)	0 (0%)						0 (0%)			0 (0%)	0 (0%)	0 (0%)	2
	2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-*P*-2)	0 (0%)						0 (0%)			0 (0%)	0 (0%)	0 (0%)	2
	2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)	0 (0%)						0 (0%)			0 (0%)	0 (0%)	0 (0%)	2
	(2-amino-3-methyl)-9*H*-pyrido[2,3-*b*]indole (MeA-α-C)	0 (0%)						0 (0%)			20 (100%)	10 (100%)	29 (100%)	3d
	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)	0 (0%)						0 (0%)			0 (0%)	0 (0%)	0 (0%)	2
	3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-*P*-1)	0 (0%)						0 (0%)			20 (100%)	10 (100%)	29 (100%)	3d
	1-methyl-3-amino-5*H*-pyrido[4,3-*b*]indole (Trp-*P*-2)	0 (0%)						0 (0%)			20 (100%)	10 (100%)	29 (100%)	3d
metals	arsenic						27 (100%)				10 (50%)	1 (10%)	0 (0%)	3d
	beryllium					24 (85%) - Lab 1 2 (100%) - Lab 2	27 (100%)				0 (0%)	0 (0%)	0 (0%)	3d
	cadmium						27 (100%)				20 (100%)	10 (100%)	29 (100%)	1
	chromium					28 (100%) - Lab 1 2 (100%) - Lab 2	27 (100%)				0 (0%)	0 (0%)	0 (0%)	3d
	cobalt					28 (100%) - Lab 1 2 (100%) - Lab 2	27 (100%)				0 (0%)	0 (0%)	0 (0%)	3d
	lead					28 (100%) - Lab 1 2 (100%) - Lab 2	27 (100%)				15 (75%)	4 (40%)	3 (10%)	3b

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Table 2. continued

HPHC Group	HPHCs	filler								smoke				HPHC category
		cigarettes	cigarillos	large cigars	little cigars	pipes	RYOs	STPs	waterpipes	cigarettes	cigarillos	large cigars	little cigars	
	mercury					28 (100%) - Lab 1 2 (100%) - Lab 2	27 (100%)		10 (53%)					3b
	nickel						27 (100%)							1
	polonium-210					27 (96%)	23 (100%)		0 (0%)		18 (90%)	10 (100%)	28 (97%)	3d
	selenium					28 (100%) - Lab 1 2 (100%) - Lab 2			4 (21%)		4 (20%)	3 (30%)	0 (0%)	3d
	uranium-235					0 (0%)			0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
	uranium-238	27 (100%)				0 (0%) - Lab 1 2 (100%) - Lab 2	27 (100%)	30 (100%)	0 (0%)	0 (0%)	8 (40%)	5 (50%)	7 (24%)	3d
nitrosamines	*N*-nitrosodiethylamine (NDEA)	0 (0%)				0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (30%)	0 (0%)	3c
	N-nitrosodiethanolamine (NDELA)					0 (0%)	1 (4%)		0 (0%)					3c
	N-nitrosodimethylamine (NDMA)					0 (0%)	0 (0%)		0 (0%)					2
	N-ethylmethylamine (NMEA or NEMA)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		18 (95%)	10 (100%)	7 (24%)	3d
	N-nitrosomorpholine (NMOR)	0 (0%)				0 (0%)	0 (0%)		0 (0%)		1 (5%)	1 (10%)	0 (0%)	3c
	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)						27 (100%)							1
	N-nitrosonornicotine (NNN)						27 (100%)							1
	N-nitrosopiperidine (NPIP)	0 (0%)					0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
	N-nitrosopyrrolidine (NPYR)	0 (0%)				0 (0%)	0 (0%)		0 (0%)		19 (100%)	10 (100%)	26 (90%)	3d
	N-nitrososarcosine (NSAR)	0 (0%)	20 (100%)	10 (100%)	29 (100%)	0 (0%)	14 (58%)	20 (74%)	0 (0%)		4 (21%)	1 (10%)	0 (0%)	3d
phenols	catechol	1 (4%)	2 (10%)	0 (0%)	0 (0%)	13 (46%)	6 (25%)	5 (19%)						3c
	m-cresol		2 (10%)	0 (0%)	1 (3%)	3 (11%)	4 (17%)							3c
	o-cresol		2 (10%)	0 (0%)	1 (3%)	3 (11%)	4 (17%)							3c
	p-cresol		2 (10%)	0 (0%)	1 (3%)	3 (11%)	4 (17%)							3c
	phenol		5 (25%)	1 (10%)	1 (3%)	7 (25%)	4 (17%)							3a
polycyclic aromatic hydrocarbons (PAHs)	5-methylchrysene	0 (0%)	4 (21%)	1 (10%)	1 (3%)	4 (13%)	2 (9%)		0 (0%)		11 (55%)	8 (80%)	10 (34%)	3c
	benzo(j)aceanthrylene + benzo(e) aceanthrylene	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	1 (5%)		14 (70%)	5 (50%)	19 (66%)	3c
	benzo(a)anthracene	26 (96%)	20 (100%)	10 (100%)	29 (100%)	28 (100%) - Lab 1 2 (100%) - Lab 3	20 (91%)		12 (63%)		20 (100%)	10 (100%)	29 (100%)	3b
	benzo(a)pyrene		20 (100%)	10 (100%)	29 (100%)	28 (100%) - Lab 1 1 (50%) - Lab 3	20 (91%)		3 (16%)					3b
	benzo(b)fluoranthene	24 (89%)	20 (100%)	10 (100%)	29 (100%)	28 (100%) - Lab 1 2 (100%) - Lab 3	19 (86%)		5 (26%)		20 (100%)	10 (100%)	29 (100%)	3b
	benzo(c)phenanthrene	16 (59%)	13 (68%)	8 (80%)	16 (55%)	24 (80%)	18 (82%)	21 (78%)	0 (0%)		20 (100%)	10 (100%)	29 (100%)	3d
	benzo(k)fluoranthene	27 (100%)	20 (100%)	10 (100%)	29 (100%)	28 (100%) - Lab 1 2 (100%) - Lab 3	22 (100%)		8 (42%)		20 (100%)	10 (100%)	29 (100%)	3b

