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Brand Variation in Oxidant Production in Mainstream Cigarette Smoke: Carbonyls and Free Radicals

Samantha M. Reilly[†], **Reema Goel**[†], **Neil Trushin**[†], **Ryan J. Elias**[‡], **Jonathan Foulds**[†], **Joshua Muscat**[†], **Jason Liao**[†], 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, PA

[‡]Department of Food Science, Pennsylvania State University, College of Agricultural Sciences, University Park, PA

Abstract

Oxidative stress/damage resulting from exposure to cigarette smoke plays a critical role in the development of tobacco-caused diseases. Carbonyls and free radicals are two major classes of oxidants in tobacco smoke. There is little information on the combined delivery of these oxidants across different cigarette brands; thus, we set out to measure and compare their levels in mainstream smoke from popular US cigarettes. Mainstream smoke from 28 different cigarette brands produced by smoking (FTC protocol) was analyzed for five important, abundant carbonyls, and levels were compared to previously determined free radical for the same brands. Overall, there were large variations (3- to 6-fold) in carbonyl levels across brands with total carbonyl levels ranging from 275 to 804 µg/cigarette, which persisted even after adjusting for ventilation. Individual carbonyl levels were highly correlated with each other (r^2: 0.40–0.95, $P<0.003$) except for formaldehyde. Both gas-phase (r^2: 0.37, $P=0.006$) and particulate-phase (r^2: 0.27, $P=0.005$) free radicals were correlated to total carbonyl content; however, this correlation disappeared after adjusting for ventilation. These data show that overall oxidant production varies widely by cigarette brand and the resulting difference in oxidant burden could potentially lead to differences in disease risk.

Graphical Abstract

[*]Corresponding Author. Department of Public Health Sciences, Pennsylvania State University Tobacco Center of Regulatory Science (TCORS), Pennsylvania State University College of Medicine, 500 University Dr. – Mail Code: CH69, Hershey, PA, USA 17033. Phone: 717-531-5381; jrichie@psu.edu.

Author Contributions
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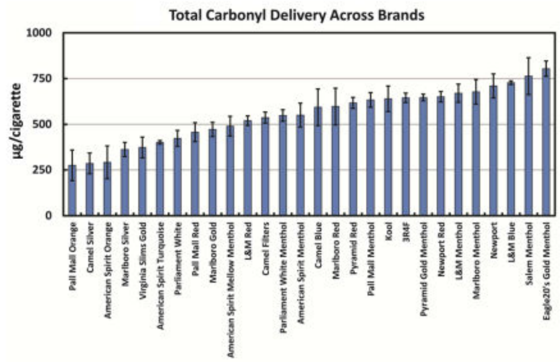
Supporting Information. Brand details and a representative chromatogram are available in the Supporting Information.



Keywords

Acetaldehyde; formaldehyde; crotonaldehyde; methyl ethyl ketone; propionaldehyde; oxidants; tobacco smoke

1. 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 (Danaei et al., 2009; U.S. Department of Health and Human Services, 2014; Xu et al., 2015). It leads to lung cancer (Doll and Hill, 1950; Wynder and Graham, 1950), chronic obstructive pulmonary disease (COPD) (Burney et al., 2014; Zaher et al., 2004), cardiovascular and many other diseases (Krupski, 1991; U.S. Department of Health and Human Services, 2014). Oxidative stress is thought to play a major role in the development of many of these tobacco-caused diseases (Church and Pryor, 1985; Pryor, 1997); thus, it is important to better understand the total oxidant burden resulting from cigarette smoke in order to gain a better assessment of a smoker's potential oxidative risk.

