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Article

Carbonyl Yields in Cigars under Three Smoking Regimens Using a Linear Smoking Machine

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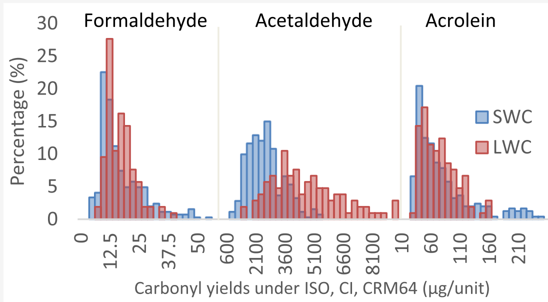
ABSTRACT: This study used standard linear smoking machines and puffing protocols to generate data on carbonyl yields in mainstream smoke from 11 unfiltered sheet-wrapped cigars (SWC), seven leaf-wrapped cigars (LWC), and two Kentucky reference cigarettes (3R4F, 1R6F). Carbonyl yields in cigar and cigarette products were determined using three different smoking regimens: International Organization for Standardization (ISO), Canadian Intense (CI), and Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Recommended Method (CRM) No. 64 (CRM64, Routine Analytical Cigar-Smoking Machine—Specifications, Definitions and Standard Conditions). Mainstream tobacco smoke was collected using a smoking machine fitted with an impinger containing 2,4-dinitrophenylhydrazine (DNPH) and carbonyl compounds quantified using liquid chromatography with an ultraviolet detector. Commercial SWC and LWC generated comparable formaldehyde yields (SWC, 9.4–28 μg/cigar [ISO], 8.2–43 μg/cigar [CI], 8.6–13 μg/cigar [CRM64]; LWC, 11–13 μg/cigar [ISO], 11–22 μg/cigar [CI], 16–21 μg/cigar [CRM64]) and acrolein yields; however, LWC generated higher acetaldehyde yields compared to SWC, using CI and CRM64 regimens. Reference cigarettes using standard puffing regimens generated carbonyl yields within reported ranges and 5–10% RSDs, whereas the CRM64 regimen generated lower carbonyl yields and 12–14% RSDs. Reference cigarettes generated higher formaldehyde yields using cigarette smoking regimens (21–28 μg/cigarette under ISO, 76–96 μg/cigarette under CI) but comparable formaldehyde yields under CRM64 (12–14 μg/cigarette). In addition, this study evaluated physical parameters (e.g., tobacco weight, length, diameter, circumference, tobacco rod density) that show the correlation between tobacco weight, tobacco rod density, and acetaldehyde yields under the three smoking regimens. Carbonyl yields in the mainstream smoke of cigar products using the three smoking regimens were highly variable; however, the CI smoking regimen may provide meaningful analytical information regarding cigar smoke constituents, with lower likelihood of self-extinguishment due to the short puffing intervals.



■ INTRODUCTION

Since the enactment of the 2009 Family Smoking Prevention and Tobacco Control Act (FSPTCA), under section 904(a)(3) of the Food, Drug, and Cosmetic Act, tobacco manufacturers are required to report harmful and potentially harmful constituents (HPHCs) in regulated tobacco products and tobacco smoke.[1] The Food and Drug Administration (FDA) established a list of HPHCs in tobacco products and tobacco smoke as well as a draft Guidance for Industry for reporting HPHC quantities in regulated tobacco products.[2,3] In August 2016, FDA finalized the "deeming rule", extending its regulatory authority to other tobacco products not covered in 2009, which therefore are now subject to FSPTCA requirements. This includes cigar products such as leaf-wrapped cigars (LWCs; also known as regular or large cigars), cigarillos (also known as small cigars, unfiltered sheet-wrapped cigars), and filtered sheet-wrapped cigars (SWCs; also known as brown cigarettes, filtered cigars, or little cigars). Few laboratories have characterized cigar products and reported about cigar smoking conditions and chemical constituents compared to the extensive studies available on cigarette tobacco products and smoke.[4−10] One of the challenges is that cigars, unlike cigarettes, are available in a wide variety of sizes, shapes, flavors, and filtration. Many commercial smoking machines are designed for cigarette research and may not be appropriate for cigar smoking due to their tobacco product holder design features; furthermore, the standardized smoking regimens used to study cigarettes may not reflect cigar smoking behaviors.

The FDA-established HPHC list includes volatile carbonyl chemical compounds typically generated during tobacco product combustion; these include formaldehyde, acetaldehyde, crotonaldehyde, methyl ethyl ketone (MEK), and acrolein. Acetaldehyde, crotonaldehyde, and formaldehyde

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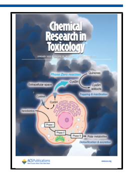

Table 1. Physical Parameters of Cigar Products and Reference Cigarettes (RSDs Shown in Parentheses)[a]