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Table 2. continued

HPHC Group	HPHCs	filler: cigarettes	filler: cigarillos	filler: large cigars	filler: little cigars	filler: pipes	filler: RYOs	filler: STPs	filler: waterpipes	smoke: cigarettes	smoke: cigarillos	smoke: large cigars	smoke: little cigars	HPHC category
	chrysene	26 (96%)	20 (100%)	10 (100%)	29 (100%)	28 (100%) - Lab 1 2 (100%) - Lab 3	20 (91%)		12 (63%)		20 (100%)	10 (100%)	29 (100%)	3b
	cyclopenta(c,d)pyrene	23 (85%)	7 (37%)	3 (30%)	4 (14%)	22 (73%)	14 (64%)	18 (67%)	3 (14%)		20 (100%)	10 (100%)	29 (100%)	3b
	dibenzo(a,h)anthracene	0 (0%)	18 (90%)	4 (40%)	22 (76%)	25 (89%) - Lab 1 0 (0%) - Lab 3	3 (14%)		0 (0%)		18 (90%)	10 (100%)	26 (90%)	3d
	dibenzo(a,e)pyrene	0 (0%)	1 (5%)	0 (0%)	0 (0%)	5 (16%)	0 (0%)		0 (0%)		11 (55%)	6 (60%)	1 (3%)	3c
	dibenzo(a,h)pyrene	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)		5 (25%)	3 (30%)	1 (3%)	3c
	dibenzo(a,i)pyrene	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)		0 (0%)		11 (55%)	6 (60%)	9 (31%)	3c
	dibenzo(a,l)pyrene	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	3c
	indeno(1,2,3-cd)pyrene	20 (74%)	20 (100%)	10 (100%)	29 (100%)	28 (100%) - Lab 1 1 (50%) - Lab 3	19 (86%)		0 (0%)		20 (100%)	10 (100%)	29 (100%)	3d
	naphthalene	0 (0%)	20 (100%)	10 (100%)	29 (100%)	28 (100%)								3d
volatiles and semivolatiles	acrylonitrile	0 (0%)						0 (0%)						2
	benzene	0 (0%)						0 (0%)						2
	2,3-benzofuran	0 (0%)					0 (0%)	0 (0%)	0 (0%)		19 (100%)	10 (100%)	29 (100%)	3d
	1,3-butadiene	0 (0%)						0 (0%)						2
	ethylbenzene	0 (0%)						0 (0%)			19 (100%)	10 (100%)	29 (100%)	3d
	ethylene oxide	0 (0%)						0 (0%)			19 (100%)	10 (100%)	29 (100%)	3d
	furan	0 (0%)						0 (0%)			19 (100%)	10 (100%)	29 (100%)	3d
	isoprene	0 (0%)						0 (0%)						2
	nitrobenzene	0 (0%)					0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
	nitromethane	0 (0%)						0 (0%)			19 (100%)	10 (100%)	29 (100%)	3d
	2-nitropropane	0 (0%)					0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
	propylene oxide	0 (0%)						0 (0%)			19 (100%)	10 (100%)	29 (100%)	3d
	quinoline	10 (37%)						18 (67%)						3a
	styrene	0 (0%)						0 (0%)			19 (100%)	10 (100%)	29 (100%)	3d
	toluene	0 (0%)						0 (0%)						2
	vinyl acetate							0 (0%)			16 (84%)	9 (90%)	15 (52%)	3c
	vinyl chloride	0 (0%)						0 (0%)						2
others	aflatoxin B1	24 (89%)	0 (0%)	0 (0%)	0 (0%)	0 (0%) - Lab 1 & 2	6 (22%)	5 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3c
	ammonia						27 (100%)							1
	caffeic acid	27 (100%)					22 (100%)	9 (33%)	19 (90%)		14 (74%)	1 (10%)	20 (69%)	3b
	coumarin	0 (0%)	2 (11%)	0 (0%)	3 (10%)	2 (7%) - Lab 3 0 (0%) - Lab 2	0 (0%)	14 (47%)	3 (14%)	9 (33%)	16 (84%)	7 (70%)	19 (66%)	3c

