Published in final edited form as:

Chem Res Toxicol. 2018 May 21; 31(5): 325–331. doi:10.1021/acs.chemrestox.8b00011.

Influence of Smoking Puff Parameters and Tobacco Varieties on Free Radicals Yields in Cigarette Mainstream Smoke

Reema Goel[†], Zachary T. Bitzer[‡], Samantha M. Reilly[†], Jonathan Foulds[†], Joshua Muscat[†], Ryan J. Elias[‡], and John P. Richie Jr.^{*}

[†] Department of Public Health Sciences, Pennsylvania State University Tobacco Center of Regulatory Science (TCORS), Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033, United States

[‡] Department of Food Science, College of Agricultural Sciences, Pennsylvania State University, University Park, Pennsylvania 16802, United States

Abstract

Cigarette smoke is a major exogenous source of free radicals, and the resulting oxidative stress is one of the major causes of smoking-caused diseases. Yet, many of the factors that impact free radical delivery from cigarettes remain unclear. In this study, we machine-smoked cigarettes and measured the levels of gas- and particulate-phase radicals by electron paramagnetic resonance (EPR) spectroscopy using standardized smoking regimens (International Organization of Standardization (ISO) and Canadian Intense (CI)), puffing parameters, and tobacco blends. Radical delivery per cigarette was significantly greater in both gas (4-fold) and particulate (6-fold) phases when cigarettes were smoked under the CI protocol compared to the ISO protocol. Total puff volume per cigarette was the major factor with radical production being proportional to total volume, regardless of whether volume differences were achieved by changes in individual puff volume or puff frequency. Changing puff shape (bell vs sharp vs square) or puff duration (1–5 s), without changing volume, had no effect on radical yields. Tobacco variety did have a significant impact on free radical production, with gas-phase radicals highest in reconstituted > burley > oriental > bright tobacco and particulate-phase radicals highest in burley > bright > oriental > reconstituted tobacco. Our findings show that modifiable cigarette design features and measurable user smoking behaviors are key factors determining free radical exposure in smokers.

Graphical Abstract

^{*}Corresponding Author, jrichie@psu.edu., Phone: 717-531-5381.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemres-tox.8b00011. Yields of gas- and particulate-phase radicals expressed per mg of tobacco weight (PDF)

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 cigarette emissions or free radical measurement).

INTRODUCTION

Smoking-caused oxidative stress and damage play important roles in the development of many chronic diseases such as cancer, and diseases of the heart and lung.^{1–4} Free radicals, one of the most reactive classes of oxidants in tobacco smoke, are a major source of oxidative stress from smoking.^{2,5–7} Cigarette smoke contains high levels of radical species, both in the gas-phase (nmol levels) and particulate-phase (pmol levels).⁵ While their production in cigarette smoke has been known for more than over 30 years, there have been no systematic studies that investigate factors that could impact their production in cigarettes.

Smoking behaviors (e.g., puff topography) can significantly impact toxicant delivery resulting in intraindividual variation in levels of exposure.^{8–12} These behavioral factors can include puff volume, duration, frequency, and count as well as blocking of filter vents. In addition to impacting overall volume of smoke exposure, smoking topography parameters can potentially alter the burning process and smoke chemistry and, thus, impact radical formation. In the laboratory, these topography parameters are often simulated by machine-smoking with several standardized protocols used to represent common smoking topography characteristics, such as the International Organization of Standardization (ISO) protocol and the Canadian Intense (CI) protocol.^{13,14} With ISO being less-intensive and CI being more intensive, the resulting yields under these two protocols reflects the range of toxicant deliveries occurring in a majority of smokers. Recently we developed and standardized protocols for analyzing and comparing gas- and particulate-phase free radicals in machine smoked cigarettes under ISO conditions.⁵ To our knowledge, there are no studies that have measured free radical production under other smoking conditions (e.g., the intensive CI regimen) or examined the impact of individual puff parameters on radical yields.

In addition to smoking behavior, cigarette design features can impact toxicant delivery. Previously, we have shown that levels of free radicals in mainstream smoke vary substantially (>10-fold) by cigarette brand.⁵ While the cause of this variation is unknown, there are many potential design features that may be involved. The modern cigarette is a highly engineered and complex product containing many different types of tobacco, additives, flavors, filters, and ventilation.^{15–17} Most commercial cigarettes manufactured

since the 1950s are a blend of different tobacco varieties (burley, bright, oriental and reconstituted) with specific blends providing distinct flavor and taste characteristics.^{18,19} However, different tobacco types are known to impact the delivery of many Harmful and Potentially Harmful Constituents (HPHC) in mainstream smoke, such as nicotine, carbon monoxide and aldehydes.^{19–27} To our knowledge, there have been no reports examining radical production in different tobacco varieties.