ID	Tobacco Product Brand Name	Weight (g/unit)	Length (mm)	Diameter at 15mm (mm)[1]	Diameter at 33 mm (mm)	Puff Count (/unit)		
						ISO	CI	CRM 64
1	Backwoods - Sweet Aromatic	2957 (9)	97.9 (1.3)	9.3 (2.8)	9.6 (2.6)	34.5 (12)	50.2 (22)	55.9 (13)
2	Cheyenne Cigarillo[2]	2778 (6)	112.2 (0.6)	9.8 (1.1)	9.6 (0.7)	36.36 (19)	52.3 (25)	54.4 (10)
3	Cheyenne Cigarillo	2890 (6)	112.6 (0.5)	9.8 (1.6)	9.5 (1.2)	31.21 (18)	47.2 (31)	51.6 (9)
4	Cheyenne Cigarillos - White Grape	2921 (5)	112.8 (0.5)	9.7 (1.8)	9.6 (0.9)	27 (19)	37.3 (31)	50.8 (10)
5	Dutch Masters Cigarillo	2875 (7)	110.6 (0.5)	9.9 (0.9)	10.3 (1.4)	30.14 (13)	27 (9)	54.8 (14)
6	Game - Black	2654 (8)	107.6 (0.5)	9.9 (1.4)	9.5 (1.5)	23.37 (13)	26.4 (23)	40.7 (15)
7	Phillies Natural Cigarillo	2905 (9)	110.9 (0.4)	10.4 (2.5)	10.0 (1.8)	23.71 (17)	24.5 (26)	38.3 (9)
8	Swisher Sweets Cigarillos[3]	3021 (3)	109.1 (0.6)	9.8 (0.5)	9.8 (0.6)	26.29 (18)	28.9 (34)	55.7 (15)
9	Swisher Sweets Cigarillos	2950 (3)	107.8 (0.7)	10.2 (1.5)	9.8 (1.5)	24.87 (18)	21.9 (7)	43.4 (9)
10	Swisher Sweets Cigarillos - Sticky Sweet	3038 (4)	109.1 (0.5)	9.8 (1.8)	10.1 (1.2)	25.36 (16)	23.9 (7)	47.4 (19)
11	Dutch Masters Presidents	7827 (8)	133.9 (0.6)	15.3 (1.3)	15.6 (1.2)	72.86 (10)	71.8 (16)	73.4 (7)
12	Phillies - Titans	9662 (6)	147.3 (0.4)	16.4 (1.7)	16.7 (1.5)	75.14 (9)	86.4 (16)	83.4 (8)
13	Phillies Blunt	7161 (8)	116.2 (0.8)	15.5 (1.7)	15.6 (1.7)	46.43 (19)	57 (14)	58.1 (11)
14	Swisher Sweets	6384 (5)	102.4 (0.7)	15.5 (1.3)	15.6 (1.3)	44.71 (22)	43.7 (16)	47.9 (8)
15	Swisher Sweets - Perfecto	6708 (6)	116.5 (0.5)	15.2 (0.9)	15.4 (1.0)	52.79 (17)	48.2 (12)	55.3 (9)
16	1R6F Cigarettes	606.3 (2.1)	83.0 (0.0)	7.6 (0.6)	N/A	7.66 (7)	8.4 (5)	12.1 (2)
17	3R4F Cigarettes	736.5 (1.8)	83.9 (0.3)	7.6 (0.0)	N/A	8.54 (4)	9.9 (5)	12.8 (3)

[a]N = 20 for all physical parameters in LWC products; N = 20 for cigarillo product weight, length, and diameter at 15 mm, and N = 10 for cigarillo diameter at 33 mm; N = 16 for 1R6F reference cigarettes; N = 19 for 3R4F reference cigarettes; N = 7 for puff count for each smoking regimen. (1) Diameter for reference cigarette products at 9 mm. (2) The testing laboratory did not identify Cheyenne Cigarillo as either Sweet or Dark & Mellow; thus, we assigned 7 replicates randomly to each flavor. (3) The testing laboratory did not identify Swisher Sweets Cigarillos as either Black or Regular; thus, we assigned 7 replicates randomly to each flavor.

have been classified as human carcinogens by either the International Agency for Research on Cancer (IARC), the U.S. Environmental Protection Agency (EPA), or the National Toxicology Program, and their presence in cigarette smoke causes negative health effects.[2] MEK and acrolein are carbonyl chemical compounds classified as respiratory toxicants.[2,11] Acrolein has been identified as a significant contributor to noncancer respiratory effects, such as chronic obstructive pulmonary disease (COPD).[12]

Carbonyl yields in mainstream smoke can be determined by machine-smoking combustible tobacco products using a defined smoking regimen followed by collection of the volatile chemical compounds using impingers containing derivatization reagents,[13,14] such as 2,4-dinitrophenylhydrazine (DNPH), that convert such compounds into stable, less volatile compounds that can be quantified using conventional analytical methods with ultraviolet (UV) detection. Analysis of carbonyls has been incorporated into cigarette smoke analysis methods such as the Health Canada method T-104 (Determination of Selected Carbonyls in Mainstream Tobacco Smoke) and the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) recommended method No. 74 (Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC).[15] A recent scientific study by

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Table 2. Validation Report Summary for the Quantification of Carbonyls in a Standard Solution Using CORESTA Recommended Method 74 (CRM74)

validation parameter	target value	formaldehyde	acetaldehyde	acrolein	crotonaldehyde	MEK
method accuracy (recovery)	>80% and <120%	115.1%	110.2%	106.5%	86.6%	134.2%
method precision (ISO) (N = 23) (%CV)	<15	10.9	8.8	10.5	12.8	7.8
method precision (CI) (N = 23) (%CV)	<15	13.4	7.2	7.7	not provided	not provided
total error probability[a] (ISO)	>95%	67%	85%	93%	69%	4%
total error probability[a] (CI)	>95%	63%	90%	87%	not provided	not provided
specificity (resolution)	>1.5	>2	>2	>2	0.831	>2
limit of quantification[b] (μg/unit)	<2	0.64	1.02	1.07	1.40	0.64

[a]The probability that a sample with 100% result will fall within the acceptance range of 80−120% (higher values have lower total variability). [b]Product represents a single cigarette or sheet-wrapped cigar.

Cecil et al.[10] studied the acrolein yields in the mainstream smoke of cigarettes and sheet-wrapped cigar products using International Organization for Standardization 3308:2012 (ISO) and Health Canada test method T-401 (Canadian Intense [CI]), which is equivalent to ISO 20778:2018, standard cigarette smoking regimens; variability in acrolein yields under the ISO smoking regimen was not statistically different at the 95% confidence interval and was observed to be within 15% relative standard deviations (RSDs).[10]

The present study uses a standard cigarette testing protocol to investigate the carbonyl yields in two different subcategories of cigar products: cigarillos and LWCs. The CORESTA Recommended Method No. 74 (CRM74—Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC) was designed for use with conventional cigarette products; however, because cigars are combustible products, the combustion of cigar products may generate the same HPHCs that are in cigarette smoke. This study examines the carbonyl yields of cigarillos and LWCs using three smoking regimens: two standard cigarette smoking regimens (ISO and CI) and a smoking regimen developed by CORESTA and the International Committee for Cigar Smoke Study called the CORESTA Recommended Method No. 64 (CRM64, Routine Analytical Cigar-Smoking Machine—Specifications, Definitions and Standard Conditions). The CRM64 smoking regimen has a puff profile that is different from the standard cigarette smoking regimens, simulating the puffing characteristics of smokers of cigar products. One key difference between the standard cigarette smoking regimen and the CORESTA-defined cigar smoking regimen is that the puff volume varies between cigar products based on the diameter of the cigar product to maintain a constant airspeed of 11.8 cm/s during the smoking process.[16] Also, the cigar smoking regimen has a shorter puff duration (1.5 s) compared to the two standard cigarette smoking regimens (2 s). To our knowledge, this is the first study to investigate the three smoking regimens and their impact on carbonyl yields in the two types of cigar tobacco products. Furthermore, this study investigates some of the physical characteristics of cigar products and the impact on carbonyl yields that has not been reported in previous studies.

