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Effects of Topography-Related Puff Parameters on Carbonyl Delivery in Mainstream Cigarette Smoke

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Abstract

Smoking topography parameters differ substantially between individual smokers and may lead to significant variation in tobacco smoke exposure and risk for tobacco-caused diseases. However, to date, little is known regarding the impact of individual puff parameters on the delivery of many harmful smoke constituents including carbonyls. To examine this, we determined the effect of altering individual puff parameters on mainstream smoke carbonyl levels in machine-smoked reference cigarettes. Carbonyls including formaldehyde, acetaldehyde, crotonaldehyde, propionaldehyde, methyl ethyl ketone (MEK), acrolein and acetone, were determined in cigarette smoke by HPLC after derivatization with 2,4-dinitrophenylhydrazine (DNPH). Delivery of all carbonyls were nearly 2-fold greater when cigarettes were smoked according to the more intense Health Canada Intense (HCI) protocol compared to the International Organization of Standardization (ISO) method, consistent with the 2-fold difference in total puff volume between methods (ISO: 280–315 mL; CI: 495–605 mL). When individual topography parameters were assessed, changes in puff volume alone had the greatest effect on carbonyl delivery as predicted with total carbonyls being strongly correlated with overall puff volume (r^2: 0.52 – 0.99), regardless of how the differences in volume were achieved. All seven of the carbonyls examined showed a similar relationship with puff volume. Minor effects on carbonyl levels were observed from vent blocking and changing the interpuff interval while effects of changing puff duration and peak flow rate were minimal. Overall, these results highlight the importance of considering topography, especially puff volume, when assessing the toxicant delivery and potential exposure smokers receive. The lack of an impact of other behaviors, including puff intensity and duration

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SUPPORTING INFORMATION: HPLC spectra for carbonyl analyses as well as the combined acetone-DNPH/acrolein-DNPH peak areas are provided in the Supporting Information.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

The authors declare the following competing financial interest(s): J.F. has done paid consulting for pharmaceutical companies involved in producing smoking cessation medications, including GSK, Pfizer, Novartis, J&J, and Cypress Bioscience, and has received a research grant and study drug from Pfizer (not relating to toxicant output from cigarettes). There are no competing interests to declare for other authors.

independent of volume, indicate that factors such as temperature and peak flow rate may have minimal overall effects on carbonyl production and delivery.

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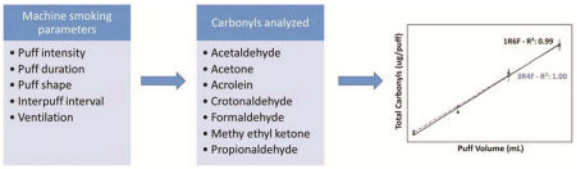

Keywords

acetaldehyde; acrolein; acetone; crotonaldehyde; formaldehyde; methyl ethyl ketone; propionaldehyde; puff volume; topography

Introduction

Cigarette smoking is the number one cause of preventable death worldwide, causing one of every five deaths in the United States (U.S.) each year.[1–3] It leads to lung cancer,[4, 5] chronic obstructive pulmonary disease (COPD),[6, 7] cardiovascular and many other diseases.[3, 8] Of the various toxicants produced in cigarette smoke, carbonyls have toxic and carcinogenic effects, linking them to a significant number of these smoking-related diseases. Many carbonyls are listed on the Food and Drug Administration's published list of 93 harmful and potentially harmful constituents (HPHC).[9] For example, formaldehyde, acetaldehyde, and crotonaldehyde are classified as carcinogens, propionaldehyde and acrolein are cardiovascular toxicants,[9] and acetone and all previously mentioned carbonyls, except crotonaldehyde, are respiratory toxicants.[9–11] Thus, understanding the delivery of these toxicants to smokers is crucial to better understanding of potential harm to which different smokers are exposed.

Differences in smoking behaviors among individual smokers are thought to be an important factor leading to differences in overall smoke exposure.[12] However, there is little known regarding variation in carbonyl delivery that smokers can receive from cigarettes based on their own puffing behavior (topography). Puff topography summarizes how a smoker inhales a cigarette, including details (puff parameters) such as how long (puff duration), how much (puff volume), how quickly (peak flow rate), and how long between each inhalation (interpuff interval). It has been previously established that nicotine and carbon monoxide exposures in smokers are heavily influenced by smoking behavior.[12–17] In terms of carbonyl delivery and thus potential exposure, the effects of puff topography are unclear. As the puff profile affects the dynamics of the tobacco burning process, we expect that it would also affect the carbonyl levels delivered to the smoker as carbonyls are produced mostly from incomplete combustion of tobacco.[3, 18] Studies that used both the International Organization of Standardization (ISO) or the Health Canada Intense (HCI) puff profiles have shown that carbonyl delivery does increase substantially from ISO to HCI for conventional cigarettes;[19–22] however, the specific puff parameter(s) are responsible for this difference in

carbonyl delivery were not examined. Our current objective was to determine what effects individual changes in puff parameters have on carbonyl delivery in machine-smoked cigarettes. To do this, we examined two different standardized research cigarettes (1R6F and 3R4F). This study could have important implications on risk assessment as it is hard to predict carbonyl delivery and thus potential exposure when a smoker's own smoking behavior can change so drastically. With the new information generated by this study, we hope to help guide development of improved predictive models for carbonyl delivery to smokers based on their own puff parameters, which can help predict potential carbonyl exposure.

Methods

Our overall approach to explore the effects of single puff parameter changes on carbonyl delivery started with first machine smoking of reference cigarettes using both the ISO and HCI methods. To test individual parameters, we modified the ISO puff profile to take only the single puff parameter of interest into account, such as puff duration (2 s changes to 1 s, 3 s, or 5 s), puff volume (35 mL changes to 17.5 mL, 55 mL, or 75 ml), puff shape (bell changes to square or sharp peak shapes), and interpuff interval (60 s changes to 15 s or 30 s). These altered profiles were programmed into the smoke machine and used to smoke cigarettes to test for carbonyl delivery. For testing ventilation effects, cigarette vents were blocked with tape and smoked with the remaining puff parameters matching the ISO method, which is referred to as the "vent blocked" method for the rest of the manuscript.

Mainstream Smoke Generation

Mainstream smoke was generated by a single-port smoking machine (Human Puff Profile Cigarette Smoking Machine (CSM-HPP), CH Technologies, NJ, USA). One cigarette was smoked at a time under the various smoking protocols, such as the ISO (35 mL volume, 2 s duration, and 60 s interpuff intervals with open vents) and Health Canada Intense (HCI; 55 mL volume, 2 s duration, and 30 s interpuff intervals with blocked vents) methods. All other protocols used were initially based on the provided ISO protocol, only changing one parameter of interest, such as puff volume, puff duration, interpuff interval, for each topography experiment. All cigarettes were smoked to the same butt length (ISO standard: 3 mm from the filter overlap).