Regulatory strategies aimed at harm reduction from tobacco products must rely, in part, upon information on their delivery of toxicants, including oxidants, which can be impacted by product design features as well as usage characteristics.²⁸ The main objective of this study was to compare free radical yields under different smoking regimens and, to assess how specific puffing parameters (volume, duration, frequency, and shape) and different tobacco varieties impact free radical generation in mainstream cigarette smoke.

MATERIALS AND METHODS

Cigarette Selection.

The 3R4F and 1R6F research cigarettes were obtained from the University of Kentucky (Lexington, Kentucky, USA) in 2014 and 2016, respectively. 3R4F is a widely used research cigarette in the literature, while 1R6F is a newer certified research cigarette developed to better reflect products currently on the market.²⁹ Four brands of king size (85 mm) cigarettes, were purchased locally (Dauphin and Lebanon Counties, PA, USA) in 2016–2017. The cigarette brand varieties were selected to represent US popular brands with varying filter ventilation (Newport Red, 3%; Camel Blue, 32%; Marlboro Silver, 46%; Pall Mall Orange, 58%).⁵ Four types of unfiltered single-blend tobacco cigarettes (bright, burley, oriental, and reconstituted) were custom-made made with the same paper and no filter ventilation, and obtained from Centers For Disease Control and Prevention (CDC), Atlanta, USA as previously described.^{20,23,27} All the cigarettes were stored in their original packaging long-term at –20 °C in airtight plastic bags.

Materials.

Analytical grade chemicals: nitron spin trap phenyl-N-*tert*-butylnitron (PBN), *tert*-butylbenzene, 2,2,6,6-tetramethyl-1-pi-peridinyloxy (TEMPO), 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL) from Sigma-Aldrich (St. Louis, MO, USA), and Suprasil EPR tubes (4 mm o.d; Wilmad-Labglass, Vineland, NJ, USA), Schlenk line (Chemglass Life Sciences, Vineland, NJ, USA), and Cambridge filters pads (CFP, Performance Systematix Inc., Grand Rapids, MI, USA) were used as supplied.

Mainstream Smoke Generation.

The cigarettes were conditioned for testing by removing them from cold storage and placing them in a constant humidity chamber (60% relative humidity and approximately 22 °C) for at least 24 h before smoking. Mainstream smoke was generated by a single-port linear smoking machine (Human Puff Profile Cigarette Smoking Machine (CSM-HPP), CH Technologies, NJ, USA). For testing the effect of puff topography, research cigarettes were smoked (one at a time) to a length 3 mm prior to the marked filter overwrap (tipping) under

the International Organization of Standardization (ISO 3308:2012)30 standard smoking regimen (35 mL puff volume, 60-s puff interval, 2-s duration, and no filter vents blocked) or Canada Intense (CI T-115:1999)31 smoking regimen (55 mL puff volume, 2-s duration, and 30-s puff interval and filter vents blocked with clear tape). In other experiments, ISO conditions were used except for individual puff parameters which were changed one at a time (Figure 1) as previously described.¹² Mainstream smoke was separated into particulate-phase and gas-phase by passing through a 47 mm Cambridge filter pad (CFP). For testing the effect of tobacco blends, the single blend cigarettes (unfiltered) were smoked 23 mm away from the end with a 55 mL puff volume, 2-s duration, and 30-s puff interval. The CFP for collection of particulate-phase was placed upstream of the pump. Gas-phase radicals were spin-trapped in an impinger containing 4 mL ice-cold *tert*-butylbenzene and 0.05 M PBN. The impinger was placed downstream of the pump to avoid contamination of the pump with solvent and prevent changes in puff characteristics. The total dead volumes for particulate and gas-phase radical collection were 89 and 127 mL, respectively, and were consistent in all experiments.

Analysis of Free Radicals.