While carbonyls are most known for their toxic and carcinogenic effects (U.S. Food and Drug Administration, 2012), carbonyls are also a major class of powerful oxidants, depleting glutathione (Wooten et al., 2006), forming adducts with DNA bases (Halliwell and Gutteridge, 2015), and producing free radicals when metabolized (Kundu et al., 2007). Together, these effects have linked carbonyls to a significant number of smoking-related diseases (U.S. Food and Drug Administration, 2012). Previous reports on carbonyls in cigarette smoke show their levels vary by brand (Bodnar et al., 2012; Counts et al., 2004; Counts et al., 2005; Ding et al., 2015; Hammond and O'Conner, 2008; Marcilla et al., 2012; Roemer et al., 2003). Potential sources of variation between cigarette brands include differences in ventilation, tobacco type, filter design, additives, and paper porosity as well as a smoker's unique puff profile (Baker et al., 2004a, b; Chen et al., 2014; Dittrich et al., 2014; Hoffmann et al., 1995; Roemer et al., 2012; Seeman et al., 2003). While carbonyl production is known to differ by brand, little is known as to how this variation may compare to the other major class of oxidants, free radicals. Thus, we sought to determine the variation in the levels of five important and abundant tobacco smoke carbonyls (formaldehyde, acetaldehyde, propionaldehyde, crotonaldehyde, and methyl ethyl ketone (MEK)) and compare their levels to those of free radicals. Since both classes of oxidants are produced by the incomplete combustion and pyrolysis of the tobacco in the cigarette (U.S. Department of Health and Human Services, 2014), we hypothesize that these levels will vary similarly amongst brand/type. Altogether, this information can be used to better understand the total oxidant burden of cigarette smoke. To this end, we recently developed standardized methodology to analyze both the highly reactive gas-phase free radical content and the stable particulate-phase free radical content of cigarette smoke and applied the method to quantify the free radical levels in 27 brands of popular US cigarettes for the first time (Goel et al., 2017). In the current paper, we report on the levels of carbonyls in the same 27 cigarette brands smoked under identical conditions (FTC protocol) and use these results to determine the relationship of these two oxidant classes across cigarette brands.

2. Materials and Methods

2.1 Cigarettes

Twenty-seven cigarette brands (all king-sized except for Virginia Slims Gold) were purchased from local retailers, chosen for their popularity in the local area (Dauphin and Lebanon Counties, PA) based on self-report from the retailers and their combined share of the US cigarette market (these brands represent approximately 70% of the US market) (Sharma et al., 2015). The 3R4F research cigarette was obtained from the University of Kentucky (Lexington, Kentucky, USA) and was used as a reference cigarette. The cigarettes were stored at −20°C in airtight plastic bags.

2.2 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, 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) and recrystallized before use to remove water, which can affect the reactivity of DNPH with carbonyls (Risner and Martin, 1994). Recrystallization was done by dissolving 17 g DNPH in 350 mL acetonitrile, heating this solution at 70°C for 60 min., and then cooling to room temperature. The crystals were collected via vacuum filtration, and then stored in a desiccator.

2.3 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, 22±1°C), for at least 48 h before smoking. Mainstream smoke was generated by a 30-port smoking machine (Jaeger-Baumgartner, CSM JB2080). Five cigarettes were smoked simultaneously on the machine under a FTC smoking protocol: 35 mL puff volume, 2 s puff duration, and 60 s puff interval. We tested for breakthrough, which is when any carbonyl passes through the capturing solution and thus is not captured, by adding a second impinger in line. Breakthrough was minimal (~2%) for all carbonyls when testing between one and five cigarettes on the smoking machine, but did become an issue when testing ten or more cigarettes. Thus, we limited our study to five or less cigarettes per collection.

2.4 Derivatization of Carbonyls

DNPH solution was made as described previously (Risner and Martin, 1994) 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 five cigarettes of each brand as described above was passed directly into an impinger containing 10 mL of DNPH solution placed after the pump. The sample was then transferred into a 20 mL scintillation vial and stored at 4°C until HPLC-UV analysis, which was performed within 5 days of collection. We performed a minimum of two replicates of each collection (n = 2–4 for all experiments). Day to day assay variation was low (Coefficient of variation (CV) = 5%) for total carbonyls in 3R4F cigarettes (Table 1). When the impinger was moved upstream of the pump, yields were increased for acetaldehyde only (15%; p-value: 0.04); however, it was difficult to maintain a consistent flow rate in this configuration, so it was not used for further experiments.

2.5 HPLC-UV Analysis

High performance liquid chromatography with ultraviolet detection (HPLC-UV) analyses were performed using a binary system consisting of two Waters (Milford, MA, USA) 510 pumps, a Waters 440 UV absorbance detector, and a Hitachi (Tokyo, Japan) D-2500 Integrator. Compounds were separated using a Bondclone C18 column (10 μm × 300 mm × 3.9 mm; Phenomenex, Torrance, CA, USA) using two mobile phases: water (A) and acetonitrile (B). The elution gradient parameters are: 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 is 1.0 mL/min. for all time steps, and the detection wavelength was 254 nm. All sample injections were 10 μL and were manually injected. All measurements were carried out at room temperature (22±1°C). Supplementary Figure 1 is a representative chromatogram. This method was found to have good precision (CV: 6–12%) for all carbonyls tested, which was determined by 12 replicate injections of a collected cigarette sample. Accuracy was determined through spiking a sample with known amounts of carbonyl standards and found to have a CV of 4%.