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Table 2. continued

HPHC Group	HPHCs	filler								smoke				HPHC category
		cigarettes	cigarillos	large cigars	little cigars	pipes	RYOs	STPs	waterpipes	cigarettes	cigarillos	large cigars	little cigars	
	ethyl carbamate	0 (0%)					3 (14%)	8 (30%)	6 (29%)		3 (16%)	0 (0%)	0 (0%)	3c
	hydrazine	0 (0%)					0 (0%)		0 (0%)		0 (0%)	0 (0%)	0 (0%)	2
	hydrogen cyanide	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)					2

[a]Respective percentage for the total number of brands examined. Carbon monoxide (CO) was not included in this table; it was not tested due to the fact that CO is well studied in smoke and literature/well-established test methods are lacking for CO in filler.

cigarette brands (category 3a, 22, 15, and 48%, respectively), while 2,6-dimethylaniline is quantifiable in the smoke of all cigar brands but not in the filler of any of the cigarette brands examined (category 3d). Aromatic amines are reported to be formed by enzymic or microbial degradation of protein or amino acid in tobacco plants or pyrolytic process during smoking.[40] The quantifiability of 2,6-dimethylaniline in the smoke of all cigar brands examined in this study and in cigarettes as reported[6] appears to be supported by the pyrolytic formation mechanism. However, 2,6-dimethylaniline was not quantifiable in all cigarette fillers examined in this study and has not been measured in cigar fillers, so it is unknown whether 2,6-dimethylaniline is quantifiable in cigar fillers through protein or amino acid degradation and transferred into cigar smoke. Additionally, the quantifiability of 1-aminonaphthalene, 2-aminonaphthalene, and 4-aminobiphenyl in fillers of some cigarette brands examined implies the protein/amino acid degradation formation. To understand the differences in the quantifiability of these HPHCs by brand, TCTP type, and matrix, more information (e.g., ingredients, tobacco types, product manufacturing processes) and further investigation may be helpful.

Carbonyls. Acetone, methyl ethyl ketone (MEK), and propionaldehyde are all quantifiable in the smoke of every cigar brand examined. MEK and propionaldehyde are quantifiable in the filler of most cigarette brands (74 and 78%, respectively, category 3b). Acetone is quantifiable in the filler of some brands of RYOs (68%), STPs (59%), and waterpipes (79%) but is not quantifiable in any cigarette brand fillers (category 3d). In addition, acrolein is not quantifiable in the filler of any cigarette or RYO brands examined but is quantifiable in the filler of one waterpipe brand examined (5%, category 3c). Carbonyls are known combustion products and are expected to be in tobacco smoke.[13,14] The quantifiability of acetone, MEK, and propionaldehydes in the smoke of all cigar brands examined along with the literature data about the quantifiability of acrolein in cigar smoke[9] supports this expectation. The quantifiability of carbonyls in the filler of some brands and not others likely arises from tobacco aging, curing, and storing processes (e.g., leaf age, curing time, drying atmosphere, storage condition).[41,42] The potential differences in these processes for different brands of TCTPs may contribute to the percent differences in the quantifiability of filler carbonyl observed in this study.

Chlorinated Dibenzo-p-dioxins and Dibenzofurans (CDD/CDF). The quantifiability of 2,3,7,8-tetraCDF, octaCDD, total heptaCDDs, total heptaCDFs, total hexaCDDs, total hexaCDFs, total pentaCDDs, total pentaCDFs, total tetraCDDs, and total tetraCDFs varies by brand (category 3c). All of these CDDs and CDFs are quantifiable in the filler of at least one brand of cigarettes (4−67%) and RYOs (9−91%), while most are in the filler of at least one brand of STP (0−74%). Conversely, waterpipe filler and cigar smoke have few CDDs and CDFs at quantifiable levels. Specifically, only one waterpipe brand (5%) and one cigar brand (5%) contain some CDDs (total hepta, total hexa, total penta, total tetra) and one CDF (total tetra) in filler or smoke at quantifiable levels. CDDs and CDFs have been attributed to a variety of sources, including contamination during tobacco growing or during product packaging and shipment.[43] More research will determine the specific sources of CDD and CDF variations by both tobacco product type and brand.