EXPERIMENTAL PROCEDURES

Reagents and Materials. The cigarillos and LWCs tested in this study represented a range of brand names that are available in the U.S. market. This study was performed in two different time periods. For the first study, one cigarillo and two LWCs were purchased in Chesapeake, VA, shipped to an ISO 17025-accredited analytical laboratory (Labstat Inc., Kitchener, Ontario) on September 19, 2016, and analyzed in November 2016. For the second study, 10 cigarillos and five LWC brands were purchased in Chesapeake, VA, shipped to the analytical laboratory on September 22, 2017, and analyzed in November 2017. The second study also analyzed two reference cigarettes (i.e., 3R4F, 1R6F) that were purchased from the University of Kentucky (Lexington, KY). The two reference cigarettes contain similar physical properties and smoke chemistry.[17] The tobacco products were kept in their original packaging under ambient conditions and stored at room temperature prior to testing. All the test products were conditioned and smoked under the environmental conditions specified in ISO 3402 (1999) "Tobacco and tobacco products—Atmosphere for conditioning and testing", which states that tobacco products are conditioned at 22 ± 1 °C and relative humidity 60 ± 3% and smoked at 22 ± 2 °C and relative humidity 60 ± 5%.

Mainstream tobacco smoke was collected using a linear smoking machine fitted with an impinger containing 80 mL of 2,4-DNPH solution. All reagents were analytical reagent grade, unless otherwise stated. The derivatized solution was syringe-filtered and diluted with 1% Trizma base aqueous acetonitrile solution. Additional solutions necessary for the validation of the derivatization efficiency (e.g., calibration solutions using formaldehyde, acetaldehyde, acrolein, crotonaldehyde chemical standards) and the analytical procedure were prepared from commercially sourced acrolein with >99% purity.

Experimental Methods. The test tobacco products were analyzed within 60 days of receipt. Three different smoking parameters were used to analyze the carbonyls in cigarillos and LWCs, including two standard cigarette smoking regimens (ISO 3308 [ISO] and Health Canada T-115 [CI]) and CRM64. The ISO smoking regimen parameters are 35 mL puff volume, 60 s puff intervals, 2 s puff duration, and allowing ventilation. The CI smoking regimen is a more intense smoking regimen with 55 mL puff volume, 30 s puff interval, 2 s puff duration, and no ventilation (i.e., ventilation holes blocked using tape). The cigar products in this study do not contain filters or ventilation holes and were tested under ISO and CI smoking regimens without the use of tape for ventilation blocking. The CRM64 smoking regimen defines the puff volume based on cigar diameter:

- If the diameter (x) is less than 12 mm, puff volume (y) is set at 20 mL.
- If the diameter (x) is greater than 12 mm, puff volume (y) is determined as $y = 0.139x^2$.

CRM64 also defines the puff interval as 40 s and puff duration as 1.5 s. The CRM64 smoking regimen does not block ventilation. The products were tested in seven replicates.

Carbonyl yields were generated using a validated method in accordance with the Health Canada Test Method T-104 and CORESTA Recommended Method No. 74. Prior to testing, the physical parameters of the tested products were measured as shown in Table 1. The unfiltered mainstream smoke sample was collected using an impinger containing 80 mL of DNPH solution connected to a linear smoking machine and analyzed using high-performance liquid chromatography (HPLC) with UV detection.[10] The method was validated using Kentucky research cigarette 3R4F, due to the lack of a well-characterized reference cigar product; a summary of the method validation is shown in Table 2. Further details on method validation

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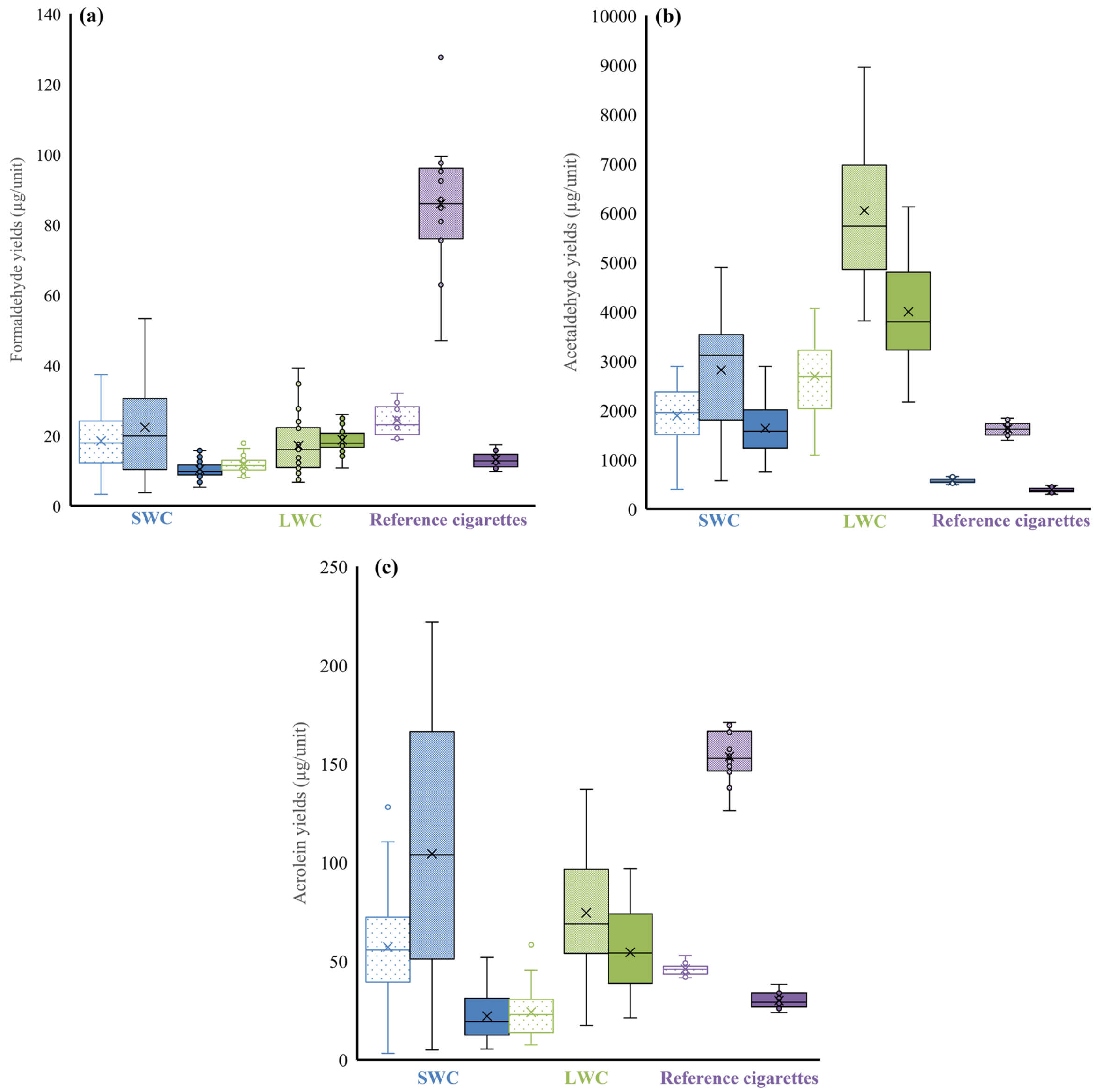