All free radicals were analyzed on a Bruker eScan R spectrometer (Bruker-Biospin, Billerica, MA, USA) operating in X-band and provided highly reproducible results for both gas-phase radicals (3.6% precision) and particulate-phase radicals (1.7% precision), as described in detail previously.⁵ Briefly, concentrations of particulate-phase radicals trapped on a CFP were obtained by direct insertion of the entire rolled-up filter into a high quality quartz tube to a predetermined point, and properly positioning the tube into the center of the EPR cavity. For gas-phase radicals, the content of the impinger were deoxygenated using a Scenk line prior to EPR analysis. Results are expressed in moles per cigarette rather than spin number to allow direct comparison to other methods of estimation³² and to other smoke toxicants.^{28,33}

Statistical Analysis.

Analyses were performed using SAS software Version 9.4 of the SAS System for Windows x64 Systems (SAS Institute Inc., Cary, NC, USA) or Microsoft Excel. Linear regression was used to determine the significance of the trends in free radical levels with puff volume. For all puff parameter and tobacco blend effects on free radicals, one-way ANOVA with Tukey contrast was used to evaluate all pairwise comparisons presented.

RESULTS

Impact of Smoking Regimens on Free Radical Yields.

Mainstream smoke levels of gas- and particulate-phase radicals were analyzed in four brands of commercial cigarettes and two types of research cigarettes under the ISO and CI smoking regimens (Figure 2). Under the ISO method, gas-phase radical delivery was 9.0 ± 3.5 (mean $\pm$ SD) nmol/cig for the commercial cigarettes and 5.7 ± 0.8 and 6.1 ± 0.9 nmol/cig for the 3R4F and 1R6F research cigarettes, respectively. Overall, the gas-phase radical yield varied 2.6-fold between the commercial brands tested. When cigarettes were smoked using the CI regimen, there was a significant ($p < 0.05$), 2–4-fold increase in yield for gas-phase radicals

over ISO conditions (commercial, 22.5 ± 3.8 nmol/cig; 3R4F, 25.5 ± 2.4 nmol/cig; 1R6F, 24.4 ± 4.6 nmol/cig). On the CI method, radical yield varied 1.4-fold between the commercial brands.

Levels of particulate-phase radicals under the ISO method were 160 ± 94 pmol/cig for the commercial cigarettes, 316 ± 36 and 259 ± 54 pmol/cig for the 3R4F and 1R6F research cigarettes, respectively. The particulate-phase radical yield varied 3.9-fold between the commercial brands tested. When cigarettes were smoked under the CI regimen, there was a significant ($p < 0.05$), 1.5 to 5.8-fold increase in yield for particulate-phase radicals (commercial: 456 ± 18 pmol/cig; 3R4F: 551 ± 56 pmol/cig; 1R6F: 571 ± 49 pmol/cig). The particulate-phase radical yield did not vary significantly between the commercial brands on the CI method.

Relative radical yields under the CI method compared to the ISO method were generally greater for cigarettes with higher filter ventilation and were also generally greater for particulate-phase than for gas-phase radicals. The free radical yields on a per gram tobacco weight compared to per cigarette did not affect the overall pattern or rank order (data provided in Supplementary Figure 1). Also, there was no pattern in rank order of cigarettes by gas-phase and particulate-phase radicals for both the ISO and CI methods.

Impact of Puff Parameters on Free Radical Yields.

Because the research cigarettes (3R4F and 1R6F) produce similar levels of both gas-phase and particulate-phase radicals as commercial cigarettes, we have used the research cigarettes to examine the role of individual puff parameters. Levels of gas- and particulate-phase radicals (Figure 3) were analyzed in mainstream smoke from 3R4F and 1R6F cigarettes by systematically modifying individual puff parameters in comparison with the standardized ISO regimen (bell puff shape, 2 s puff duration, 35 mL puff volume, 60 s puff interval; Figure 1). Modifying the shape, duration and volume of the individual puffs did not significantly change the total numbers of puffs per cigarette from the ISO method (ISO: 3R4F = 9 puffs; 1R6F = 8 puffs). However, decreasing the puff interval from 60 s to 30 and 15 significantly increased the numbers of puffs per cigarette (3R4F, 9, 14, and 19 puffs; 1R6F, 8, 12, and 16 puffs, respectively). We found that changing the puff shape from the typical bell to either square or sharp shape by modifying the peak flow profile without impacting puff volume did not significantly alter gas- and particulate-phase radical yields for either 3R4F or 1R6F research cigarette. Similarly, changing the puff duration from 2 s to either 1, 3, or 5 s without changing puff volume did not significantly impact either gas- or particulate-phase radical yields for either research cigarette. Meanwhile, increasing the puff volume (17.5, 35, 55, and 75 mL) by increasing peak flow significantly increased both gas- and particulate-phase free radical yield linearly proportional with puff volume (gas-phase, $r^2 = 0.98$ – 0.99 ; particulate-phase, $r^2 = 0.97$ – 0.98) for both 3R4F and 1R6F (Figure 4). Decreasing the interval between puffs from 60 s to either 30 or 15 s also significantly increased gas- and particulate-phase free radical yield per cigarette due to the greater number of puffs taken per cigarette and the associated higher total puff volume.