2.6 GC-MS Analysis

All peaks, except crotonaldehyde, were verified by collecting the eluting peaks from the HPLC-UV and analyzing by GC-MS. To do this, an Agilent Technologies 7890A gas chromatograph (Agilent Technolgies, Inc., Santa Clara, CA) fitted with a Gerstel MPS2 autosampler (Gerstel GMbH & Co. KG. Mülheim an der Ruhr, Germany) was used in combination with an Agilent 5975C mass selective detector. The instrument and autosampler were controlled by Agilent MassHunter GCMS Acquisition software (Version B.07.00 SP2.1654) and Gerstel Maestro 1 software (Version1.2.20.41), respectively. The GC was fitted with a J&W VF-35ms capillary column with dimensions 60 m × 0.25 mm × 0.25 μm. The inlet was fitted with a borosilicate glass single taper inlet liner with wool (6.5 mm o.d., 4.0 mm i.d., 78.5 mm long) (Restek, Bellefonte, PA). Helium (ultra-high purity) was used as the carrier gas at a constant flow rate of 1.4 mL/min. Depending upon analyte concentration within the sample, between one and three microliters of sample were introduced to an injector operated in split-less mode and maintained at a temperature of 270°C. The splitter was opened to 71:1 after 60 s. The GC oven was first held at 50°C for 2.5 minutes, then increased to 200°C at a rate of 15°C/min, and finally increased to 300°C at a rate of 5°C/min. The MS transfer line was held at 280°C. The mass spectrometer was operated in full scan mode across an ion range of m/z 35 to 350. Data analysis was performed using the Agilent MSD Enhanced ChemStation software package (Version F.01.00.1903).

2.7 Statistics

Summary statistics and boxplots are presented for the major variables of carbonyls. The associations between carbonyls and free radicals are estimated and tested using regression analyses using SAS software Version 9.4 of the SAS System for Windows x64 Systems (SAS Institute Inc., Cary, NC, USA). Linear regression was used to determine the significance of ventilation and flavor group effects and performed using R 3.3. (R Core Team, 2016) Ventilation and TPM values were those found by Goel et al. (Goel et al., 2017).

3. Results

3.1 Cigarettes

The brand information is given in Supplementary Table 1. All twenty-seven cigarette brands, the research cigarette, and their corresponding carbonyl delivery per cigarette and their respective flavors are listed in Table 1. All cigarettes were 80–85 mm, except for Virginia Slims (100 mm). Flavor refers to if the cigarettes were labelled as menthol.

3.2 Carbonyl Levels and Variation

Carbonyl levels were determined by HPLC-UV, and peak-compound assignments were matched to standards by retention times and later verified by GC-MS. Precision of peak areas were found to be between 6 – 12% for all compounds. Acrolein co-eluted with acetone on the HPLC-UV, thus individual levels could not be determined. Based on the five that were readily quantified, acetaldehyde was the most abundant and least variable, accounting for, on average, 72 ± 3% (CV =5%) of total carbonyls across all brands. This was followed by MEK at 11 ± 1% (CV = 8%) and propionaldehyde at 7 ± 1% (CV = 7%). Crotonaldehyde was the least abundant, but second most variable at 3±1% (CV = 33%). The most variation was observed with formaldehyde levels, which were approximately 7 ± 4% (CV = 54%) of total carbonyls.

There is a large variation in carbonyl production across brands (Table 1). For individual carbonyls, levels ranged between 3- and 6-fold between brands with the greatest variation observed for formaldehyde (6-fold) and crotonaldehyde (6-fold) and least variation observed for MEK (3-fold) and propionaldehyde (3-fold). Total carbonyl levels ranged three-fold (275 ± 84 μg/cigarette for Pall Mall Orange to 804 ± 42 μg/cigarette for Eagle20's Gold Menthol).

3.3 Correlations between Carbonyls

To examine for potential associations between carbonyls, coefficients of determination (r^2) were calculated (Table 2). Acetaldehyde, propionaldehyde, and MEK were highly correlated with one another (r^2: 0.90–0.95, P<0.001). Correlations, albeit weaker, were also observed between each of these carbonyls and crotonaldehyde (r^2: 0.36–0.51, P<0.01). Formaldehyde was the sole carbonyl that was not correlated with the rest, which could be predicted by the high variation we observed for formaldehyde.