Figure 1. (a) Formaldehyde, (b) acetaldehyde, and (c) acrolein in cigarillos (SWCs), LWCs, and reference cigarette tobacco products under ISO (20% shaded), CI (50% shaded), and CRM64 (solid) smoking regimens (N = 7).

are described in detail elsewhere.[10] Carbonyl yields were also generated using the CRM64 smoking regimen. No deviations were made to the standard operating procedure for cigar product analysis. Method validation for accuracy and precision was only performed using ISO and CI smoking regimens using a reference cigarette (i.e., Kentucky reference cigarette 3R4F) and was not determined for the cigarillo and cigar products. The analytical laboratory did not verify analytical method suitability using the cigar smoking regimen but stated that no loss of carbonyl compounds in the mainstream smoke was anticipated from the large cigar products despite the greater number of puffs necessary to consume them compared to the reference cigarette (Table 1). Table 2 summarizes the figures of merit for the carbonyl analysis using Kentucky reference cigarette 3R4F.

For all compounds analyzed except for MEK, accuracy (i.e., spike recovery) and precision were within the laboratory's target values. The average recovery exceeded the laboratory acceptable range. Total error probability was determined to evaluate the method validation and fitness-for-purpose analysis. The total error was calculated using the accuracy and precision reported for ISO and CI smoking regimens, with the decision level set at a 95% confidence level to fall within a 20% total error window. For all compounds tested, the total error probability suggests that variability of the results would generally make a decision at a 95% confidence level difficult. Acetaldehyde and acrolein results achieved a >85% confidence level. For formaldehyde, crotonaldehyde, and MEK, the variability in method accuracy and method precision suggests that the probability of the sample to fall within a 20% total error window is low. Furthermore, the method precision under the CI smoking regimen was not reported for crotonaldehyde and MEK. Due to the poor resolution observed for crotonaldehyde, lack of method validation under the CI smoking regimen, and poor total error probability for crotonaldehyde and MEK, this study focuses on the acetaldehyde, acrolein, and formaldehyde yields in mainstream cigarette and cigar smoke.

The Kentucky reference cigarette 3R4F does not contain certified values; however, carbonyl yields in 3R4F mainstream smoke using the ISO smoking regimen have been reported as follows: formaldehyde, 18.8−25.0 µg/cigarette (6.3−69% RSD); acetaldehyde, 469−538 µg/cigarette (4.3−33% RSD); crotonaldehyde, 10.7−12.1 (8.1−119% RSD); and acrolein, 47.6−52.2 µg/cigarette (6.1−50% RSD).[18,19] Furthermore, Eldridge et al.[15] reported that 3R4F yielded 72.2 µg/cigarette (8.0% RSD) of formaldehyde, 1235 µg/cigarette (3.1% RSD) of acetaldehyde, 46.4 µg/cigarette (7.8% RSD) of crotonaldehyde, and 124.9 µg/cigarette (6.8% RSD) of acrolein in the mainstream smoke under the CI smoking regimen. Figure 1 shows

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Table 3. Carbonyl Yields in Cigarillo and LWC Tobacco Products Tested in 2016 and 2017 under CRM64 Smoking Regimen ($N = 7$)

	2016				2017			
product brand name	product weight (mg/unit)	formaldehyde mean (RSD)	acetaldehyde mean (RSD)	acrolein mean (RSD)	product weight (mg/unit)	formaldehyde mean (RSD)	acetaldehyde mean (RSD)	acrolein mean (RSD)
Dutch Masters Cigarillo	2484 (9)	16.7 (34)	2232 (9)	46.2 (30)	2879 (9)	9.8 (16)*[a]	2259 (23)	23.1 (32)*
Dutch Masters Presidents	7538 (3)	11.8 (12)	4855 (7)	49 (16)	7603 (5)	16.3 (9)*	3913 (17)*	34.5 (22)*
Phillies Blunt	6611 (6)	9.6 (15)	3152 (4)	35.8 (25)	6931 (4)	19.8 (18)*	4145 (20)*	64.6 (33)*

[a]* indicates statistically different constituent yield for the tobacco product analyzed in 2016 and 2017 ($p < 0.05$).

that the carbonyl yields in 3R4F mainstream smoke generated under ISO smoking conditions are consistent with the values reported in the literature; however, under the CI smoking regimen, acetaldehyde and acrolein yields were higher than those reported in the literature. The controls for the two sets of studies performed 12 months apart show that the two data sets are consistent between each other.