Impact of Tobacco Variety on Free Radical Yields.

Levels of gas- and particulate-phase radicals (Figure 5) were analyzed in mainstream smoke from unfiltered single-blend tobacco cigarettes (burley, bright, oriental, and reconstituted) under the CI regimen (filter-vent blocking was unnecessary since cigarettes were unfiltered). Gas-phase radical yields per cigarette for the single-blend tobacco cigarettes varied greatly (7.5-fold) between the different tobacco types as follows: bright (8 ± 1 nmol) < oriental (18 ± 7 nmol) < burley (55 ± 8 nmol) < reconstituted (63 ± 13 nmol). Thus, cigarettes made with either burley tobacco or reconstituted tobacco generated significantly ($p < 0.05$) more gas-phase radicals than cigarettes made with oriental or bright tobacco. On the other hand, the variation in particulate-phase radicals yields per cigarette (1.5-fold) was substantially lower and differed in order of yield: reconstituted (1065 ± 93 pmol) < oriental (1222 ± 51 pmol) < bright (1483 ± 160 pmol) < burley (1635 ± 128 pmol). Overall, cigarettes made with burley tobacco generated significantly ($p < 0.05$) more particulate-phase radicals than cigarettes made with oriental or reconstituted tobacco. Of note, the number of puffs taken by the cigarettes varied, with reconstituted (10.0 ± 1.0) < burley (14.3 ± 0.6) < bright (20.3 ± 1.1) < oriental (28.7 ± 1.1). Similarly, the weight of tobacco filler varied between the single-blend cigarettes, with burley (1.08 ± 0.01 g) < reconstituted (1.25 ± 0.03) < bright (1.34 ± 0.04) < oriental (1.46 ± 0.02). While there was variance in the puff count and tobacco filler weight across tobacco types, there was no change in the rank order when the gas- or particulate-phase free radical yields were calculated on a per puff basis and per gram tobacco filler weight. The data for the free radical yields per gram tobacco are provided in the Supplementary Figure 2.

DISCUSSION

While smoking topography varies widely among individual smokers, laboratory evaluation of toxicants generated from cigarettes under the different smoking regimens allows researchers and regulators to compare products and estimate the range of toxicant exposure in smokers.^{13,14} The ISO is a nonintense smoking regimen that underestimates most toxicant exposures while the CI protocol is more intensive and more reflective of a typical modern smoker's behavior using filtered and vented cigarettes. The WHO Study Group on Tobacco Product Regulation recommends reporting toxicants using both the ISO and CI methods.³⁴ Previously, we have developed protocols for the direct and quantitative assessment of cigarette smoke free radicals by electron paramagnetic resonance spectroscopy (EPR).⁵ In this study, we measured free radical yield under the CI method for the first the time and compared levels for the same cigarettes under the ISO method. Free radicals levels are in the ranges observed by us⁵ and others.^{35,36} We chose four popular commercial cigarette brands with varying filter ventilation (3–58%) and two research cigarettes 3R4F (38%) and 1R6F (33%). As expected, the relative effect of ventilation blocking was greatest for those cigarettes with highest ventilation. We found four-fold more gas-phase radicals and six-fold more particulate-phase radicals being produced per cigarette for the CI protocol compared to the ISO protocol. The higher yields under CI can be due to a complex interplay of various factors such as the larger puff volume and frequency, and blocked filter vents.