Interestingly, the brand with the highest delivery differed for each individual carbonyl (formaldehyde [Camel Silver], acetaldehyde [Eagle20's Gold Menthol], propionaldehyde [L&M Blue], crotonaldehyde [Marlboro Red], and MEK [Salem]) while all but one of the lowest values were from the same brand (Camel Silver). However, Camel Silver is not the lowest in total carbonyls despite being lowest in four of the five individual carbonyls as it produced the highest value for formaldehyde, stressing the importance of monitoring all carbonyls individually.

3.4 Ventilation, TPM, and Menthol

We conducted regressions to determine if levels of carbonyls were associated with ventilation and TPM (using ventilation and TPM data from Goel et al., 2017). We did find significant correlations between carbonyls and TPM, with the exception of formaldehyde (r^2: 0.22–0.44; $P \le 0.01$; Table 2; Figure 2). We find that ventilation has a significant negative relationship with all carbonyl deliveries, except for formaldehyde (r^2: 0.41–0.59; $P \le 0.002$; Table 2). In addition, TPM and ventilation also have a significant negative correlation with each other (r^2: 0.56; $P < 0.001$).

We find, on a per cigarette basis, menthol cigarettes tended to produce significantly higher levels for all carbonyls than non-menthol brands (Figure 1). Menthol brands themselves only varied 2-fold for each carbonyl and total carbonyls compared to 3- to 6-fold among non-menthol brands and all brands together. After adjustment for ventilation, acetaldehyde, propionaldehyde, MEK, and total carbonyls remain significant ($P \le 0.05$), suggesting that while our menthol brands are higher from containing less ventilation overall, it is not the sole contribution affecting the differences in carbonyl delivery.

3.5 Correlation with Free Radicals

We performed a correlational analysis with each carbonyl along with total carbonyls and both gas-phase and particulate-phase free radicals (Table 2; (Goel et al., 2017). Significant rank correlations were observed between all carbonyls, except formaldehyde, and gas-phase free radicals. For particulate-phase free radicals significant correlations were only observed with acetaldehyde and propionaldehyde. When total carbonyls were considered, significant correlations were observed for both gas-phase (r^2: 0.37; P: 0.006) and particulate-phase (r^2: 0.27; P: 0.005) free radicals (Figure 2). After adjusting for ventilation, which was found to be significantly correlated with free radicals and carbonyls, we find that acetaldehyde and propionaldehyde remain significantly but weakly correlated with gas-phase free radicals (r^2: 0.06; $P \le 0.048$). In addition, no carbonyls were significantly correlated with tar-phase radicals, suggesting most of the effects were driven by differences in ventilation (Table 3).

4. Discussion

Here we observe the levels of five major carbonyls in mainstream smoke from 27 popular brands of cigarettes. We observe a wide variation in levels of carbonyls examined. The overall variation in levels across brands remains quite large even after adjusting for ventilation, which accounts for 40 to 60% of the variation depending on the carbonyl. From comparing these results with those for free radicals (Goel et al., 2017), we find that carbonyl levels are significantly correlated with the levels of free radicals, the other major class of oxidants found in cigarette smoke, across cigarette brands.

Among all the carbonyls measured, acetaldehyde was the most abundant and varied 4-fold across brands, similar to differences previously observed between other US cigarette brands and types (Counts et al., 2005; Pazo et al., 2016; Roemer et al., 2003). Crotonaldehyde, propionaldehyde, and MEK also had significant variation across brands (3–6-fold), also similar to previous reports (Counts et al., 2005; Pazo et al., 2016; Roemer et al., 2003). Carbonyl levels were highly correlated with one another (except formaldehyde), suggesting that their production during pyrolysis may involve similar factors which may differ across brands based on differences in cigarette design and/or tobacco blends. Thus, smokers who smoke certain products may be exposed to higher levels of carbonyls and, thus, may have a greater negative health impact especially when these doses are extrapolated over a lifetime of smoking. Acrolein, another important carbonyl which is thought to play an important role in several smoking-induced illnesses, was not measured due to its co-elution with acetone on the HPLC., However, acrolein levels in tobacco smoke have previously been shown to be highly correlated with the other major carbonyls (except formaldehyde) across tobacco blends (Ding et al., 2015).