Unlike the Kentucky reference cigarette, 3R4F, the Kentucky reference cigarette, 1R6F, contains certified values for carbonyl compounds under the ISO and CI smoking regimen. The carbonyl yields of 1R6F reported in this study are consistent with the certified values reported for 1R6F under the ISO smoking regimen (acetaldehyde, 366−678 μg/unit; acrolein, 5−81 μg/unit; formaldehyde, 0−55 μg/unit) and CI smoking regimen (acetaldehyde, 677−2427 μg/unit; acrolein, 41−267 μg/unit; formaldehyde, 0−228 μg/unit) with 95% confidence.[20]

RESULTS

The carbonyl yields for 10 cigarillos, 5 LWCs, and 2 reference cigarette tobacco products from the 2017 study using the ISO, CI, and CRM64 smoking regimens are shown in Figure 1. Under the ISO smoking regimen, commercial cigarillos yielded mean ranges of 9.4−27.8 μg/unit of formaldehyde, 1198−2459 μg/unit of acetaldehyde, and 31.1−93.6 μg/unit of acrolein. LWCs yielded mean ranges of 10.8−13.4 μg/unit of formaldehyde, 1915−3401 μg/unit of acetaldehyde, and 19.5−30.8 μg/unit of acrolein. Under the CI smoking regimen, cigarillos resulted in mean ranges of 8.2−43.2 μg/unit of formaldehyde, 1368−4672 μg/unit of acetaldehyde, and 27.7−187.2 μg/unit of acrolein. For LWCs under the CI smoking regimen, mean yield ranges were 11.4−22.3 μg/unit of formaldehyde, 4523−8031 μg/unit of acetaldehyde, and 63.2−90.7 μg/unit of acrolein. The carbonyl yields obtained for the cigarillo products using the CRM64 method resulted in lower mainstream smoke yields of formaldehyde (8.6−12.9 μg/unit), acetaldehyde (1082−2259 μg/unit), and acrolein (10.6−36.2 μg/unit) compared to the ISO and CI smoking regimens; however, the mainstream smoke yields of carbonyls in the LWCs under the CRM64 smoking regimen (16.3−20.8 μg/unit of formaldehyde, 3018−5541 μg/unit of acetaldehyde, and 34.5−75.8 μg/unit of acrolein) were higher compared to the yields generated under the ISO smoking regimen but lower than the yields generated under the CI smoking regimen.

Select cigarillo and LWC products were analyzed in the two studies using the CRM64 smoking regimen. Formaldehyde, acetaldehyde, and acrolein yields are summarized in Table 3. In the November 2016 study, carbonyl analysis in two LWCs found 9.6−11.8 μg/unit in formaldehyde, 3152−4854 μg/unit in acetaldehyde, and 35.8−49.0 μg/unit in acrolein. Analysis of a cigarillo product resulted in an average mean of 16.7 μg/unit in formaldehyde, 2232 μg/unit in acetaldehyde, and 46.2 μg/unit in acrolein. The study products contained statistically different ($p < 0.05$) yields of most, or all, carbonyls analyzed across the two study periods; this demonstrates that the same cigar products from different batches vary greatly in carbonyl mainstream smoke yields.

For both cigar products, the results showed high variability: 11−52% RSDs for formaldehyde, 8−44% RSDs for acetaldehyde, and 17−69% RSDs for acrolein under the ISO smoking regimen; 22−72% RSDs for formaldehyde, 3−48% RSDs for acetaldehyde, and 10−100% RSDs for acrolein under the CI smoking regimen; and 9−34% RSDs for formaldehyde, 4−34% RSDs for acetaldehyde, and 16−55% RSDs for acrolein under the CRM64 smoking regimen. Of the carbonyls analyzed, acrolein yields had the highest variability. Statistical analysis indicated that the variability of the three standard smoking regimens was not significantly different ($p > 0.05$), except for the variability of formaldehyde using the CRM64 method compared to the ISO and CI smoking regimens.

DISCUSSION

Comparison of the Smoking Regimens for Evaluating Mainstream Smoke Constituents in Cigars. The main difference between the CRM64 smoking regimen used for cigarillos and LWCs and the ISO and CI smoking regimens for cigarettes is that, under CRM64, puff volume is determined by the diameter of the tobacco product. The CRM64 smoking regimen used a 20 mL puff volume for cigarillo smoking because the cigarillo diameter is <12 mm, whereas the puff volume range for LWCs, with an average diameter of 15.2−16.4 mm, was 33−39 mL.

To further investigate the differences between the cigar and cigarette smoking regimens, the three different smoking regimens were used to determine carbonyl yields of the 3R4F and 1R6F Kentucky reference cigarettes. Under the CRM64 smoking regimen, the 1R6F acetaldehyde, acrolein, and formaldehyde yields were significantly lower than those measured under ISO and CI smoking conditions. This could be because, as with the cigarillo products, the reference cigarettes were smoked using a 20 mL puff volume and a different puff profile, which may affect the carbonyl yields in mainstream smoke. Furthermore, the results for 1R6F under ISO and CI smoking regimens resulted in lower variability (5−10% RSD) compared to the CRM64 smoking regimen (12−14% RSD). These results indicate that the ISO and CI smoking regimens may offer higher precision compared to the CRM64 smoking regimen. The analytical method for carbonyl determination was only validated using the ISO and CI smoking regimens (see Table 2). However, smoke collected from 1R6F using CRM64 did not exceed the range of the analytical method (in terms of concentration) and, thus, effectively verified that the CRM64 is suitable for the products tested.

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The CRM64 puff profile is different from the standard cigarette smoking regimens to accommodate the wide range of shapes and sizes of cigar products creating a constant puff velocity for cigars with different diameters. However, this smoking regimen may not be appropriate for cigarillos and LWCs. For example, although a 40 s puff interval is defined to limit self-extinguishment during machine smoking, one of the study cigarillo products self-extinguished and thus was not included in this study. Notably, acrolein yields for the study cigarillo product resulted in the lowest acrolein yields compared to the other cigarillo products and had high variability (43% RSD). Cigar product self-extinguishment during testing might affect the product chemistry and the HPHCs present in cigarillo mainstream smoke. Furthermore, the scientific literature has shown that cigarillo and cigarette users have a similar puffing profile[21,22] (i.e., puff duration, puff interval, puff volume, puff velocity), especially among dual users. The CI smoking regimen using a 30 s puff interval appears to be a more suitable method for evaluation of combustible cigar products without self-extinguishment.