Heterocyclic Aromatic Amines (HAAs). 2-Amino-9*H*-pyrido[2,3-*b*]indole (A-α-C), (2-amino-3-methyl)-9*H*-pyrido[2,3-*b*]-

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Table 3. Categorization of the Quantifiability of HPHCs in Tobacco Products Examined[a]

		category 3: quantifiability varies by TCTP brands, types, and/or matrices (74 HPHCs, 63%)			
category 1: quantifiable in all brands for all TCTP types and matrices examined (X = 100%) (11 HPHCs, 9%)	category 2: not quantifiable in any brand of any TCTP type and matrix examined (X = 0%) (33 HPHCs, 28%)	category 3a: quantifiable in some brands for every TCTP type and matrix examined ($0 < X < 100\%$) (5 HPHCs, 4%)	category 3b: quantifiable in some brands for some TCTP types and matrices ($0 < X < 100\%$) and quantifiable in all brands of other TCTP types and matrices examined (X = 100%) (14 HPHCs, 12%)	category 3c: quantifiable in only some brands for some TCTP types ($0 < X < 100\%$) and not quantifiable in any brand for other TCTP types and matrices examined (X = 0%) (28 HPHCs, 24%)	category 3d: quantifiability varies among TCTP types and matrices examined ($0 \le X \le 100\%$) (27 HPHCs, 23%)
nicotine	crotonaldehyde	1-aminonaphthalene	anabasine	acrolein	2,6-dimethylaniline
acetamide	1,2,3,4,6,7,8-hepta CDD	2-aminonaphthalene	nornicotine	2,3,7,8-tetraCDF	acetone
o-anisidine	1,2,3,4,6,7,8-hepta CDF	4-aminobiphenyl	acrylamide	octaCDD	A-α-C
o-toluidine	1,2,3,4,7,8,9-hepta CDF	phenol	MEK	total hepta CDD	MeA-α-C
acetaldehyde	1,2,3,4,7,8-hexa CDD	quinoline	propionaldehyde	total hepta CDF	Trp-*P*-1
formaldehyde	1,2,3,4,7,8-hexa CDF		benzo(a)pyrene	total hexa CDD	Trp-*P*-2
cadmium	1,2,3,6,7,8-hexa CDD		benzo(b)fluoranthene	total hexa CDF	arsenic
nickel	1,2,3,6,7,8-hexa CDF		benzo(a)anthracene	total penta CDD	beryllium
NNK	1,2,3,7,8,9-hexa CDD		benzo(k)fluoranthene	total penta CDF	chromium
NNN	1,2,3,7,8,9-hexa CDF		chrysene	total tetra CDD	cobalt
ammonia	1,2,3,7,8-penta CDD		cyclopenta(c,d)pyrene	total tetraCDF	polonium-210
	1,2,3,7,8-penta CDF		caffeic acid	NDEA	selenium
	2,3,4,6,7,8-hexa CDF		mercury	NDELA	uranium-238
	2,3,4,7,8-penta CDF		lead	NMOR	NMEA/NEMA
	2,3,7,8-tetra CDD			catechol	NPYR
	octa CDF			*m*-cresol	NSAR
	Glu-*P*-1			*o*-cresol	benzo(c)phenanthrene
	Glu-*P*-2			*p*-cresol	dibenzo(a,h)anthracene
	IQ			5-methylchrysene	indeno(1,2,3-cd)pyrene
	PhIP			benzo(j)aceanthrylene + benzo(e)aceanthrylene	naphthalene
	uranium-235			dibenzo(a,e)pyrene	2,3-benzofuran
	NDMA			dibenzo(a,h)pyrene	ethylbenzene
	NPIP			dibenzo(a,i)pyrene	ethylene oxide
	acrylonitrile			dibenzo(a,l)pyrene	furan
	benzene			vinyl acetate	nitromethane
	1,3-butadiene			aflatoxin B1	propylene oxide
	isoprene			coumarin	styrene
	nitrobenzene			ethyl carbamate	
	2-nitropropane				
	toluene				
	vinyl chloride				
	hydrazine				
	hydrogen cyanide				

[a]X: percentage of brands that contain the HPHC for a specific TCTP type and matrix; CDD: chlorodibenzo-*p*-dioxin; CDF: chlorodibenzofuran.

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indole (MeA-α-C), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]-indole (Trp-*P*-1), and 1-methyl-3-amino-5*H*-pyrido[4,3-*b*]-indole (Trp-*P*-2) are quantifiable in the smoke of every cigar brand examined. However, they are not quantifiable in any cigarette and STP brands examined (category 3d). Tobacco combustion generates HAAs,[44−46] as supported by the result of this study. Similarly, it is likely that the other four HAAs in category 2 (Glu-*P*-1, Glu-*P*-2, IQ, PhIP) are in cigar smoke but at yields below LOD/LOQ. It may be beneficial to examine these four HAAs again when more sensitive analytical methods become available.