Cigarettes

The 3R4F and 1R6F research cigarettes were obtained from the University of Kentucky (Lexington, Kentucky, USA). These two cigarettes were also chosen because 3R4F is the most widely used reference cigarette in the literature whereas 1R6F is a newly developed to better reflect products currently on the market. The cigarettes were stored at −80°C in airtight plastic bags. The cigarettes were conditioned for testing by placing them in a constant humidity chamber (60% relative humidity, 22±1°C), for at least 24 h before use.

Materials

Acetonitrile (ACN) and concentrated hydrochloric acid (12N HCl) were purchased from Fisher Scientific (Pittsburgh, PA, USA) and used as supplied. Diglyme and

dinitrophenylhydrazones of formaldehyde, acetaldehyde, acetone, acrolein, crotonaldehyde, propionaldehyde, and MEK were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as supplied. 2,4-Dinitrophenylhydrazine (DNPH) was purchased from BOC Sciences (Shirley, NY, USA). DNPH was recrystallized before use to remove water.[23] In brief, 17 g DNPH was dissolved in 350 mL acetonitrile (ACN), heating at 70°C for 1 hour, and then cooled. Once at room temperature, the crystals were collected by vacuum filtration and stored in a desiccator.

Derivatization of Carbonyls

DNPH solution was made as described previously[23] by dissolving 1.0 g recrystallized DNPH in a mixture of 50 mL diglyme, 360 μL 12N HCl, and 150 mL ACN. Mainstream smoke generated from one cigarette was passed into an impinger containing 10 mL of DNPH solution. The sample was then transferred into a scintillation vial and stored at 4°C until HPLC-UV analysis. We performed a minimum of two replicates of each collection (n=2–4 for all experiments). Although samples were found to be stable up to 2 weeks under these conditions (after which crotonaldehyde begins to degrade), all HPLC-UV analyses were performed within 3 days of collection to allow ample time for reanalysis if deemed necessary.

HPLC-UV Analyses

For analysis of formaldehyde, acetaldehyde, crotonaldehyde, propionaldehyde, and MEK, high performance liquid chromatography with ultraviolet detection (HPLC-UV) analyses were performed as previously published.[24] Briefly, we used a binary system consisting of two Waters (Milford, MA, USA) 510 pumps, a Waters 440 ultraviolet absorbance detector, and a Hitachi (Tokyo, Japan) D-2500 Integrator. The carbonyl-DNPH derivatives were separated by a Phenomenex Bondclone C18 column (10 μm × 300 mm × 3.9 mm; Torrance, CA, USA) using water (A) and acetonitrile (B) mobile phases. The elution gradient was: 0 min., 90% A, 10% B; 20 min., 10% A, 90% B; 25 min., 10% A, 90% B, 27 min., 90% A, 10% B; and 37 min. 90% A, 10 % B. The flow rate was 1.0 mL/min.The detection wavelength was 254 nm. All sample injections were 10 μL and injected with a Hewlett Packard (Palo Alto, CA, USA) Series 1050 autosampler. All measurements were carried out at room temperature (22±1°C). Under these conditions, the carbonyls eluted at the following retention times: formaldehyde (18.5 min), acetaldehyde (19.7 min), crotonaldehyde (21.9 min), propionaldehyde (21.3 min), MEK (22.3 min), and acrolein/acetone (co-eluted at 20.9 min). A representative HPLC spectrum is given in Supplemental Figure 1A. Using this methodology, recovery values for known amounts of authentic standards added to samples prior to processing were >98% and CV values for replicate (n=12) analyses were between 6–12% for all carbonyls. Calibration curves based on DNPH derivative standards (0.6, 5, and 25 μg/ml) were used to determine concentration of the peaks of interest.

In order to measure acetone and acrolein, additional experiments were performed where pyridine (500 μl) was added to the DNPH solution (10 ml) immediately after collection to stabilize the acrolein-DNPH derivative. The method for HPLC separation used was based on the CORESTA method as follows: HPLC-UV analyses were performed using a binary system consisting of two Waters (Milford, MA, USA) 510 pumps, a Shimadzu (Kyoto,

Japan) SPD-10A VP UV-Vis Detector, and a Hitachi (Tokyo, Japan) D-2500 Integrator. The carbonyls were separated by a Phenomenex Bondclone C18 column (10 μm × 300 mm × 3.9 mm; Torrance, CA, USA) using 30% acetonitrile, 10% tetrahydrofuran, and 1% isopropanol (A) and 65% acetonitrile, 1% tetrahydrofuran, and 1% isopropanol (B) mobile phases. The elution gradient was: 0 min., 100% A; 8 min., 70% A; 16 min., 60% A; 20 min., 54% A; 22 min., 40% A; 25 min., 100% A; and 31 min. 100% A. The flow rate was 1.5 mL/min. The detection wavelength was set at 365 nm. All sample injections were 20 μL and injected with a Hewlett Packard (Palo Alto, CA, USA) Series 1050 autosampler. All measurements were carried out at room temperature (22±1°C). Under these conditions, the following retention times were obtained: acrolein (15.5 min) and acetone (14.6 min). A representative HPLC spectrum is given in Supplemental Figure 1B. Recovery and precision values for this method were >98% and ~12%, respectively. Calibration curves based on DNPH derivative standards (0.6, 5, and 25 μg/ml) were used to determine concentration of the peaks of interest.

Statistical Analysis

Linear regression was used to determine the significance of the trends in carbonyl delivery with puff volume and puff duration. For all carbonyls in all methods tested, one-way ANOVAs with Tukey contrasts were used to evaluate all pairwise comparisons presented. Krushal-Wallis tests were also performed and in agreement with the ANOVAs. All statistical analyses were generated using SAS software Version 9.4 of the SAS System for Windows ×64 Systems (SAS Institute Inc., Cary, NC, USA).

Results

Standard Smoking Protocols and Blocking Ventilation

We examined the two reference cigarettes, 3R4F and 1R6F, by both ISO and HCI methods (Figure 1). The concentrations of all carbonyls of interest increased when using HCI compared to ISO. For both methods, we found that acetaldehyde was the most abundant carbonyl followed by acetone, acrolein, methyl ethyl ketone (MEK), propionaldehyde, formaldehyde, and crotonaldehyde, which matches well with previous literature.[19–22] This order of abundance was maintained through all puff parameters tested. Acetaldehyde, MEK, and crotonaldehyde increased significantly from ISO to HCI for both cigarettes. Propionaldehyde only increased significantly for the 1R6F cigarette while formaldehyde did not significantly change between methods. All cigarettes were smoked to the same butt length (ISO standard: 3 mm from the filter overlap). The number of puffs taken using the HCI method (3R4F: 11 puffs; 1R6F: 9 puffs) was significantly greater than that for the ISO method (3R4F: 9 puffs; 1R6F: 8 puffs). These values are similar to those reported by the University of Kentucky,[25] with the exception of acetaldehyde, which was lower in both smoking protocols, possibly due to differences in smoke collection and/or analytical methodologies. Thus, the greater delivery of carbonyls observed per puff for the HCI protocol is magnified when expressed on a per cigarette basis (data not shown). While there was a trend for increased carbonyl delivery by “vent blocked” method for some of the most abundant carbonyl species, these differences were only significant ($P \leq 0.05$) for acetaldehyde (ISO is 34–39% lower than ventilation blocked method; Figure 1). Vent blocking had no effect on puff number.