Evaluation of the Carbonyl Yields in Cigar Tobacco Products. Although the CRM74 analytical method was validated using reference cigarettes (e.g., 3R4F), due to the lack of consistent, well-characterized reference cigar tobacco products, we believe the data presented here provide a meaningful comparison between different cigar products in the same category. Additionally, two reference cigarettes were included in the study to ensure that the smoking machine was working properly. The formaldehyde yields for most of the cigarillo and LWC products showed overlapping mean ranges under the three smoking regimens. The key differences between the ISO and CI smoking regimens are the ventilation blocking, puff volume, and puff interval. Formaldehyde yields in smoke are generally affected by coal temperature; formaldehyde generally forms during pyrolysis at coal temperatures of 250−300 °C.[23] Since cigar tobacco products are generally unventilated, formaldehyde yields should not be affected greatly when cigars are smoked using either the ISO or the CI smoking regimen. However, this was not the case for certain cigar products tested (products 5, 9, and 15 of Table 1), which resulted in 56−105% higher ($p < 0.05$) formaldehyde yields under the CI compared to the ISO smoking regimen (see Supporting Information Table S1 for carbonyl yields for each tested product). Differences in the smoking regimens other than ventilation blocking (e.g., puff volume, puff interval) may have caused the higher formaldehyde yields in these cigar products. Except for product 1, the study cigar products generated higher acetaldehyde yields under the CI compared to the ISO and CRM64 smoking regimens. Reilly et al.[24] reported that increased puff volume results in higher aldehyde yields in cigarette smoke, which is also the case for the study cigar products. Similarly, with the exception of products 1 and 2, the study cigar products yielded higher acrolein yields under the CI smoking regimen compared to the ISO smoking regimen, which is consistent with studies performed on cigarette products.[25] However, the mean acrolein yields in the cigarillos were higher compared to the mean acrolein yields in the LWCs under the ISO and CI smoking regimens (i.e., 19−31 μg/unit [ISO], 63.2−90.8 μg/unit [CI] acrolein for LWCs; 31−94 μg/unit [ISO], 27.7−187.2 μg/unit [CI] acrolein for cigarillos). One of the reasons for lower acrolein yields under the ISO and CI regimen could be the large diameters of the LWCs compared to the cigarillos. The larger diameter may hinder combustion and result in lower acrolein yields.[26]

Formaldehyde and acrolein yields in the mainstream smoke of Kentucky reference cigarettes 1R6F and 3R4F are comparable to the formaldehyde and acrolein yields in mainstream smoke of the studied cigar products tested under the ISO and CRM64 smoking regimens ($p > 0.05$). However, higher mainstream smoke yields of formaldehyde were reported in 1R6F and 3R4F compared to those of the study cigar tobacco products under the CI smoking regimen. Differences in the product design, puffing parameters, and smoking regimens may influence the formation of certain HPHCs. For example, the reference cigarettes are composed of tobacco blends including flue-cured, burley, oriental, and reconstituted tobacco wrapped in a porous cigarette paper, whereas cigar products are generally composed of only burley, air-cured tobacco wrapped with binders and wrappers that lack porosity to allow the oxygen needed for combustion. Also, the reference cigarettes contain filters with ventilation holes that, when blocked, result in an increased burn rate and harsher burning conditions, resulting in higher carbonyl yields.[25]

Under the three standard smoking regimens, acetaldehyde yields of the reference cigarette products were lower on a per-unit basis compared to those of the cigar products. When the carbonyl yields were normalized on a per-puff basis, the formaldehyde and acrolein yields per puff were lower in the cigar products compared to the reference cigarettes. In the case of formaldehyde, the reference cigarettes yielded significantly higher amounts of formaldehyde in mainstream smoke on a per-puff basis [2.5−3.6 μg/puff (ISO), 7.7−11.4 μg/puff (CI), and 0.93−1.2 μg/puff (CRM64)], exceeding the formaldehyde yields in cigar products on a per-puff basis [0.14−1.1 μg/puff (ISO), 0.14−1.6 μg/puff (CI), and 0.17−0.44 μg/puff (CRM64)]. The U.S. National Institutes of Health, National Cancer Institute Monograph[27] on cigar smoke constituents compared the gas phase constituents in cigar and cigarette mainstream smoke and reported that the combustion during puff drawing from cigars is less complete compared to cigarettes; this could be due to the lack of porosity of the cigar binder and wrapper compared to cigarette paper, resulting in lower concentrations of oxygen and higher concentrations of carbon monoxide in cigar smoke. It has been reported that the temperature ranges for cigars are higher (1139−1160 °C) during puffing and between puffs compared to cigarettes (944−970 °C), which may affect the generation of carbonyl compounds and other chemical constituents.[27] Although the carbonyl yields in cigar products are generally comparable or lower on a per-puff and per-unit basis, the "tar" yields in cigar products, on a per gram basis, are generally higher in cigars compared to cigarettes due to the less complete combustion generating particulate phase constituents.[28]

Furthermore, we analyzed the correlation between tobacco weight and carbonyl yields in cigar products. The cigarillo product weights ranged from 2363 to 2879 mg/unit with no noticeable correlation with carbonyl yields. This could be because the weights of the tested cigarillo products are similar and contain varying amounts of additives, such as sugars (e.g., fructose, glucose, sucrose), propylene glycol, glycerol, and flavors, which could impact the carbonyl yields so that no correlation was observed for the tested cigarillo products. In contrast, LWCs tend to contain fewer additives (in comparison to cigarettes), and the tested LWCs vary in weight ranges