Metals. Lead and mercury are in category 3b. Both metals are quantifiable in the filler of all pipe and RYO brands examined. Some cigar brands (10−75%) contain lead at quantifiable levels in smoke, while only 53% of waterpipe brands contain mercury at quantifiable levels in filler. Lead is reported to be quantifiable in tobacco (cigarette,[47] cigar[48]) and transfers up to 35% from cigarette filler to smoke under CI due to its low volatility.[19,47] Therefore, the lead content in cigar smoke depends on both the filler lead content and its transfer rate, which may explain why lead is not quantifiable in the smoke of all cigar brands examined. As mercury accumulates in tobacco from soils and the environment,[49,50] the different percentages observed regarding the quantifiability of mercury in filler by TCTP type and brand may arise from using different amounts of tobacco and tobacco from different regions.

The quantifiability of arsenic, beryllium, chromium, cobalt, polonium-210 (P210), selenium, and uranium-238 (U238) varies across TCTP types and matrices (category 3d). Arsenic (RYOs), beryllium (RYOs), chromium (pipes, RYOs), cobalt (pipes, RYOs), P210 (RYOs), selenium (pipes), and U238 (cigarettes, RYOs, STPs) are quantifiable in the filler of all brands examined. P210 is quantifiable in the filler of 96% of pipe brands, while beryllium, selenium, and U238 are quantifiable in the filler of 87% of pipe brands, 21% of waterpipe brands, and 7% of the pipe brands examined, respectively. However, P210 and U238 are not quantifiable in any waterpipe brands examined. The quantifiability of metals in the filler of most TCTP brands is expected, as tobaccos in cigarette fillers are reported to contain metals.[51−54] The "not quantifiable" results for P210 and U238 in all waterpipe fillers and of U238 in most pipe fillers are new findings. The not quantifiable of these radioactive metals in waterpipes may relate to tobacco types or tobacco amounts used due to the unique product form for waterpipes. However, the not quantifiable of U238 in pipes arose because of the higher method detection limit for Lab 1 (0.005 Bq/g = 400 ng/g,[55] 28 pipe brands tested) compared to that for Lab 2 (2.2 ng/g, 2 pipe brands tested). Results from one pipe brand tested by both laboratories showed that Lab 2 finds U238 at quantifiable levels, while Lab 1 does not, supporting the postulation. Further testing using the higher sensitivity test method may result in a different finding.

The trends for these seven metals in smoke are more complex than in filler. Beryllium, chromium, and cobalt are not quantifiable in the smoke of any brand of any TCTP examined. However, the smoke of 50 and 20% cigarillo brands and 10 and 30% large cigar brands has arsenic and selenium, respectively, but neither metal is quantifiable in any little cigar brands examined. The smoke of some cigarillo (40%), little cigar (24%), and large cigar (50%) brands contains U238, but none of the smoke from cigarette brands does. P210 is quantifiable in the smoke of most TCTPs examined (90−100% across cigar brands). Many metals transfer from filler to smoke.[56,57] However, the transfer efficiency is dependent on the volatility of the metals, temperatures, and product designs (e.g., filter ventilation, product diameter, filter materials).[47] For example, up to 20 and 30% of cadmium transfer from the filler of cigarettes with and without the activated carbon filter, respectively, to smoke.[47] Therefore, the percentage difference of the smoke metals between the examined TCTP types and matrices could arise from differences in the maximum temperature reached by each type of combustible tobacco products,[58] the product designs, and the metal quantities in the relevant tobacco fillers.

Nitrosamines. *N*-Ethylmethylamine (NMEA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrososarcosine (NSAR) differ in brand percentages by TCTP types and matrices (category 3d). No filler of any TCTP brand examined has quantifiable amounts of NMEA or NPYR. However, NMEA is quantifiable in the smoke of 24% little cigar, 95% cigarillo, and all large cigar brands examined, while NPYR is quantifiable in the smoke of all cigar brands examined except for three little cigar brands. NMEA and NPYR are reported to be thermal decarboxylation products of *N*-nitrosamino acids [e.g., 3-(*N*-nitrosomethylamino)propionic acid, *N*-nitrosoproline] found in TCTP fillers.[59,60] Thus, the differences in smoke NMEA and NPYR by cigar brands may arise from the differences in the amount of these precursors available to be degraded in some TCTP types and brands compared to others. However, NPYR is reported to be in cigar[9] and STP filler.[6,61,62] Further investigation of causes (e.g., fermentation)[61] may be helpful to understand how the NPYR content varies in filler by TCTP types. Contrasting NMEA and NPYR, NSAR is quantifiable in the filler of all cigars, 57% RYO, and 74% STP brands examined. However, it is not quantifiable in the filler of any cigarette, pipe, and waterpipe brands examined. The NSAR content varied across different STPs,[63,64] and its formation may be impacted by tobacco treatment.[61] Sensitivity difference due to test method and sample matrix may also play a role in this content variation. Therefore, it may be helpful to investigate why the NSAR content of cigarettes, pipes, and waterpipes differs from other TCTP types. Additionally, NSAR is quantifiable in the smoke of 21% cigarillo, 10% large cigar, and none of the little cigar brands examined, although it is quantifiable in all cigar fillers. The low volatility of NSAR[62] may contribute to this lower percentage of quantifiable smoke NSAR compared to that of fillers.