In comparison to the other carbonyls, formaldehyde also varied greatly in levels across brands (6-fold), but they did not correlate with the levels of other carbonyls. The range of formaldehyde levels across brands was similar to that previously observed (Counts et al., 2005; Roemer et al., 2003). While it is not known why formaldehyde differs from the other carbonyls, it is possible much of the formaldehyde in tobacco smoke may result from formaldehyde present in the tobacco leaf itself and not simply as a product of combustion as are the other carbonyls. To further support this, Ding et al. (Ding et al., 2015) reported that formaldehyde in mainstream smoke differs greatly (4-fold) between bright and burley tobacco, which are the main two blends in U.S. cigarettes (Hoffmann et al., 2001). Thus, altering the amounts of bright and burley slightly between cigarettes could cause great differences in formaldehyde produced. In addition, formaldehyde is near equally split between the particulate phase (39%) and gas phase (61%) of cigarette smoke while the other four are almost entirely in the gas phase (85–99%) (Pang and Lewis, 2011). Overall, these findings demonstrate the need to consider formaldehyde as distinctly different from other carbonyls in terms of production in cigarette smoke.

In our studies, the lowest levels for almost all carbonyls were consistently observed for American Spirit sub-brands. While this is consistent with the free radical findings, it does contrast with a previous study (Pazo et al., 2016) that found an American Spirit sub-brand to be one of their highest. However, the single sub-brand used in that study was not in our study (American Spirit Blue; personal correspondence), thus the difference might arise from that particular sub-brand. When we compare our results to those observed by Pazo et al. (Pazo et al., 2016) for the sub-brands that are the same, our levels are similar for most carbonyls; however, the levels of acetaldehyde are less than they observed (e.g., 3R4F: 610±170 vs. 489±20 ug acetaldehyde/cig.). The difference likely arises from the different methods used for analysis (gas bag sampling method vs. impinger-derivatization method) as the values reported by CORESTA, which also uses an impinger-derivatization method, also report lower values, more similar to what we report (3R4F: 538±28 ug acetaldehyde/cig.) (CORESTA, 2014).

As with the individual carbonyls, total carbonyls varied greatly across brands (3-fold; Table 1). While the nature of this variation is unknown, it could result from a variety of design differences across brands including tobacco blend, ventilation, and flavor additives. Tobacco blend is intrinsically tied to carbonyl yields, has been previously shown with different types of tobacco yielding dissimilar amounts of carbonyls (Ding et al., 2015). Filter ventilation differs amongst cigarettes and allows for differences in combustion temperature and dilution of the delivered smoke (Kozlowski et al., 1998; O'Connor et al., 2008). We found a negative correlation between ventilation and carbonyl delivery (r^2: 0.41–0.60), consistent with previous findings where carbonyl delivery was increased by blocking ventilation (Pazo et al., 2016).

We found higher levels for all carbonyls in menthol compared to non-menthol brands (Figure 1). A previous paper reported a trend toward higher carbonyl values for menthol cigarettes; however, these differences were not significant (Bodnar et al., 2012). Menthol cigarettes differ from non-menthol brands in numerous ways including a higher menthol content (Celebucki et al., 2005) and lower level of ventilation (Pazo et al., 2016). While the majority of menthol brands tested were not highly ventilated, most carbonyls remained significantly correlated after adjusting for ventilation. Thus, it remains possible that in addition to ventilation, menthol flavoring itself may be playing a role. Indeed, the potential impact of menthol as a risk factor for tobacco-related illnesses remains under debate (Heck, 2010; Hoffman, 2011; Hoffman and Simmons, 2011; Salgado and Glantz, 2011; Werley et al., 2007).

Results also showed that TPM was correlated with most carbonyl levels, helping to explain ~50% of the variation observed between brands (Table 2). TPM, which consists of tar and water formed during combustion, contains many harmful compounds as well, such as polyaromatic hydrocarbons and tobacco-specific nitrosamines. The one exception was formaldehyde, which was not correlated with TPM, further suggesting its levels are affected differently than the other carbonyls. Since levels of TPM are generally impacted by combustion, this association supports shared mechanisms for the formation of TPM constituents and carbonyls tied to the pyrolysis of the plant material. TPM can also be influenced by ventilation as noted by the strong correlation we observe between the two, suggesting that the differences in TPM should be, at least partially, corrected for by correcting for ventilation.

Our study establishes, for the first time, that total carbonyls are significantly correlated with free radicals by comparing our carbonyl levels to Goel et al.'s data on free radicals in the same 27 brands smoked under identical experimental conditions. This is important as these are the two major classes of oxidants produced in cigarette smoke from the process of pyrolysis and incomplete combustion while also being considered the source of most oxidative damage in smokers. By understanding how these levels relate across brands, we can gain insight into the potential total oxidant harm to which a smoker is exposed from the pyrolysis and incomplete combustion of tobacco.