Puff Volume

Puff volume was altered in two ways. First, we examined the effects of puff volume (17.5, 35, 55, and 75 mL) by changing puff intensity (peak flow rate) while maintaining puff duration at 2 s and interpuff interval at 60 s (Figure 2A inset), and then measuring carbonyl delivery. Under these conditions, carbonyls increased with increasing volume with significant differences being observed between 17.5 mL and 75 mL for all carbonyls for both cigarettes and for acetaldehyde and MEK at each volume for both cigarettes (Figure 2A). Next, we examined the effects of puff volume (17.5, 35, and 70 mL) by changing puff duration (1, 2, and 4 s) while maintaining puff intensity (peak flow rate: 26 mL/s; average flow: 17.5 mL/s) and interpuff interval at 60 s (Figure 2B inset), and then measuring carbonyl delivery. Under these conditions, carbonyls concentrations increased with increasing volume, with significant differences observed between 17.5 mL and 70 mL for all carbonyls for 3R4F and all carbonyls except formaldehyde for 1R6F (Figure 2B). Under both methods of examining puff volume, carbonyl delivery was linearly, significantly, and positively proportional to puff volume ($r^2 = 0.52 – 0.99$; $p<0.01$) for all carbonyls for both cigarettes (Figure 3). As expected, significantly fewer puffs ($p < 0.05$) were observed from 70–75 mL than from 17.5 mL for both cigarettes in both methods (3R4F: 8 to 7; 1R6F, 9 to 7 puffs); however, as these changes were small, the trend of carbonyl delivery increasing with increasing puff volume did not change if we examined on a per cigarette basis (data not shown). The effects of changing puff volume on delivery of acetone and acrolein were similar to those for the other carbonyls (Figure 2A) with proportional changes observed between levels of both carbonyls and puff volume (Figure 3A).

Puff Shape

We changed puff shape from the typical bell to a square or sharp shape (Figure 4 inset) to test the effects of changing the peak flow rate (i.e., how quickly/intensely one inhales) without changing puff duration (2 s), puff volume (35 mL), or interpuff interval (60 s). No significant differences in trends of carbonyl delivery were observed for either cigarette (Figure 4), and the combined acrolein/acetone peak did not significantly change between different puff shapes (Supplemental Table 1). While formaldehyde delivery did appear higher for the bell shape with the 3R4F cigarette, it was not significant. In addition, this increase was not reproduced with the 1R6F. In addition, there were no significant differences in puff count for these topographies for either cigarette.

Puff Duration

Four different puff durations were tested (1, 2, 3, and 5 s) while maintaining puff volume (35 mL) and interpuff interval (60 s; Figure 5 inset). Carbonyl delivery was not significantly different between the durations tested for any carbonyl (Figure 5); however, the linear trend observed for acetaldehyde is significant ($p < 0.05$) for both cigarettes ($r^2 = 0.49$ for 3R4F and 0.53 for 1R6F), increasing as duration extends. The combined acrolein/acetone peak did not significantly change between different durations (Supplemental Table 1). No differences were found in puff number.

Interpuff Interval

Carbonyl delivery was accessed with three different interpuff intervals (15 s, 30 s, and 60 s) while maintaining puff volume (35 mL) and puff duration (2 s). Few differences were observed between carbonyls and interpuff interval for both cigarettes on a per puff basis (Figure 6A). For 3R4F cigarettes, the carbonyl deliveries on the 15 s and 30 s interpuff interval methods were significantly higher than 60 s on a per puff basis for crotonaldehyde and MEK while acetaldehyde was different for 1R6F cigarettes. Between different interpuff intervals, the combined acrolein/acetone peak did not significantly change when analyzing the results on a per puff basis (Supplemental Table 1). As significant differences in puff number were observed for all groups for both cigarettes (3R6F: 19, 14, and 9 puffs; 1R6F: 16, 12, and 8 puffs, respectively), delivery was also examined on a per cigarette basis (Figure 6B). Almost all carbonyls decreased with longer interpuff intervals with only formaldehyde being an exception. As interpuff interval increases, the total volume per cigarette decreases (i.e., 15 s: 560 mL, 30 s: 420 mL, 60 s: 280 mL for 3R4F), further suggesting that volume is the driving factor in carbonyl delivery.

Discussion

It is well known that there are wide-ranging differences in smoking behavior patterns between individual smokers, including potentially important parameters such as puff intensity and puff volume. In the laboratory, these puff topography behaviors are tested on cigarette smoking machines by altering puff shape, puff intensity, puff duration, inter-puff interval, and ventilation. By changing these puff parameters, we introduce other profile changes including puff volume, total volume per cigarette, and the temperature dynamics of each puff. Given the potential importance of these factors on carbonyl production/delivery, we sought to examine in the laboratory the impact of altering puff profiles on mainstream smoke carbonyl levels using a smoking machine to better estimate how an individual's puff profile may impact their potential exposure to carbonyls based on the amount of carbonyls delivered from a cigarette.

First, we examined the effects of changing puff intensity alone, which impacts three parameters: puff volume, total volume pulled through the cigarette, and flow and temperature dynamics. We found that increasing puff intensity while maintaining puff shape and duration significantly increased carbonyl delivery, an effect that may be accounted for by an increase in puff volume and/or change in the temperature and dynamics of the combustion process. In order to help distinguish between these two possibilities, we also examined the effects of changing puff volume by changing puff duration but not puff intensity. Since similar increases in carbonyl delivery were found regardless of whether changes in volume were achieved by increasing puff duration or peak flow rate, we confirm the major role of puff volume in modulating carbonyl delivery. When examined further, both ways of changing puff volume exhibited a directly proportional effect further supporting that puff volume is the main puff parameter driving carbonyl delivery to the smoker.