While the ISO and CI smoking protocols provide information on the range of free radicals produced, they do not provide specific information on the wide variation that can occur in individual puff parameters that are affected by smoking behavior. These puff parameters include puff volume, shape, number, and frequency. For example, men tend to take larger puffs of longer duration, while women tend to take smaller, more frequent puffs.¹⁴ To understand the role of each of these puff parameters on free radical production, we altered them individually in a systematic fashion from the ISO regimen. The chosen range of puff parameters reflects the realistic range expected in humans.^{13,14} We selected the 1R6F and 3R4F research cigarettes instead of commercial cigarettes to examine the role of individual puff parameters due to the availability of data on the physical and smoke characteristics of these cigarettes. The research cigarettes were quite similar in design and produced similar levels of free radicals to those observed in commercial cigarettes. We found that total volume drawn through a cigarette is the key puff parameter that influences both gas-phase and particulate-phase radicals, regardless of whether differences were driven by differences in puff frequency or peak puff flow (intensity). In all cases, free radical levels increased proportionally with total puff volume. Interestingly, total puff volume is also the most important variable for many other important tobacco smoke HPHCs, including nicotine, carbon monoxide, tobacco-specific nitrosamines, hydrogen cyanide, and carbonyls.^{37,38} Thus, our results, along with others, emphasize the importance estimating total volume of smoke inhaled by a smoker to allow for a more accurate estimate of toxicant exposure. The measurement of total puff volume in clinical studies traditionally involves the use of smoking topography instrumentation, which can be problematic, particularly for use in large scale studies. Another, potentially more feasible method involves measurement of solanesol, a product of tobacco combustion efficiently trapped by the cigarette filter, in used cigarettes butts as it has been previously shown to be highly correlated to puff volume.^{38,39}

The increase in radical yield observed with increased total puff volume might be partly driven by changes in smoke chemistry in the combustion and pyrolysis zones of the cigarette resulting from changes in flow and burn rates or temperature.^{38,40} Tobacco pyrolysis experiments suggest that radical species can differ with temperatures ranging from 250–450 °C.⁷ However, there are no data for temperatures observed in the burning cigarette which can reach 600 °C in between puffs and 900 °C during puffs in the combustion zone. Our finding that radical yield is proportional to total puff volume regardless of puff frequency or flow rate changes suggests that factors such as temperature of the burning cone that are above particular threshold likely have a minimal impact on free radical production.

Modern commercial cigarettes are a result of sophisticated engineering and design. An important cigarette feature is the tobacco blend. Most American cigarettes are made by blending various tobacco types, including reconstituted and expanded tobacco, although much of that information remains proprietary. It has been estimated that most US cigarettes are thought to contain approximately 40% burley, 30% bright, 15% oriental, and 15% reconstituted tobacco and research cigarettes are designed to mimic this American blend.²¹ The 3R4F cigarette contains 21% burley, 35% bright, 12% oriental, and 29% reconstituted tobacco while the 1R6F cigarette contains 24% burley, 34% bright, 12% oriental, 20% reconstituted tobacco, 7% expanded bright, and 3% expanded burley. Expanded tobacco is commonly used in the manufacture of cigarettes to increase tobacco filling capacity. All

commercial brands have an undisclosed proportion of expanded tobacco in the overall blend. Tobacco blend influences delivery of nicotine, carbon monoxide, carbonyls, polycyclic aromatic hydrocarbons, and tobacco-specific nitrosamines.^{19–27} Carbon-centered radicals in gas-phase of smoke were found to be highest in oriental > bright > burley.³² Here we report for the first time that tobacco blends have a significant impact on overall free radical production. Gas-phase radical delivery per cigarette varied 7.5-fold, while particulate-phase radical delivery varied 1.5-fold between the tobacco types. The highest levels of gas-phase radicals were observed for burley and reconstituted tobaccos, even though the number of puffs taken by these cigarettes was significantly fewer than for oriental and bright cigarettes. Burley, oriental, and bright tobacco have similar combustion temperatures,⁴⁰ again suggesting that temperatures above a certain threshold do not impact free radical generation. Overall, these results support previous work that tobacco variety is a modifiable cigarette design feature that can be manipulated to minimize toxicant exposure.

The present results indicate the importance of cigarette design features in its delivery of free radicals. Also, many nontobacco additives are added to cigarettes such as menthol, but we have previously shown that menthol did not appear to affect free radical yields.⁵ However, there are many other potential design features that will need to be studied in this regard, including rod length and circumference, filter material and length, paper porosity, tobacco weight, tobacco cut-width and packing density, and other cigarette additives. Also, it is likely that agricultural practices, stalk position and curing methods impact free radical generation as indicated by others.^{7,32} We are currently investigating if, along with total radical concentration, individual radical species might differ by smoking regimen or tobacco blend. There are also other major hurdles for cigarette smoke free radical research that have yet to be crossed. For example, it is not known if the dose delivered to the lung or other target site is similar to the levels measured directly from the cigarette, particularly given the highly reactive nature of the gas-phase radicals. Further, the specific nature of the biological harm from particulate-phase and gas-phase–phase radical species still remains unclear. Despite this, the importance of tobacco-derived free radicals and oxidative stress is well-accepted as a mechanism for disease development.