The gas-phase radicals correlated with each carbonyl, except formaldehyde, perhaps reflecting a common mechanism of formation involving combustion. We further examined this correlation through adjusting for ventilation, finding that only weak, if any, correlations remain (Table 3); this strongly suggests that ventilation is the main common factor that these two oxidants are affected by. Ventilation, however, does not explain the wide variation in the brands in terms of oxidants as it accounts for ~55% of the variation for carbonyls and only 30% of the variation in free radicals levels. Knowing the relationship of these oxidant classes and the main cigarette design feature that affects it will help us better estimate the relative harm of these products.

On the other hand, the particulate-phase radicals show a moderate correlation with carbonyls, observing significant correlations with three of the five carbonyls: acetaldehyde, propionaldehyde, and MEK (Table 2), and these correlations disappear when adjusting for ventilation (Table 3). This lack of relationship for some carbonyls could be a result of the low overall variation in particulate-phase radicals compared to gas phase radicals, changing only 2-fold over all the brands compared to the 12-fold variation in gas phase radicals. Using the present results, a better prediction of the total oxidant exposure from pyrolysis to a smoker of different brands can be made. The strong correlation between these different classes of oxidants suggests that there is wide brand-to-brand variation in overall oxidant delivery which may be associated with potential difference in harm.

Overall, this study helps underline the importance of understanding and acknowledging the variation in delivery of oxidants between cigarette brands. We display clearly that carbonyls and free radicals do share a positively correlated relationship, suggesting that reductions in total oxidant delivery may be possible. This is important as it has been proposed previously (Burns et al., 2007) that limitations be placed on the amounts of toxicants a cigarette can produce, which the FDA can impose as a part of a total harm reduction strategy. Here we provide new information on the relative oxidant levels produced by commercially available cigarettes, which can help guide potential future regulatory strategies. More information is needed as some brands might be low in oxidants, yet be high in other dangerous toxicants. We also do not include other oxidants produced from combustion, such as polyaromatic hydrocarbons, or that are present in the tobacco itself, such as metals. Finally, we acknowledge puff topography factors, such as puff volume and duration, can have a direct impact on the delivery of smoke constituents. While the ISO protocol used in this study is more representative of light smokers, topography profiles for a majority of smokers fall along a continuum between ISO and more intense protocols such as the Health Canada Intense (HCI) method. Thus, differences in carbonyl and free radical delivery under conditions of different puffing topography will need to be examined in future investigations. We do not fully understand all the factors contributing to brand variation in oxidant production, and so further research is required to guide potential future regulatory recommendations. However, the magnitude of the brand variations found here suggests that such research may eventually help identify ways to reduce the oxidant delivery of cigarettes and so reduce smoking-caused disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

TPM	total particulate matter
CV	coefficient of variation
ρ	Spearman's rank correlational coefficient

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Highlights

- Carbonyls and free radicals are two of the major classes of oxidants in tobacco smoke.
- Carbonyls varied widely (3-6-fold) across cigarette brands with ventilation accounting for only half of this variation.
- Carbonyls are correlated with gas- and particulate-phase free radicals.
- However, these correlations are eliminated when adjusting for ventilation.

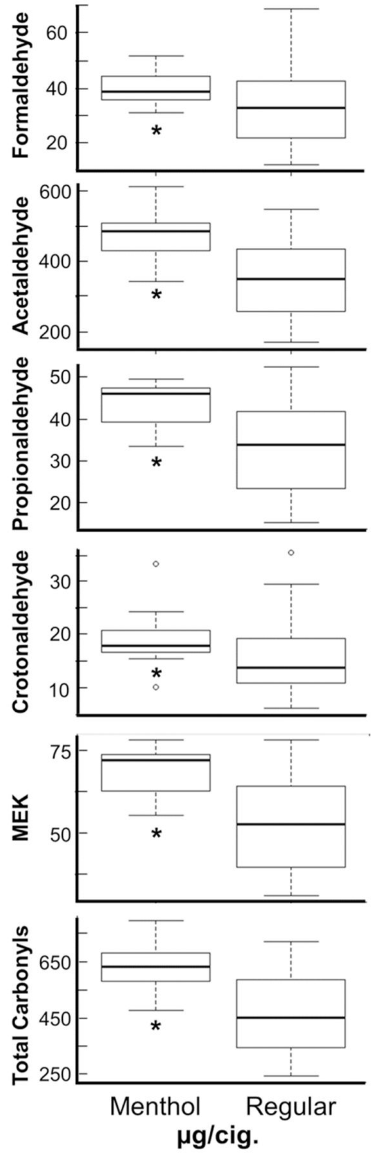

Figure 1.