N-Nitrosodiethanolamine (NDELA), *N*-nitrosodiethylamine (NDEA), and *N*-nitrosomorpholine (NMOR) are in category 3c. NDELA is not quantifiable in the filler of any pipe or waterpipe brands but is quantifiable in the filler of one RYO brand. NDELA forms during tobacco curing from the nitrosation of diethanolamine (DEA), which is an added solubilizing agent for a major tobacco sucker growth regulator.[65] Controlling the DEA use in tobacco farming may reduce the NDELA content in finished TCTPs. NDEA and NMOR are not quantifiable in the filler of any brands examined. However, NDEA is quantifiable in the smoke of 30% large cigar brands but not of other cigar brands examined, while NMOR is quantifiable in the smoke of 5% cigarillo and 10% large cigar brands but none of little cigar brands examined. Nitrosamines form from the reaction of nitrogen-containing species created by combustion with amines (diethylamine,[66] morpholine[67]) in tobacco, similar to NDMA.[68] The amount of filler amines and the nitrogen-containing species generated during smoking may determine the quantity of nitrosamines in smoke. However, further investigation of these three nitrosamines may be helpful to understand why only a small percentage of TCTP types and

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brands have quantifiable amounts in filler (NDELA) and in smoke (NDEA, NMOR).

Phenols. Catechol, *m*-cresol, *o*-cresol, and *p*-cresol are in category 3c. Specifically, catechol is quantifiable in the filler of 1 cigarette (4%), 2 cigarillo (10%), 13 pipe (46%), 6 RYO (25%), and 5 STP (19%) brands but no little and large cigar brands (0%). All three cresols are quantifiable in 1 little cigar (3%), 2 cigarillo (10%), 3 pipe (11%), and 4 RYO (17%) brands but no large cigar brands (0%). Phenol is quantifiable in the filler of some brands (25% cigarillo, 10% large cigar, 3% little cigar, 25% pipe, and 17% RYO) of all TCTP types examined (category 3a). Low levels of catechol, cresols, and phenol have been noted in tobacco leaf and TCTP fillers, with the authors suggesting that they are either natural in plants or resulted from heat exposure during tobacco processing.[69−71] Therefore, more product information, such as ingredients, tobacco types, and TCTP manufacturing processes used for each examined brand, may be needed to understand why these five phenols were quantifiable in only some brands. It would be helpful to assess the causes that lead to the differences in the quantifiability of these phenols by brands, TCTP types, and matrices.

Polycyclic Aromatic Hydrocarbons (PAHs). Benzo(a)-anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)-fluoranthene, chrysene, and cyclopenta(c,d)pyrene are quantifiable in fillers of some brands of some TCTP types and all brands of other examined (14−100%, category 3b). All six PAHs are quantifiable at lower percentages in waterpipe filler (14−63%) compared to other TCTPs (14−85% for cyclopenta(c,d)pyrene, 86−100% for other five PAHs), implying potential unique waterpipe characteristic(s) that results in fewer brands having these PAHs. All five PAHs examined in smoke (benzo(a)pyrene not included) are quantifiable in all cigar brands examined (100%). 5-Methylchrysene, benzo(j)aceanthrylene + benzo(e)-aceanthrylene, dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, and dibenzo(a,l)pyrene are quantifiable in some brands of some TCTP types and not in any brand of other types examined (category 3c). These PAHs are quantifiable in fewer brands in filler (0−21%) compared to smoke (0−80%). Conversely, the quantifiability of benzo(c)phenanthrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, and naphthalene differs (category 3d) by brand, TCTP type, and matrix (0−100% across the four PAHs). PAHs are well-known incomplete combustion products and can also develop from tobacco curing processes or environmental pollutants.[53,72−74] Therefore, the quantifiability of PAHs in smoke is expected. Also, the high variation of PAHs in filler could arise from differences in tobacco curing processes. Understanding the exact sources of the differences for each PAH among different TCTP types, brands, and matrices may be helpful.