We also examined the impact of reducing ventilation by blocking of vents. In this scenario, puff volume remained unchanged; however, the contribution of air entering from the vent holes and diluting the mainstream smoke is reduced from about 30–33%[25, 26] to 0% after

blocking. The increase observed in acetaldehyde when comparing the vent blocked method to the ISO was consistent with the reduction in smoke dilution as well as the observed associations of puff volume with carbonyl delivery. As such, it would be expected that larger differences would be observed with more ventilation, an effect predicted previously from comparisons of ISO and HCI methods between full-flavored and ultralight cigarettes.[19]

To further examine potential effects of combustion temperature, we examined the impact of altering puff shape and puff duration (Figure 4 & 5 insets) without changing puff volume on carbonyl delivery. Using these protocols, changes in temperature dynamics of the burning coal of the cigarette would be expected as a previous study has shown.[27] Because changes in puff shape in the absence of changes in puff volume can have a direct impact on flow rate and, in turn, the dynamics of combustion, it could potentially have an important impact on the production and delivery of tobacco combustion products including carbonyls. Since there was no clear trend in carbonyl delivery observed when changing puff shape and only minimal changes were observed when changing puff duration, we conclude that changing the flow and temperature dynamics without change puff volume has minimal effect on the delivery of carbonyls.

In order to distinguish between effects of differences in individual puffs and total puff volume per cigarette, the impact of altering interpuff interval on carbonyl delivery was examined. In this way, total puff volume per cigarette was impacted without changing the volume of individual puffs. Previously, it had been reported that shorter interpuff intervals can impact the temperature dynamics of the burning cigarette by changing the starting temperature of subsequent puffs.[28] Changing interpuff interval has little change on carbonyl delivery on a per puff basis, again suggesting that temperature changes were playing a relatively minor role in carbonyl delivery. However, on a per cigarette basis, larger differences in carbonyl delivery were observed, similar to those found with puff volume, further suggesting that volume is the most critical role.

Lastly, we observed significantly higher carbonyl delivery for cigarettes smoked by the HCI method compared the standard ISO method. Previous reports indicate that ISO, the most common method used for smoking machine testing, typically underestimates smoking exposure,[29, 30] and that the HCI method typically estimates more extreme topographies with the true exposure in most smokers (~86%) occurring between these two extremes.[30, 31] By comparing these methods, we can observe the effects of changing multiple parameters at once as it changes puff volume (35 to 55 mL), interpuff interval (60 to 30 s), and ventilation (open to closed). The higher levels observed with HCI are similar to the increase observed increasing puff volume from 35 to 55 mL (Figure 2A), suggesting that the difference in puff volume between the two methods is playing a more significant role than the other parameters, including interpuff interval and ventilation. In addition, the delivery of all carbonyls were nearly 2-fold greater when cigarettes were smoked by HCI protocol compared to the ISO method, which matches very closely to the differences in volume on both methods as well (ISO: 280–315 mL; CI: 495–605 mL). However, the volume differences associated with these later two topography parameters may account for the remaining differences in carbonyl delivery.

Thus, the single parameter with the largest effect appears to be the total volume inhaled and, by relation, puff volume. A given total volume can be achieved by changing various parameters, puff duration, puff intensity, or interpuff interval, but all these methods show similar effects on carbonyl delivery. Temperature increases without a coupled increase in air inhaled does not appear to have an effect itself based on the experiments here. Unfortunately, there are a few cigarette design changes that could be implemented to lower the volume a cigarette can deliver, and those that could be used (shortened cigarette length and ventilation) can all be overcome with increased cigarette consumption or have been shown be ineffective already at lowering harm. A limitation of this study is that we used two research cigarettes and not commercial cigarettes, which can limit the generalizability of these results. However, given the strong associations we observed in both products tested, particularly for puff volume, we suspect that similar relationships would occur for commercial products as well.

Altogether, this laboratory study elucidates the effects of systematically changing individual puff parameters linked to human topography on the delivery of toxic and/or carcinogenic carbonyls in mainstream tobacco smoke. Based on comparison of HCI to each parameter individually, the carbonyl delivery on the HCI method appears to be most affected, on a per puff basis, by the change in puff volume with more minor effects from blocking vents and changing the interpuff interval. Puff duration and peak flow were found to have minimal affect when puff volume is kept constant. Together, these findings on carbonyl delivery suggest that, puff duration and peak flow are not as important as puff volume when interpreting human smoking topography data for potential carbonyl exposure from cigarettes. Most importantly, these results can help us better understand and guide analyses of human topography data for predicting potential carbonyl exposure by showing that puff volume and total volume are two parameters with the greatest effect on carbonyl delivery, thus the most important to consider when grouping and simplifying the data. This further supports that other measures strongly correlated with total volume, such as solanesol, should be able to be used to estimate a smoker’s potential exposure to harmful carbonyls.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

DNPH	2,4-Dinitrophenylhydrazine
HCI	Health Canada Intense method

ISO	International Organization of Standardization Method
MEK	Methyl Ethyl Ketone

References

1. Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJ, Ezzati M. The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med. 2009; 6:e1000058. [PubMed: 19399161]
2. Xu X, Bishop EE, Kennedy SM, Simpson SA, Pechacek TF. Annual Healthcare Spending Attributable to Cigarette Smoking: An Update. Am J Prev Med. 2015; 48:326–333. [PubMed: 25498551]
3. U.S. Department of Health and Human Services. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; Atlanta: 2014.
4. Doll R, Hill AB. Smoking and carcinoma of the lung. Br Med J. 1950; 2:739. [PubMed: 14772469]
5. Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma: a study of six hundred and eighty-four proved cases. JAMA. 1950; 143:329–336.
6. Zaher C, Halbert R, Dubois R, George D, Nonikov D. Smoking-related diseases: the importance of COPD. Int J Tuberc Lung Dis. 2004; 8:1423–1428. [PubMed: 15636487]
7. Burney P, Jithoo A, Kato B, Janson C, Mannino D, Nižankowska-Mogilnicka E, Studnicka M, Tan W, Bateman E, Koçabas A, Vollmer WM, Gislason T, Marks G, Koul PA, Harrabi I, Gnatiuc L, Buist S. Chronic obstructive pulmonary disease mortality and prevalence: the associations with smoking and poverty—a BOLD analysis. Thorax. 2014; 69:465–473. [PubMed: 24353008]
8. Krupski WC. The peripheral vascular consequences of smoking. Ann Vasc Surg. 1991; 5:291–304. [PubMed: 2064925]
9. U.S. Food and Drug Administration. [Accessed: Oct. 27, 2016] Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List. 2012. Webpage: http://www.fda.gov/TobaccoProducts/Labeling/RulesRegulationsGuidance/ucm297786.htm.
10. Wynder EL, Goodman DA, Hoffmann D. Ciliatoxic Compounds in Cigarette Smoke. II. Carboxylic Acids and Aldehydes. Cancer. 1965; 18:505–509. [PubMed: 14278048]
11. Moghe A, Ghare S, Lamoreau B, Mohammad M, Barve S, McClain C, Joshi-Barve S. Molecular Mechanisms of Acrolein Toxicity: Relevance to Human Disease. Toxicol Sci. 2015; 143:242–255. [PubMed: 25628402]
12. Krebs NM, Chen A, Zhu JJ, Sun DX, Liao J, Stennett AL, Muscat JE. Comparison of Puff Volume With Cigarettes per Day in Predicting Nicotine Uptake Among Daily Smokers. Am J Epidemiol. 2016; 184:48–57. [PubMed: 27313218]
13. Koszowski B, Rosenberry ZR, Viray LC, Potts JL, Pickworth WB. Make Your Own Cigarettes: Toxicant Exposure, Smoking Topography, and Subjective Effects. Cancer Epidem Biomar. 2014; 23:1793–1803.
14. Hammond D, Fong GT, Cummings KM, Hyland A. Smoking Topography, Brand Switching, and Nicotine Delivery: Results from an *In vivo* Study. Cancer Epidemiol Biomarkers Prev. 2005; 14:1370–1375. [PubMed: 15941943]
15. Bridges RB, Combs JG, Humble JW, Turbek JA, Rehm SR, Haley NJ. Puffing Topography as a Determinant of Smoke Exposure. Pharmacol Biochem Behav. 1990; 37:29–39. [PubMed: 2263664]
16. Herning RI, Jones RT, Benowitz NL, Mines AH. How a Cigarette Is Smoked Determines Blood Nicotine Levels. Clin Pharmacol Ther. 1983; 33:84–90. [PubMed: 6848303]
17. Zacny JP, Stitzer ML, Brown FJ, Yingling JE, Griffiths RR. Human Cigarette Smoking: Effects of Puff and Inhalation Parameters on Smoke Exposure. J Pharmacol Exp Ther. 1987; 240:554–564. [PubMed: 3806411]
18. Miyake T, Shibamoto T. Quantitative analysis by gas chromatography of volatile carbonyl compounds in cigarette smoke. J Chromatogr A. 1995; 693:376–381.