Drastic changes have occurred over the years in smoking behaviors and tobacco composition that have increased the incidence of certain cancers.³ Our findings offer a new way to evaluate free radical exposure of smokers and set a framework for free radical evaluation from modified risk tobacco products such as heat-not-burn cigarettes. Smoking behavior and tobacco blend impact free radical levels and thus could be targets for development of regulatory strategies and risk assessment models. Public education efforts are warranted to make smokers aware that individual exposure depends not only on how many, but also on how the product is smoked.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We would like to thank the Centers for Disease Control and Prevention, Atlanta, USA for providing us the single tobacco blend cigarettes.

Funding

This work was supported in part by the National Institute on Drug Abuse of the National Institutes of Health and the Center for Tobacco Products of the U.S. Food and Drug Administration (under Award No. P50-DA-036107). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Food and Drug Administration.

ABBREVIATIONS

CFP	Cambridge filter pad
EPR	electron paramagnetic resonance spectroscopy
ISO	International Organization of Standardization
CI	Canada Intense

REFERENCES

- (1). Pryor WA (1997) Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ. Health Perspect* 105 (Suppl 4), 875–882. [PubMed: 9255574]
- (2). Church DF, and Pryor WA (1985) Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ. Health Perspect* 64, 111–126. [PubMed: 3007083]
- (3). U.S. Department of Health and Human Services. (2010) How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease, pp 13–22, Centers for Disease Control and Prevention, WA.
- (4). Fearon IM, Phillips G, Carr T, Taylor M, Breheny D, and Faux SP (2011) The Role of Oxidative Stress in Smoking-Related Diseases. *Mini-Rev. Org. Chem.* 8, 360–371.
- (5). Goel R, Bitzer Z, Reilly SM, Trushin N, Foulds J, Muscat J, Liao J, Elias RJ, and Richie JP Jr (2017) Variation in Free Radical Yields from U.S. Marketed Cigarettes. *Chem. Res. Toxicol.* 30, 1038–1045. [PubMed: 28269983]
- (6). Stone K, Bermudez E, Zang LY, Carter KM, Queenan KE, and Pryor WA (1995) The ESR properties, DNA nicking, and DNA association of aged solutions of catechol versus aqueous extracts of tar from cigarette smoke. *Arch. Biochem. Biophys.* 319, 196–203. [PubMed: 7771784]
- (7). Dellinger B, Khachatryan L, Masko S, and Lomnicki S (2011) Free Radicals in Tobacco Smoke. *Mini-Rev. Org. Chem.* 8, 427–433.
- (8). Baker RR, and Lewis LS (2001) A Review of the Incidence and Consequences of Cigarette Filter Vent Blocking Among Smokers. *Beitr Tabakforsch* 19, 209–228.
- (9). Gregg E, Bachmann T, Bito R, Cahours X, McEwan M, Nelson P, Prasad K, Scherer G, and Stiles M (2013) Assessing Smoking Behaviour and Tobacco Smoke Exposure: Definitions and Methods. *Beitr Tabakforsch* 25, 685–699.
- (10). Zacny JP, Stitzer ML, Brown FJ, Yingling JE, and Griffiths RR (1987) Human cigarette smoking: effects of puff and inhalation parameters on smoke exposure. *J. Pharmacol Exp Ther* 240, 554–564. [PubMed: 3806411]
- (11). Bridges RB, Combs JG, Humble JW, Turbek JA, Rehm SR, and Haley NJ (1990) Puffing topography as a determinant of smoke exposure. *Pharmacol., Biochem. Behav.* 37, 29–39. [PubMed: 2263664]