Volatiles and Semivolatiles (VSVs). The fillers of 37 and 67% of cigarette and STP brands, respectively, have quantifiable quinoline quantities (category 3a). Other than quinoline, an environmental contaminant,[75] and a pyrolysis product reported to be in both tobacco and smoke,[40] no VSVs are found quantifiable in tobacco fillers examined. However, vinyl acetate is quantifiable in the smoke of some brands for all cigar types examined (52−90%; category 3c), while 2,3-benzofuran, ethylbenzene, ethylene oxide, furan, nitromethane, propylene oxide, and styrene are quantifiable in the smoke of all cigar brands examined (category 3d). As VSVs are primarily combustion products, the lack of quantifiability of these VSVs in the filler is expected.[76−83] It may be beneficial to understand the sources of the differences (e.g., testing method sensitivity, HPHC formation mechanism) of why nitrobenzene and 2-nitropropane are not quantifiable in the smoke of any cigar and cigarette brand examined, while all other examined VSVs were quantifiable in the smoke of most cigar brands. Conflicting results for nitrobenzene and 2-nitropropane in cigarette smoke had been reported.[6,9,84−87] Test method sensitivity likely contributes to the conflicts. Further investigation may be helpful.

Other HPHCs. Caffeic acid is in category 3b. It is quantifiable in the filler of 33−100% of brands and in the smoke of 10−74% of brands across TCTP types examined. This finding is supported by the reports that identified caffeic acid in tobacco.[88,89]

Aflatoxin B1, coumarin, and ethyl carbamate are in category 3c. Aflatoxin B1 is quantifiable in the fillers of some brands of some TCTP types (89% cigarettes, 22% RYOs, 17% STPs) and not quantifiable in the filler of other brands (i.e., cigars, pipes, waterpipes) examined. However, aflatoxin B1 is not in the smoke of any brand of TCTP types examined. Aflatoxin B1 did not transfer from filler to smoke at a quantifiable level; it was quantifiable in the filler of 89% of cigarette brands yet not detected in the smoke of any cigarette brands examined. Thus, for combustible tobacco products (cigarettes, cigars, RYOs), smoke aflatoxin B1 may be less likely a chemical of public health concern as it is unlikely to reach users. However, aflatoxin B1 is quantifiable in 17% of STP brands examined, with the measured highest quantity being 0.27 ng/g (LOQ = 0.007 ng/g). Aflatoxin B1 is a secondary metabolite (mycotoxin) produced by fungi that frequently contaminate agricultural commodities and has been linked to certain STPs.[90] As such, STP users may be exposed to aflatoxin B1, making aflatoxin B1 a potential chemical of public health concern for STPs.

Coumarin is quantifiable in 0−47% brands of TCTP fillers examined and is quantifiable in 33−84% brands of TCTP smoke examined. The difference in fillers among brands may relate to product composition as coumarin is reported to be higher in fire-cured tobacco compared to other tobacco types[91] and present in flavoring ingredients like clove, cinnamon, and vanilla extract.[83,92] However, it is unknown why the percent of brands containing coumarin in the smoke in each TCTP type is higher (33% cigarettes, 66−84% cigars) compared to the corresponding filler (0% cigarettes, 0−11% cigars), as coumarin is considered a plant-derived chemical[83] and not a typical combustion product. The differences in coumarin between matrices may arise from methodology differences. Further investigation into the cause of this difference may be helpful.

Ethyl carbamate (EC) is quantifiable in the filler of 0% cigarette, 14% RYO, 30% STP, and 29% waterpipe brands examined and in the smoke of 16% cigarillo and 0% little and large cigar brands examined. EC is converted from ethanol via nitrogenous compound in tobacco, and the formation is temperature-dependent.[93−95] EC has been found in both tobacco and smoke.[9,94] The percent quantifiable differences observed may come from differences in tobacco treatment, product composition, and product processing. Future research investigating the sources of these differences may be helpful.

Challenges and Future Directions. This study faces five challenges. One challenge leads to a study limitation. The HPHC categorization used in this study is based solely on the data collected from the TCTP brands, types, and matrices examined in the course of the study. The inclusion of new data may change the HPHC categories and potentially change the current interpretation of the study results. This limitation is ever present, as new data emerges, addressing knowledge gaps

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including the remaining ones this study was not able to address. In addition, including the established data listed in Table S1 also affects the HPHC categorization regarding whether an HPHC is quantifiable or not quantifiable in a specific tobacco type and product matrix.