19. Pazo DY, Moliere F, Sampson MM, Reese CM, Agnew-Heard KA, Walters MJ, Holman MR, Blount BC, Watson C, Chambers DM. Mainstream Smoke Levels of Volatile Organic Compounds in 50 US Domestic Cigarette Brands Smoked with the ISO and Canadian Intense Protocols. Nicotine Tob Res. 2016; 18:1886–1894. [PubMed: 27113015]
20. Miller JH IV, Gardner WP, Gonzalez RR. UHPLC Separation with MS Analysis for Eight Carbonyl Compounds in Mainstream Tobacco Smoke. J. Chromatogr. Sci. 2010; 48:12–17. [PubMed: 20056029]
21. Ding YS, Xizheng Y, Wong J, Chan M, Watson CH. In Situ Derivatization and Quantification of Seven Carbonyls in Cigarette Mainstream Smoke. Chem Res Toxicol. 2015; 29:125–131.
22. Uchiyama S, Tomizawa T, Inaba Y, Kunugita N. Simultaneous determination of volatile organic compounds and carbonyls in mainstream cigarette smoke using a sorbent cartridge followed by two-step elution. J Chromatogr A. 2013; 1314:31–37. [PubMed: 24054423]
23. Risner CH, Martin P. Quantitation of formaldehyde, acetaldehyde, and acetone in sidestream cigarette smoke by high-performance liquid chromatography. J Chromatogr Sci. 1994; 32:76–82. [PubMed: 8200918]
24. Reilly SM, Goel R, Trushin N, Elias RJ, Foulds J, Muscat J, Liao J, Richie JP Jr. Brand variation in oxidant production in mainstream cigarette smoke: Carbonyls and free radicals. Food Chem Toxicol. 2017; 106:147–154. [PubMed: 28528972]
25. University of Kentucky Center for Tobacco Reference Products. [Accessed: Nov. 22, 2016] Certificate of Analysis - 1R6F Certified Reference Cigarette. 2016. Webpage: https://ctrp.uky.edu/resources/pdf/webdocs/CoA_1R6F.pdf.
26. University of Kentucky Center for Tobacco Reference Products. [Accessed: Nov. 22, 2016] 3R4F Preliminary Analysis. 2016. Webpage: https://ctrp.uky.edu/resources/pdf/webdocs/3R4F%20Preliminary%20Analysis.pdf.
27. Lendvay AT, Laszlo TS. Cigarette Peak Coal Temperature Measurements. Beitr Tabakforsch Int. 1974; 7:276–281.
28. Li B, Pang HR, Zhao LC, Wang B, Liu C, McAdam K, Luo DS. Quantifying Gas-Phase Temperature Inside a Burning Cigarette. Ind. Eng. Chem. Res. 2014; 53:7810–7820.
29. Hammond D, Fong GT, Cummings KM, O'Conner RJ, Giovino GA, McNeill A. Cigarette Yields and Human Exposure: a Comparison of Alternative Testing Regimens. Cancer Epidemiol Biomarkers Prev. 2006; 15:1495–1501. [PubMed: 16896039]
30. Roemer E, Carchman RA. Limitations of Cigarette Machine Smoking. Toxicol Lett. 2011; 203:20–27. [PubMed: 21354281]
31. Jackson KJ, Schroeder MJ, Hoffman AC. Mouth Level Exposure and Similarity to Machine-smoked Constituent Yields. Tob Regul Sci. 2016; 2:3–8. [PubMed: 27034970]