Boxplot results comparing flavor groups on a per cigarette basis. All units are µg/cigarette. After adjustment for ventilation, acetaldehyde, propionaldehyde, MEK, and total carbonyls remain significant. *P ≤ 0.05

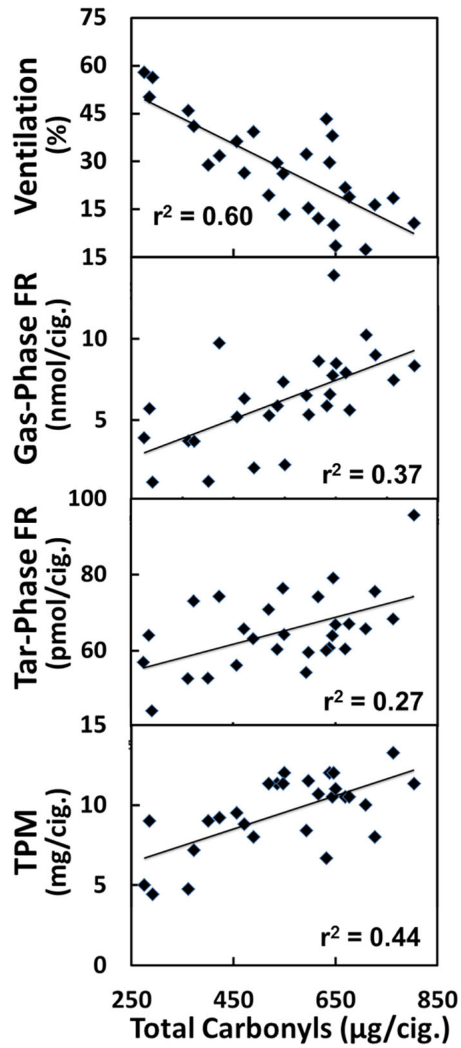

Figure 2.

Correlations between cigarette ventilation, gas-phase free radicals, particulate-phase free radicals, and TPM with total carbonyls. All correlations are statistically significant ($P \leq 0.05$).

Table 1

Carbonyl delivery by cigarette brand[a]

Brands	Flavor[b]	Form-aldehyde (µg/cigarette)	Acetaldehyde (µg/cigarette)	Propion-aldehyde (µg/cigarette)	Croton-aldehyde (µg/cigarette)	Methyl Ethyl Ketone (µg/ cigarette)	Total Carbonyls (µg/cigarette)
3R4F	R	19 ± 5	489 ± 20	46 ± 5	19 ± 3	72 ± 7	644 ± 26
American Spirit Mellow Menthol	M	52 ± 32	345 ± 22	33 ± 2	10 ± 1	50 ± 6	490 ± 54
American Spirit Menthol	M	39 ± 22	397 ± 56	38 ± 5	16 ± 1	61 ± 7	550 ± 65
American Spirit Orange	R	69 ± 42	173 ± 29	15 ± 7	6 ± 1	28 ± 1	292 ± 90
American Spirit Turquoise	R	42 ± 18	270 ± 19	27 ± 3	11 ± 4	51 ± 6	401 ± 10
Camel Blue	R	45 ± 4	435 ± 82	43 ± 8	13 ± 4	58 ± 16	593 ± 100
Camel Filters	R	38 ± 33	391 ± 2	36 ± 1	14 ± 4	57 ± 3	536 ± 30
Camel Silver	R	23 ± 28	201 ± 24	21 ± 3	7 ± 1	34 ± 4	286 ± 57
Eagle20's Gold Menthol	M	45 ± 17	611 ± 36	49 ± 7	24 ± 5	76 ± 18	804 ± 42
Kool	M	35 ± 15	471 ± 61	46 ± 6	18 ± 10	69 ± 13	639 ± 70
L&M Blue	R	29 ± 4	546 ± 2	52 ± 3	23 ± 1	78 ± 2	728 ± 10
L&M Menthol	M	37 ± 9	489 ± 34	47 ± 1	22 ± 2	75 ± 10	670 ± 50
L&M Red	R	43 ± 24	377 ± 17	37 ± 1	13 ± 1	51 ± 2	520 ± 26
Marlboro Gold	R	19 ± 10	352 ± 27	34 ± 3	13 ± 1	54 ± 1	472 ± 39
Marlboro Menthol	M	44 ± 12	494 ± 50	48 ± 4	20 ± 1	72 ± 6	677 ± 67
Marlboro Red	R	53 ± 18	407 ± 80	36 ± 5	36 ± 8	66 ± 10	597 ± 100
Marlboro Silver	R	33 ± 43	251 ± 6	23 ± 1	14 ± 9	41 ± 2	362 ± 39
Newport	M	31 ± 17	521 ± 43	50 ± 12	33 ± 13	75 ± 5	710 ± 66
Newport Red	R	33 ± 24	483 ± 26	44 ± 3	22 ± 14	69 ± 5	651 ± 29
Pall Mall Menthol	M	43 ± 17	460 ± 26	41 ± 6	17 ± 1	73 ± 5	633 ± 40
Pall Mall Orange	R	22 ± 34	194 ± 42	19 ± 5	7 ± 1	34 ± 4	275 ± 84
Pall Mall Red	R	21 ± 4	323 ± 47	28 ± 2	30 ± 1	55 ± 5	457 ± 52
Parliament White	R	12 ± 7	312 ± 33	30 ± 4	16 ± 8	53 ± 5	423 ± 44
Parliament White Menthol	M	35 ± 3	404 ± 22	37 ± 3	17 ± 2	55 ± 3	548 ± 31