The other four challenges do not pose limitations to this study. First, the literature search was comprehensive but not intended to be exhaustive due to the tremendous number of chemicals and matrices in the study scope. If limited or no literature was found, the relevant HPHC was included in the study scope for testing. Second, the availability of a specific validated test method for an HPHC in a specific TCTP type and matrix at the time when the study was designed determined which laboratory was qualified for being contracted to conduct an analysis. The method LOD/LOQ for the same HPHCs but different TCTP types and matrices may differ within and between laboratories, resulting in challenges in defining HPHC quantifiability when using different LOQs and in comparing across TCTP types when HPHC levels are near LOQs (see method LOD/LOQ in Tables S4 and S5). For example, U238 for one pipe tobacco brand was measured at two laboratories; one tested it correctly as pipe and the other tested it mistakenly as RYO. Each laboratory has a remarkably different LOD for U238, resulting in different results for the single overlapping brand. Third, the brand market availability differed slightly between laboratories. Also, two manufacturer self-defined pipe brands were mistakenly purchased by the acquisition contractor and assessed by the laboratories for relevant HPHCs as RYOs. However, during data processing, this error was corrected, and the two brands were included with pipes. Thus, the total number of brands for each TCTP type sometimes varies by HPHC. Fortunately, the impact is limited as these differences are small for all TCTP types. All results agree for the brands across laboratories except for U238, as discussed above. The error with the two brands indicates that universally defining RYO and pipe tobacco may be helpful for the general public. Lastly, different laboratories worked on the project at separate times (Lab 1: 2016−2018, Lab 2: 2014−2016, Lab 3: 2016−2020), so TCTPs of each brand may not be identical across this time frame (e.g., variations from tobaccos of different regions and years). Such a variation could potentially affect the HPHC quantities or yields. However, the potential product variation(s) should not affect the conclusion of this work (e.g., if HPHCs are quantifiable or not) but may impact further analyses of the data (e.g., causal correlations of HPHCs in filler and smoke).

Future work will compare the HPHCs in TCTPs in this study to those in other tobacco products (e.g., ENDS containing nicotine extracted from tobacco or nontobacco nicotine). In addition, future work will compare the difference in quantities and yields among tobacco product types, especially those in category 3 where quantifiability was variable across TCTP types. Lastly, as this work shows differences in HPHCs by tobacco product types, future studies may investigate the sources for the HPHCs that do not have a clear cause for their variation as well as what drives differences in HPHCs between the smoke and corresponding filler.

CONCLUSIONS

In conclusion, 11 (9%) HPHCs are quantifiable in all brands for all TCTP types and matrices examined, 33 (28%) HPHCs are not quantifiable in any brands of any TCTP type and matrix, and 74 (63%) HPHCs are quantifiable only in some brands across TCTP types or matrices examined. Additional investigation of HPHCs, specifically the origins and development of validated analytical methods, will be useful for understanding the quantifiability of an HPHC in a given TCTP brand or matrix and its potential impact on public health.

This work addresses knowledge gaps regarding whether an HPHC is quantifiable in a specific tobacco product type and matrix and the measurement of quantifiable amounts. As a result, this work can inform the scientific basis for regulators and manufacturers to determine, at present, which HPHCs should be reported, which HPHCs may be difficult to report, and, when considered alongside tobacco user behaviors, what specific tobacco product type and matrix could pose public health concerns due to potential health risks. The findings can also inform public communications regarding the health risks of tobacco products.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02646.

Results of the literature search and HPHC data gap identification in tobacco products and which laboratories analyzed which HPHCs in which TCTPs and matrices (PDF)

Method instrumentation used at each lab for each HPHC in each matrix and the HPHC quantities and yields determined from tobacco-containing products by type and brand (XLSX)

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Notes

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ABBREVIATIONS

A-α-C, 2-amino-9*H*-pyrido[2,3-*b*]indole; CDC, Centers for Disease Control and Prevention; CDD, chlorodibenzo-*p*-dioxin; CDF, chlorodibenzofuran; CI, Canadian Intense smoking regime; CORESTA, Cooperation Centre for Scientific Research Relative to Tobacco; CRM, CORESTA Recommended Method; DEA, diethanolamine; EC, ethyl carbamate; ENDS, electronic nicotine delivery systems; FDA, U.S. Food and Drug Administration; FD&C Act, U.S. Federal Food Drug and Cosmetic Act; HAA, heterocyclic aromatic amines; Glu-*P*-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-*P*-2, 2-aminodipyrido[1,2-*a*:3′,2-*d*]imidazole; HPHC, harmful and potentially harmful constituents; HTP, heated tobacco product; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; ISO, International Organization for Standardization; LOD, limit of detection; LOQ, limit of quantification; MeA-α-C, 2-amino-3-methyl)-9*H*-pyrido[2,3-*b*]indole; MEK, methyl ethyl ketone; NDEA, *N*-nitrosodiethylamine; NDELA, *N*-nitrosodiethanolamine; NDMA, *N*-nitrosodimethylamine; NMEA, *N*-ethylmethylamine; NMOR, *N*-nitrosomorpholine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *n*-nitrosonornicotine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; NSAR, *N*-nitrososarcosine; P210, polonium-210; PAH, polycyclic aromatic hydrocarbon; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; RYOs, roll-your-own tobaccos; STPs, smokeless tobacco products; TCTP, tobacco-containing tobacco product; TPM, total particulate matter; Trp-*P*-1, 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole; Trp-*P*-2, 1-methyl-3-amino-5*H*-pyrido[4,3-*b*]-indole; U238, uranium-238; VSVs, volatiles and semivolatiles

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