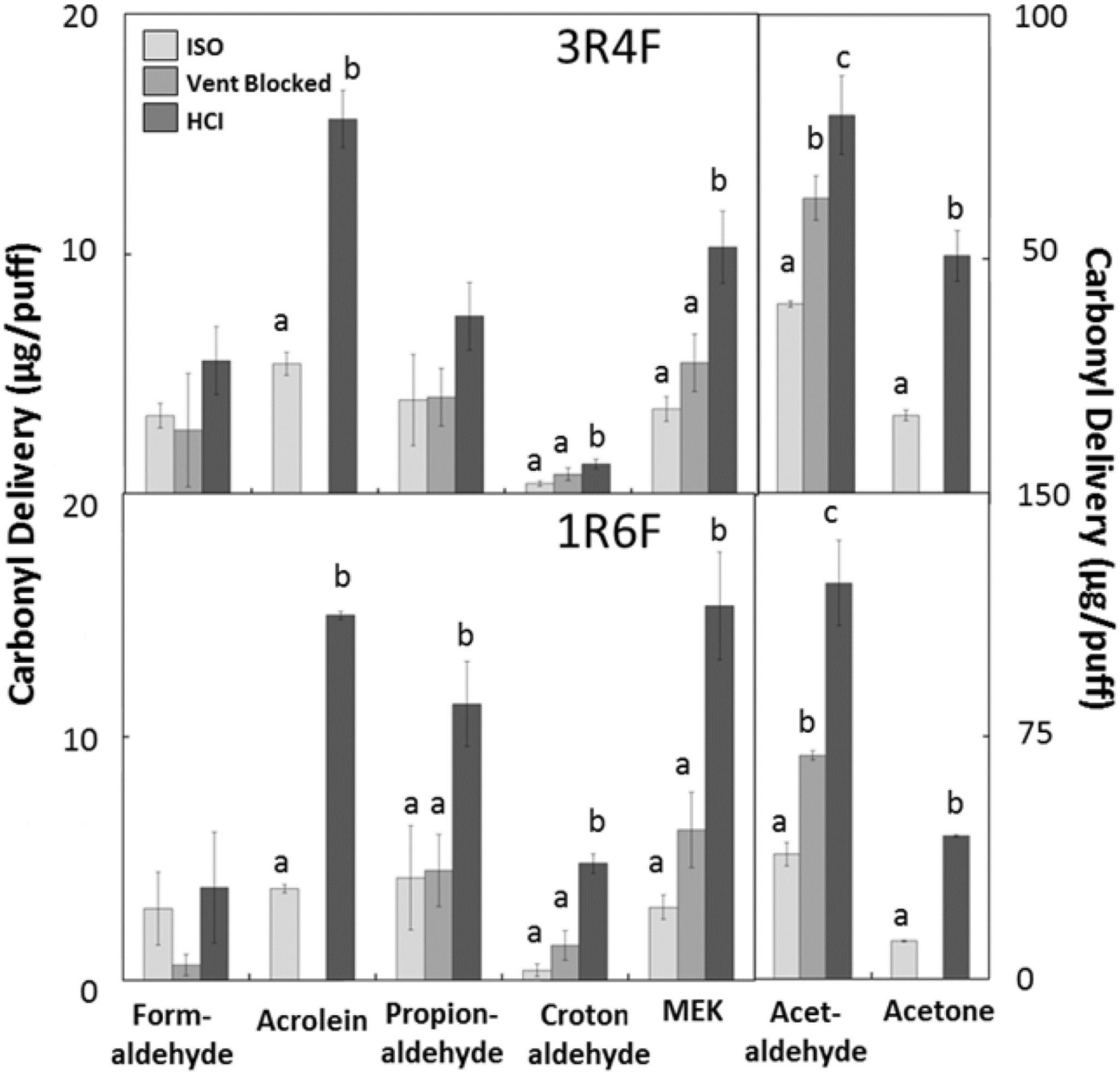

Figure 1.

Effect of smoking protocols on carbonyl delivery. Carbonyl levels in mainstream smoke from 3R4F (upper) and 1R6F (lower) research cigarettes generated by different smoking protocols (light gray: ISO method, gray: “vent blocked” method, dark gray: Health Canada Intense (HCI) method) were analyzed. Within carbonyl groups, values without a common letter differ significantly ($p < 0.05$). The acetone/acrolein peak did not change significantly between ISO and vent blocked method (Supplemental Table 1); thus, acetone and acrolein were not determined for the “vent blocked method”.

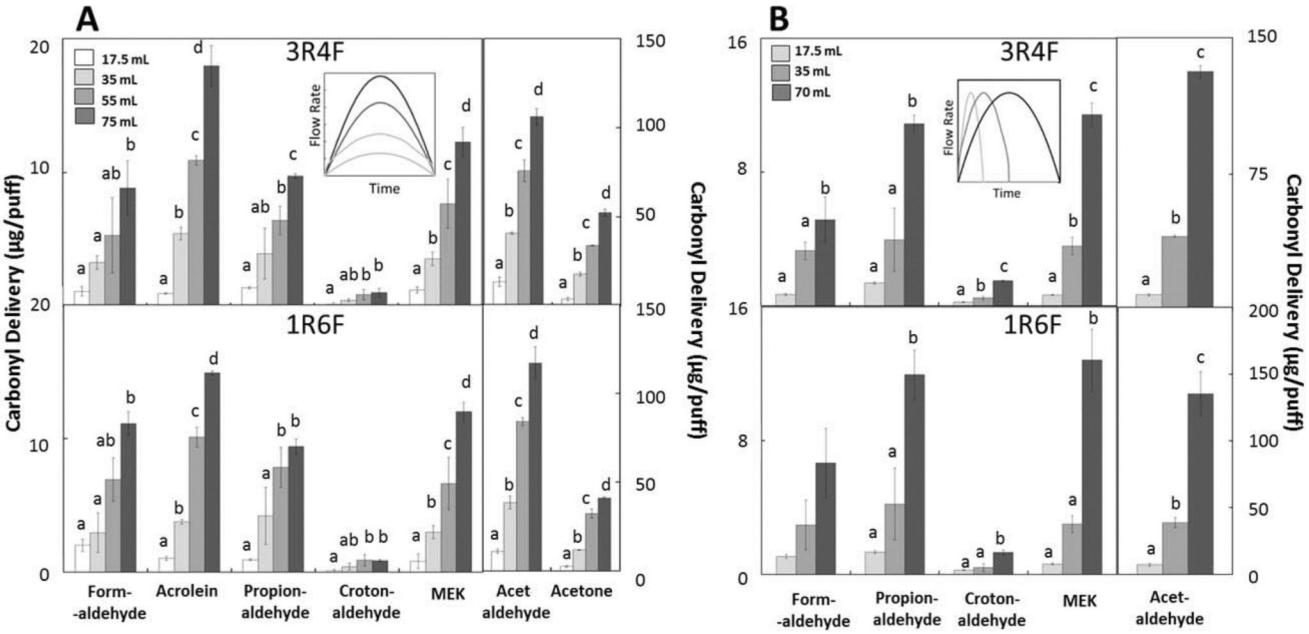

Figure 2.

Effect of puff volume on carbonyl delivery. Carbonyl levels were measured in mainstream smoke from 3R4F (upper) and 1R6F (lower) research cigarettes smoked under conditions of differing puff volume. Differences in puff volume were achieved by altering (A) puff intensity (white: 17.5 mL, light gray: 35 mL, gray: 55 mL, dark gray: 75 mL) and (B) puff duration (light gray: 17.5 mL, gray: 35 mL, dark gray: 70 mL). Inset: Puff profiles used for each corresponding volume. Within carbonyl groups, values without a common letter differ significantly ($p < 0.05$). The acetone/acrolein peak did not change significantly between methods (Supplemental Table 1); thus, acetone and acrolein were not determined for the volume by altering puff duration method (B).