Brands	Flavor[b]	Form-aldehyde (µg/cigarette)	Acetaldehyde (µg/cigarette)	Propion-aldehyde (µg/cigarette)	Croton-aldehyde (µg/cigarette)	Methyl Ethyl Ketone (µg/ cigarette)	Total Carbonyls (µg/cigarette)
Pyramid Gold Menthol	M	36 ± 4	485 ± 10	45 ± 2	16 ± 4	64 ± 1	647 ± 18
Pyramid Red	R	54 ± 26	448 ± 32	42 ± 3	17 ± 4	57 ± 9	617 ± 30
Salem	M	52 ± 1	568 ± 84	47 ± 14	19 ± 2	78 ± 6	764 ± 101
Virginia Slims Gold	R	40 ± 22	260 ± 37	22 ± 3	10 ± 4	41 ± 4	373 ± 57

Note: All cigarettes were from king-sizes hard packs (boxes), except 3R4F (soft pack) and Virginia Slims Gold (100s).

[a]Values are mean ± SD (n=2–4)

[b]R = Regular; M = Menthol

Table 2

Correlations between carbonyl and free radical deliveries across different brands of cigarettes.

	Formaldehyde	Acetaldehyde	Propionaldehyde	Crotonaldehyde	Methyl Ethyl Ketone (MEK)	Total Carbonyls	Ventilation
Formaldehyde							
Acetaldehyde	0.009 (0.64)						
Propionaldehyde	0.001 (0.87)	0.953 (< 0.001)					
Crotonaldehyde	(−) 0.005 (0.72)	0.395 (0.003)	0.356 (0.008)				
MEK	0.000 (0.97)	0.909 (< 0.001)	0.895 (< 0.001)	0.510 (< 0.001)			
Free Radicals (Gas Phase)	(−) 0.107 (0.09)	0.416 (0.002)	0.418 (0.002)	0.209 (0.01)	0.327 (0.002)	0.370 (0.006)	(−) 0.336 (0.001)
Free Radicals (Particulate Phase)	0.012 (0.50)	0.311 (0.002)	0.236 (0.009)	0.054 (0.23)	0.172 (0.03)	0.268 (0.005)	(−) 0.313 (0.002)
TPM	0.0003 (0.93)	0.440 (< 0.001)	0.421 (0.002)	0.225 (0.01)	0.401 (0.003)	0.435 (< 0.001)	(−) 0.564 (< 0.001)
Ventilation	0.009 (0.63)	(−) 0.589 (< 0.001)	(−) 0.589 (< 0.001)	(−) 0.411 (0.002)	(−) 0.526 (< 0.001)	(−) 0.600 (< 0.001)	

[a]Values are r^2 with negative sign added to indicate a negative correlation and corresponding p-values in parentheses.

Table 3

Correlations between carbonyl and free radical deliveries across different brands of cigarettes adjusted for ventilation.

	Formaldehyde	Acetaldehyde	Propionaldehyde	Crotonaldehyde	Methyl Ethyl Ketone (MEK)	Total Carbonyls
Free Radicals (Gas Phase)	0.120 (0.06)	0.060 (0.048)	0.062 (0.046)	0.010 (0.50)	0.035 (0.17)	0.039 (0.12)
Free Radicals (Particulate Phase)	0.050 (0.26)	0.050 (0.22)	0.005 (0.60)	0.023 (0.32)	0.0001 (0.94)	0.010 (0.42)

[a]Values are partial r^2 value with corresponding p-values in parentheses.