- (12). Reilly SM, Goel R, Bitzer Z, Elias RJ, Foulds J, Muscat J, and Richie JP Jr (2017) Effects of Topography-Related Puff Parameters on Carbonyl Delivery in Mainstream Cigarette Smoke. *Chem. Res. Toxicol.* 30, 1463–1469. [PubMed: 28648066]
- (13). Hammond D, Fong GT, Cummings KM, O'Connor RJ, Giovino GA, and McNeill A (2006) Cigarette yields and human exposure: a comparison of alternative testing regimens. *Cancer Epidemiol., Biomarkers Prev.* 15, 1495–1501. [PubMed: 16896039]
- (14). Marian C, O'Connor RJ, Djordjevic MV, Rees VW, Hatsukami DK, and Shields PG (2009) Reconciling human smoking behavior and machine smoking patterns: implications for understanding smoking behavior and the impact on laboratory studies. *Cancer Epidemiol., Biomarkers Prev.* 18, 3305–3320. [PubMed: 19959678]
- (15). U.S. Food & Drug Administration. (2016) How a Cigarette Is Engineered, U.S. FDA <https://www.fda.gov/TobaccoProducts/Labeling/ProductsIngredientsComponents/ucm527462.htm> (accessed November 10, 2017).
- (16). Holbrook JH (1981) The changing cigarette. *Western journal of medicine* 134, 353. [PubMed: 7245738]
- (17). Thielen A, Klus H, and Muller L (2008) Tobacco smoke: unraveling a controversial subject. *Exp. Toxicol. Pathol.* 60, 141–156. [PubMed: 18485684]
- (18). Leffingwell J (1999) Leaf Chemistry - Basic Chemical Constituents of Tobacco Leaf and Differences among Tobacco Types, Leffingwell and Associates.
- (19). Hoffmann D, Djordjevic MV, and Brunnemann KD (1995) Changes in Cigarette Design and Composition Over Time and How They Influence the Yields of Smoke Constituents. *NCI smoking and tobacco control monograph* 7, 15–37.
- (20). Lawler TS, Stanfill SB, DeCastro RB, Lisko JG, Duncan BW, Richter P, and Watson CH (2017) Surveillance of Nicotine and pH in Cigarette and Cigar Filler. *Tob Regul Sci.* 3, 101–116. [PubMed: 28989950]
- (21). Hoffmann D, Djordjevic MV, and Hoffmann I (1997) The changing cigarette. *Prev. Med.* 26, 427–434. [PubMed: 9245661]
- (22). Bombick DW, Putnam K, and Doolittle DJ (1998) Comparative cytotoxicity studies of smoke condensates from different types of cigarettes and tobaccos. *Toxicol. In Vitro* 12, 241–249. [PubMed: 20654406]
- (23). Ding YS, Zhang L, Jain RB, Jain N, Wang RY, Ashley DL, and Watson CH (2008) Levels of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. *Cancer Epidemiol., Biomarkers Prev.* 17, 3366–3371. [PubMed: 19064552]
- (24). Piade JJ, Wajrock S, Jaccard G, and Janeke G (2013) Formation of mainstream cigarette smoke constituents prioritized by the World Health Organization-yield patterns observed in market surveys, clustering and inverse correlations. *Food Chem. Toxicol.* 55, 329–347. [PubMed: 23357567]
- (25). Adam T, Mitschke S, Streibel T, Baker RR, and Zimmermann R (2006) Puff-by-puff resolved characterisation of cigarette mainstream smoke by single photon ionisation (SPI)-time-of-flight mass spectrometry (TOFMS): comparison of the 2R4F research cigarette and pure Burley, Virginia, Oriental and Maryland tobacco cigarettes. *Anal. Chim. Acta* 572, 219–229. [PubMed: 17723481]
- (26). Djulancic N, Radojicic V, and Sbrinowska M (2013) The influence of tobacco blend composition on carbon monoxide formation in mainstream cigarette smoke. *Arh Hig Rada Toksikol* 64, 107–113. [PubMed: 23612614]
- (27). Ding YS, Yan X, Wong J, Chan M, and Watson CH (2016) Situ Derivatization and Quantification of Seven Carbonyls in Cigarette Mainstream Smoke. *Chem. Res. Toxicol.* 29, 125–131. [PubMed: 26700249]
- (28). Hecht SS (2012) Research opportunities related to establishing standards for tobacco products under the Family Smoking Prevention and Tobacco Control Act. *Nicotine Tob. Res.* 14, 18–28. [PubMed: 21324834]