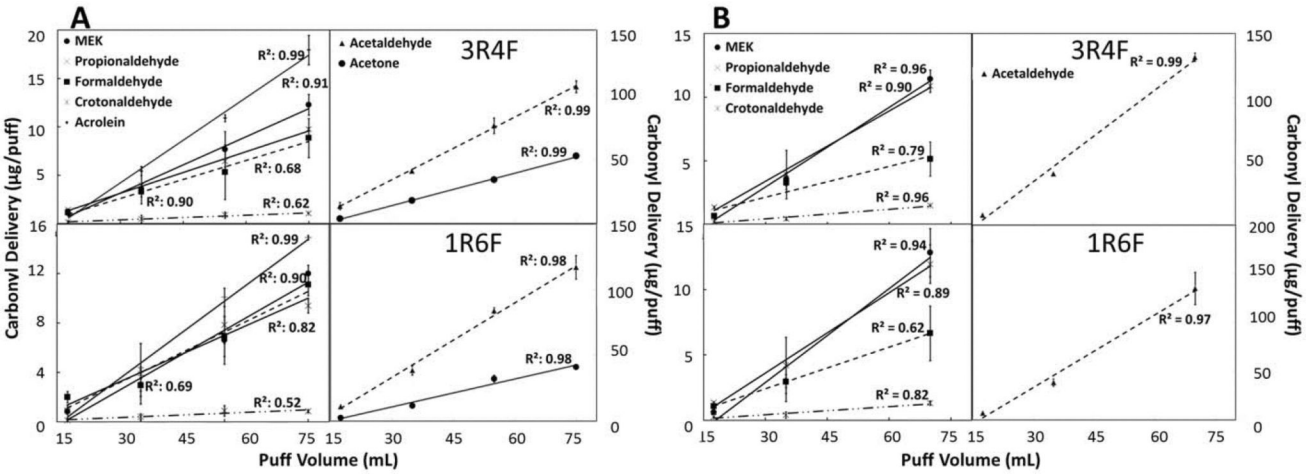

Figure 3.

Correlation of carbonyl delivery with puff volume. Carbonyl levels were assessed in mainstream smoke from 3R4F (top panel) and 1R6F (bottom panel) and correlated with (A) puff intensity and (B) puff duration. All trends are significant by linear regression analysis ($p < 0.01$).

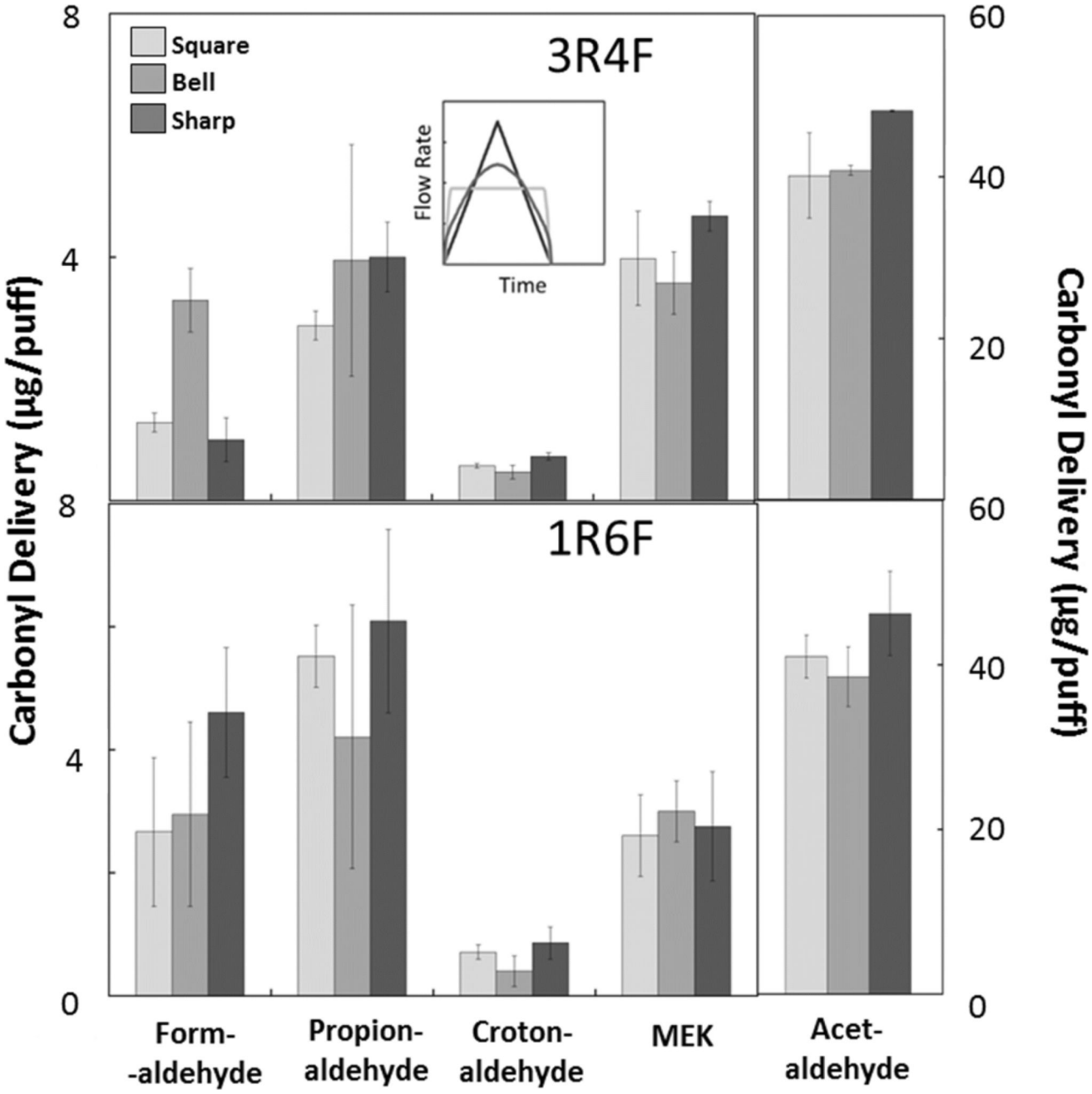

Figure 4.

Effect of puff shape on carbonyl delivery. Carbonyl levels were measured in mainstream smoke from 3R4F (upper) and 1R6F (lower) smoked under conditions of differing puff shape (light gray: square, gray: bell, dark gray: sharp). Inset: Puff profiles used for each corresponding shape.