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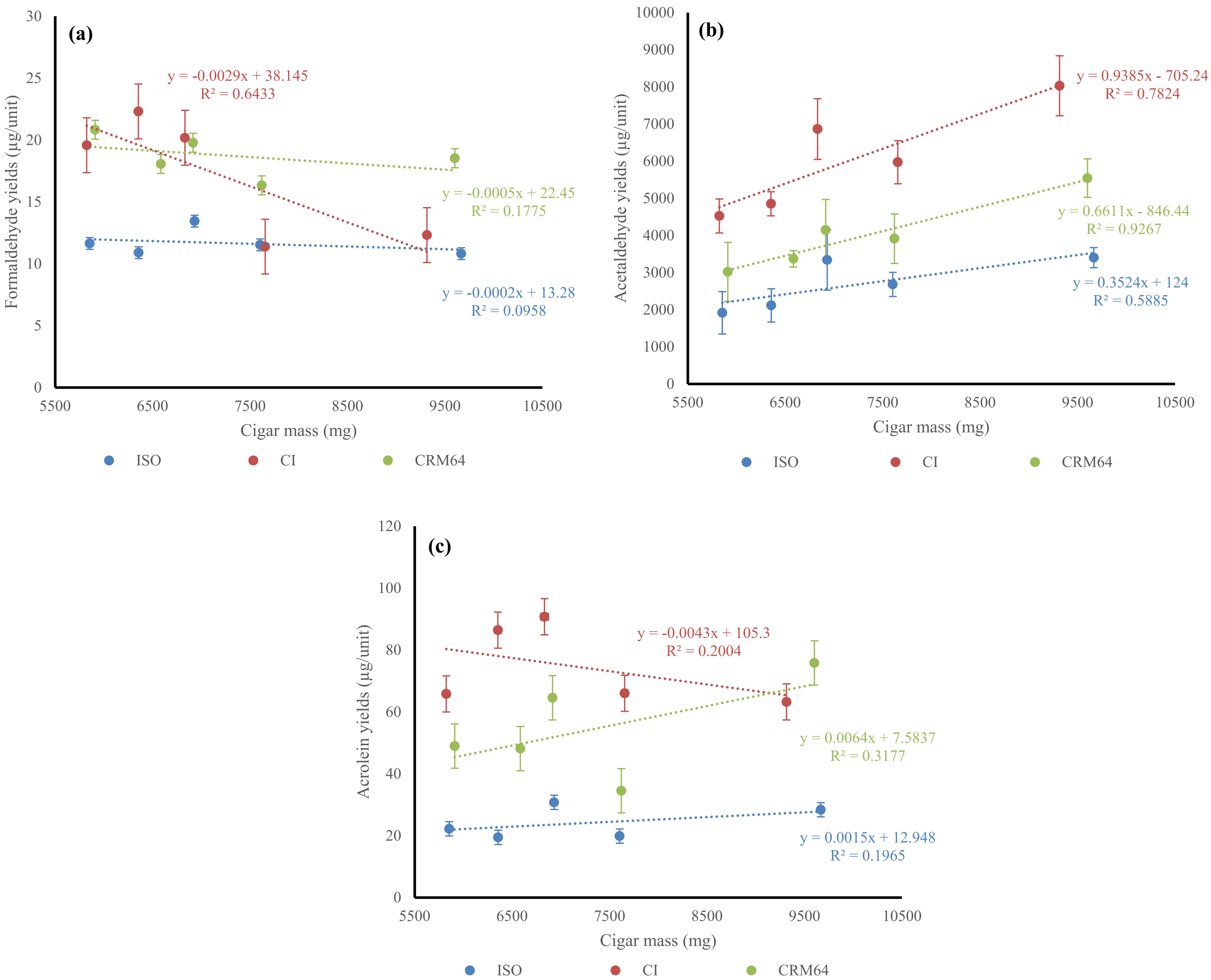

Figure 2. (a) Formaldehyde, (b) acetaldehyde, and (c) acrolein in LWCs relative to cigar weight (N = 7).

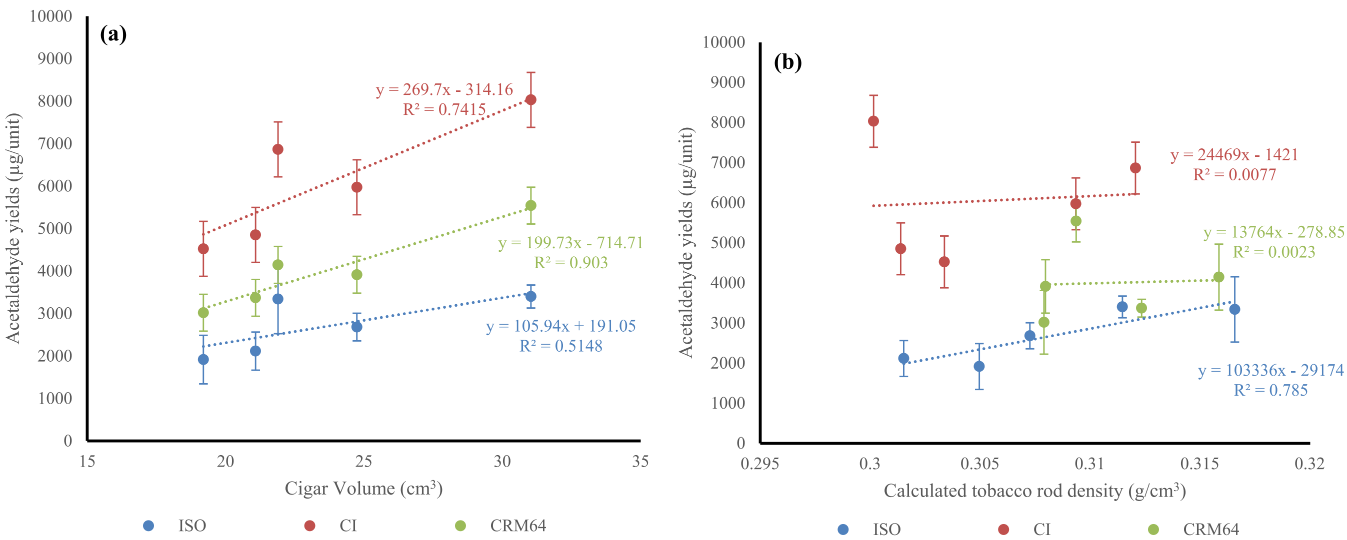

Figure 3. Acetaldehyde yields in LWCs relative to (a) cigar volume and (b) tobacco rod density (calculated using the diameter at 15 mm) (N = 7).