- (29). University of Kentucky. Center for Tobacco Reference Product. (2016) 1R6F Certified Reference Cigarette, University of Kentucky https://ctrp.uky.edu/resources/pdf/webdocs/CoA_1R6F.pdf (accessed March 20, 2017).
- (30). International Organization for Standardization. (2012) ISO 3308:2012. Routine Analytical Cigarette-Smoking Machine- Definitions and Standard Conditions, ISO, London.
- (31). Tobacco Control Programme. (1999) Determination of “Tar”, Nicotine, and Carbon Monoxide in Mainstream Tobacco Smoke. Official Publication T-155, Tobacco Control Programme.
- (32). Bartalis J, Zhao YL, Flora JW, Paine JB, and Wooten JB (2009) Carbon-centered radicals in cigarette smoke: acyl and alkylaminocarbonyl radicals. *Anal. Chem.* 81, 631–641. [PubMed: 19093757]
- (33). Wright C (2015) Standardized methods for the regulation of cigarette-smoke constituents. *TrAC, Trends Anal. Chem.* 66, 118–127.
- (34). WHO. (2015) Report on the scientific basis of tobacco product regulations: Fifth report of a WHO study group, World Health Organ Tech Rep, 1–234, WHO.
- (35). Baum SL, Anderson IGM, Baker RR, Murphy DM, and Rowlands CC (2003) Electron spin resonance and spin trap investigation of free radicals in cigarette smoke: development of a quantification procedure. *Anal. Chim. Acta* 481, 1–13.
- (36). Pryor WA, Prier DG, and Church DF (1983) Electron-spin resonance study of mainstream and sidestream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. *Environ. health perspect* 47, 345–355. [PubMed: 6297881]
- (37). Schlotzhauer SW, and Chortyk OT (1983) Effects of varied smoking machine parameters on deliveries of total particulate matter and selected smoke constituents from an ultra low-tar cigarette. *J. Anal. Toxicol* 7, 92–95. [PubMed: 6855211]
- (38). Purkis SW, Troude V, and Hill CA (2013) Effect of puffing intensity on cigarette smoke yields. *Regul. Toxicol. Pharmacol.* 66, 72–82. [PubMed: 23523712]
- (39). Polzin GM, Wu W, Yan X, McCraw JM, Abdul-Salaam S, Tavakoli AD, Zhang L, Ashley DL, and Watson CH (2009) Estimating smokers’ mouth-level exposure to select mainstream smoke constituents from discarded cigarette filter butts. *Nicotine Tob. Res.* 11, 868–874. [PubMed: 19541951]
- (40). Lendvay AT, and Laszlo TS (1974) Cigarette Peak Coal Temperature Measurements. *Beitr Tabakforsch* 7, 276.

Figure 1.

Graphical representation of changes made in individual puff parameters from the ISO smoking regimen to modify the peak flow rate by changing puff shape (bell changes to square or sharp peak shapes), the puff duration (2 s changes to 1, 3, or 5 s), or the puff volume (35 mL changes to 17.5 mL, 55 mL, or 75 mL).

Figure 2.

Comparison of gas- and particulate-phase free radical yields per cigarette under ISO and CI standardized smoking regimens. ISO = Machine smoked with 35 mL puff volume, 60 s puff interval, 2 s puff duration, filter vents open; CI = machine smoked with 55 mL puff volume, 30 s puff interval, 2 s puff duration, filter vents 100% blocked. Values are mean $\pm$ standard deviation ($n = 3-4$). * indicates $p < 0.05$ compared to the ISO method for the same brand.

Figure 3.

Effect of smoking puff parameters on gas- and particulate-phase free radical yields per cigarette. Puff parameters were individually modified from the ISO method. Puff volume was kept constant under conditions of different puff durations by compensatory changes in peak puff flow (intensity). Values are mean $\pm$ standard deviation ($n = 2-5$). * indicates $p < 0.05$ for both 3R4F and 1R6F compared to the ISO puff parameters (35 mL puff volume, 2 s puff, and 60 s puff interval).

Figure 4.

Delivery of gas- and particulate-phase radicals are linearly dependent on puff volume. Puff volume was independently modified from the standard ISO method by altering peak flow rate (puff intensity). Values are mean $\pm$ standard deviation ($n = 3$).

Figure 5. Free radical yields per cigarette from unfiltered single-blend tobacco cigarettes. Cigarettes were machine smoked with the following conditions: 55 mL puff volume, 30 s puff interval, and 2 s puff duration. Bars are mean $\pm$ standard deviation ($n = 3$).