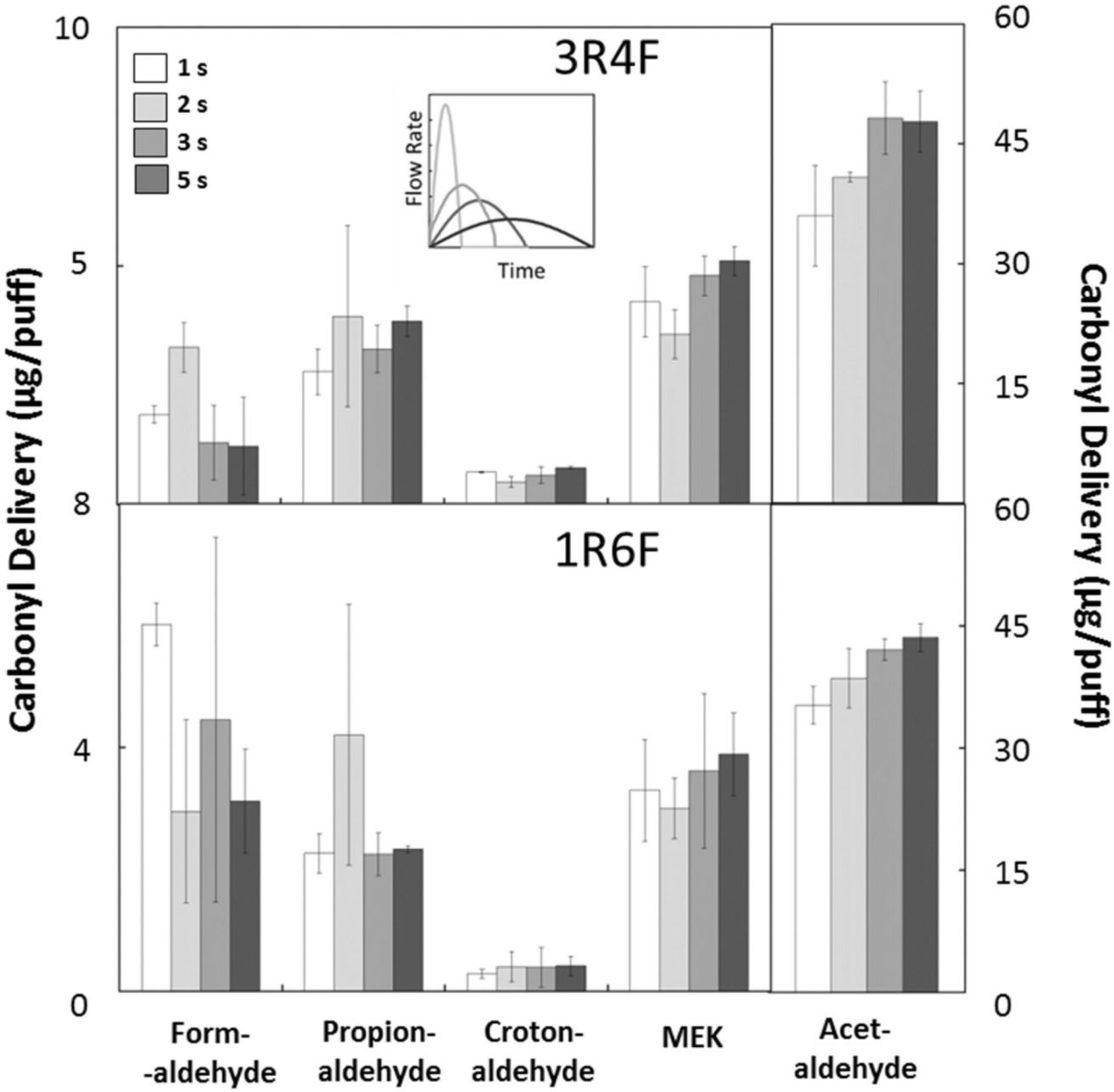

Figure 5.

Effect of puff duration on carbonyl delivery. Carbonyl levels were measured in mainstream smoke from 3R4F (upper) and 1R6F (lower) smoked under conditions of differing puff duration (white: 1 s, light gray: 2 s, gray: 3 s, dark gray: 5 s). Inset: Puff profiles used for each corresponding duration. A trend of increasing acetaldehyde with longer puff duration was observed for both cigarettes ($r^2 = 0.49$ for 3R4F and 0.53 for 1R6F; $p < 0.05$).

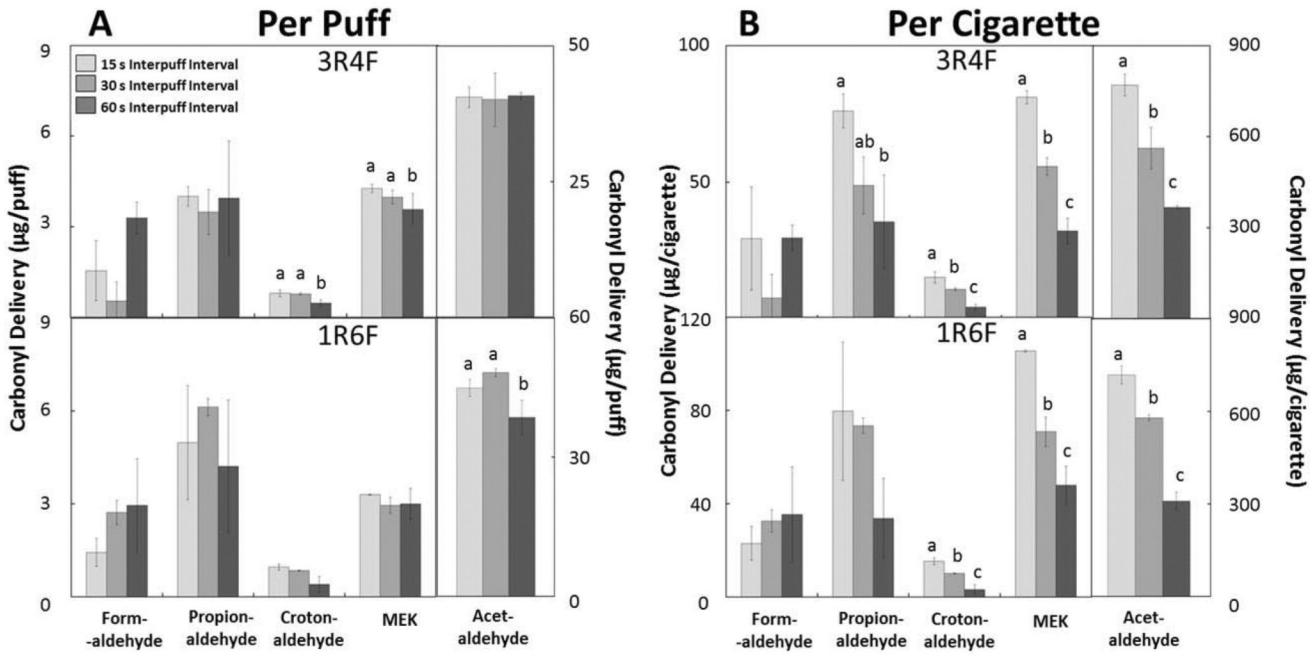

Figure 6.

Effect of interpuff interval on carbonyl delivery. Carbonyl levels were measured in mainstream smoke from 3R4F (upper) and 1R6F (lower) smoked under conditions of differing interpuff interval (light gray: 15 s interpuff interval, gray: 30 s interpuff interval, dark gray: 60 s interpuff interval). Within carbonyl groups, values without a common letter differ significantly ($p < 0.05$).