(5855−9667 mg/unit), which may provide useful information about tobacco weight and carbonyl yields. Figure 2 shows the regression curves for formaldehyde, acetaldehyde, and acrolein in comparison to tobacco weight for LWCs. This shows that

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formaldehyde and acrolein yields may be independent based on tobacco weight; however, the acetaldehyde yields seem to be correlated with tobacco weight. The coefficient of determination (R^2) for the linear regression of tobacco weight and acetaldehyde yields under the CRM64 smoking regimen resulted in a value >0.9. Although the R^2 value for the correlation of tobacco weight and acetaldehyde yields under ISO and CI resulted in a value <0.9, omission of product 13 would result in a regression constant value of >0.9. Chepiga et al.[29] reported on the mainstream smoke yields of acetaldehyde for three different "tar" ranges of commercial cigarettes that demonstrate that acetaldehyde yields directly correlate with "tar" yields. Similarly, Richet et al. tested the "tar" yields of cigars that showed the highest "tar" yield for large cigars due to the larger amount of tobacco, followed by small cigars. The "tar" and acetaldehyde yield relationship in cigarettes may also apply to cigars. Although cigarettes and cigars contain differences in product design, tobacco blend, additives, and other properties, both are combusted products that contain qualitatively similar toxic and carcinogenic compounds. Similar to cigarettes,[26] acetaldehyde yields were found to be independent of cigar circumference. A further linear correlation of the cigar volume calculated using the geometric calculation at a diameter of 15 mm from the butt of the cigar and acetaldehyde yields from the three smoking regimens were investigated, which resulted in similar results as the correlation with tobacco weight (Figure 3a): R^2 of >0.9 for cigar volume and acetaldehyde yields under CI and CRM64 smoking regimens; and R^2 of >0.7 for the linear correlation of cigar volume and acetaldehyde yields under the ISO smoking regimen or >0.9 if product 13 is omitted. Since large cigars comprise primarily tobacco and contain lower additive compared to cigarettes, greater amount of tobacco mass may result with larger product volume, thus resulting in higher acetaldehyde yields due to larger amount of tobacco combusted. On the other hand, no correlation was observed for calculated tobacco rod density and carbonyl yields (see Figure 3b for acetaldehyde and tobacco rod density regression curves). Several factors may impact the smoke delivery in terms of tobacco rod density that resulted in no direct correlation for the calculated tobacco rod density and carbonyl yields. For example, higher tobacco rod density results in a higher amount of tobacco mass combusted and would therefore deliver a higher amount of HPHCs, including carbonyls; on the other hand, the higher tobacco rod density results in increased packed tobacco that may act as a filter, affecting the filtration efficiency through the tobacco rod and impacting the smoke chemistry.[30] One of the limitations of this study is the small number of tested products to formulate a general conclusion on physical parameters and carbonyl yields for the LWCs. Moreover, additional factors, such as presence of additives, other than the physical parameters recorded for the tested cigar products, may impact the HPHC mainstream smoke yields. In addition, even though the tested LWCs contain differences in tobacco mass and tobacco rod volume to provide a linear correlation on acetaldehyde yields relative to tobacco mass or tobacco rod volume, the calculated tobacco rod densities were in similar ranges (0.300−0.3112 g/cm^2) to provide a general correlation on tobacco rod density and carbonyl yields. Although the preliminary findings of this study suggest similar observations as cigarette smoke in terms of acetaldehyde yields and tobacco mass or product volume in cigar products, further studies are needed to determine the impact on different physical parameters and product composition on the impact of the delivery of carbonyls and other HPHCs.

Limitations. In the present study, the analysis of carbonyls in the mainstream smoke of cigar products using three smoking regimens showed high levels of variability. Several factors may contribute to the high variability. First, cigars are generally more variable tobacco products than cigarettes: machine-made cigars are manufactured using less sophisticated machines; cigar tobacco is typically blended using a single crop, and cigars are wrapped in tobacco or tobacco sheet instead of cigarette paper.[31] Second, the smoking machines in this study contain cigarette holders designed specifically for cigarettes, which may not be able to accommodate the different diameters and shapes of cigar products.[32,33] Third, the smoking regimen may result in self-extinguishment of the cigar product, which may affect the amount of HPHCs in mainstream smoke. The variability in cigar products is in agreement with reported studies[32,34] that show that RSDs for carbonyls are higher in cigar smoke than in cigarette smoke, with RSDs ranging up to 25% for formaldehyde, acrolein, and acetaldehyde using three smoking regimens (i.e., ISO, CI, CRM64). Furthermore, Jablonski et al.[35] report up to 37% RSDs for cigars using the CRM64 smoking regimen.

The scientific literature has reported that puff topography for sheet-wrapped cigar users (41.5 mL puff volume, 2.0 s puff duration, 20.8 s puff interval) and cigarillo users (57.0 mL puff volume, 2.8 s puff duration, 24.5 s puff interval) generally has parameters that more closely resemble the CI smoking regimen than the ISO or CRM64 smoking regimens.[21,36] Cigarettes are currently analyzed using standard smoking regimens (ISO and CI), which reveal a range of HPHC levels present in mainstream cigarette smoke. The CI smoking regimen may provide meaningful analytical information regarding cigar smoke constituents, with lower likelihood of self-extinguishment due to the short puffing intervals and, therefore, lower variability. However, the development of cigar reference products would provide additional insight on smoking machine parameter suitability and contribute to the validation of standard methods for analyzing cigar mainstream smoke.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.2c00322.

Average carbonyl yields (i.e., formaldehyde, acetaldehyde, acrolein) in the tested products of this study using the three smoking regimens (PDF)

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■ ABBREVIATIONS

CI, Canadian Intense smoking regimen; Health Canada method T-115, equivalent to ISO 20778:2018; COPD, chronic obstructive pulmonary disease; CORESTA, Cooperation Centre for Scientific Research Relative to Tobacco; CRM64, Cooperation Centre for Scientific Research Relative to Tobacco Recommended Method No. 64 (Routine Analytical Cigar-Smoking Machine—Specifications, Definitions and Standard Conditions); CRM74, Cooperation Centre for Scientific Research Relative to Tobacco Recommended Method No. 74 (Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC); DNPH, 2,4-dinitrophenylhydrazine; EPA, U.S. Environmental Protection Agency; FDA, U.S. Food and Drug Administration; FSPTCA, Family Smoking Prevention and Tobacco Control Act; HPHC, harmful and potentially harmful constituent; IARC, International Agency for Research on Cancer; ISO, International Organization for Standardization 3308:2012; LWC, leaf-wrapped cigars; MEK, methyl ethyl ketone; RSD, relative standard deviation; SWC, sheet-wrapped cigars

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