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Targeted Characterization of the Chemical Composition of JUUL Systems Aerosol and Comparison with 3R4F Reference Cigarettes and IQOS Heat Sticks

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Abstract: Aerosol constituent yields have been reported from a wide range of electronic nicotine delivery systems. No comprehensive study has been published on the aerosol constituents generated from the JUUL system. Targeted analyses of 53 aerosol constituents from the four JUUL products currently on the US market (Virginia Tobacco and Menthol flavored e-liquids in both 5.0% and 3.0% nicotine concentration by weight) was performed using non-intense and intense puffing regimens. All measurements were conducted by an ISO 17025 accredited contract research organization. JUUL product aerosol constituents were compared to published values for the 3R4F research cigarette and IQOS Regular and Menthol heated tobacco products. Across the four JUUL products and two puffing regimes, only 10/53 analytes were quantifiable, including only two carbonyls (known propylene glycol or glycerol degradants). The remaining analytes were primary ingredients, nicotine degradants and water. Average analyte reductions (excluding primary ingredients and water) for all four JUUL system aerosols tested were greater than 98% lower than 3R4F mainstream smoke, and greater than 88% lower than IQOS aerosol. In summary, chemical characterization and evaluation of JUUL product aerosols demonstrates a significant reduction in toxicants when compared to mainstream cigarette smoke from 3R4F reference cigarettes or aerosols from IQOS-heated tobacco products.

Keywords: JUUL; aerosol; HPHC; GC-MS

1. Introduction

A number of compounds in tobacco smoke have been recognized by the US Food and Drug Administration (FDA) as harmful and potentially harmful constituents (HPHCs) [1] and the Agency has required the reporting of these toxicant levels in mainstream cigarette smoke [2]. These compounds have toxicities relevant to a number of tobacco related diseases such as cancer, and cardiovascular and respiratory diseases [3]. In contrast to combustible cigarettes, electronic nicotine delivery system/s (ENDS) are designed to deliver nicotine without combustion [4]. The devices themselves consist of a battery, a heating element (most often a coil), and a reservoir for storing e-liquid. A number of studies have investigated whether this is reflected in a reduced toxicant profile of ENDS aerosol and concluded that compounds such as carbonyls [5–12], tobacco-specific nitrosamines [13–15], polycyclic aromatic hydrocarbons (PAH) [14,16,17], volatile organic compounds [8,14,18], and others [19,20] are significantly reduced in comparison to the levels in mainstream cigarette smoke. Correspondingly, a number of scientific bodies have concluded that completely substituting ENDS products for combustible cigarettes may reduce a smoker's exposure to toxicants, including carcinogens [21,22].

However, some publications have also reported the production of elevated levels of carbonyl analytes and other HPHCs in ENDS aerosol. This has included reports of

formaldehyde levels in ENDS aerosols that have approached, or even exceeded, levels in mainstream cigarette smoke [9,10,23–25]. Kosmider and colleagues analyzed the aerosol of a second generation ENDS device, the eGo-3, and reported that formaldehyde content was dependent on e-liquid formulation and device power [26]. When the variable power battery was raised to its highest setting, carbonyl content approached that of mainstream cigarette smoke. Jensen et al. reported similar findings with an unnamed tank system ENDS [27]. The authors reported the detection of no formaldehyde at low voltage, but formaldehyde content exceeding that of mainstream cigarette smoke at high voltage.

More recently, formaldehyde, acetaldehyde, and acrolein levels have been shown to be highly dependent on the power levels of the ENDS device [24,28]. A recent study examining the effects of high-power settings illustrated that for every 5 watts of increase above manufacturer recommended power settings, carbonyl generation increased by up to 20 times [28]. An additional study found that higher than manufacturer recommended power settings not only increase the carbonyl content of ENDS aerosol, but are aversive to the sensory experience of the user [25]. Previous work has established that higher coil temperatures lead to increased thermal degradation of the e-liquid, leading to the excess production of a range of HPHCs [29]. The wide range of HPHC levels reported in ENDS indicates that device performance, including power delivered to the coil and coil temperature, have a substantial impact on the production of HPHCs in the ENDS aerosols.

There are a large variety of ENDS products with a vast array of device characteristics on the market. Over the last 14 years, ENDS products have undergone several significant changes in design. The fourth generation of ENDS device, the “pod mod” or simply “pod”, generally consist of a small, rechargeable battery and an insertable pod which comes pre-filled with e-liquid. Most e-liquids consist of humectants such as propylene glycol (PG) and vegetable glycerol (VG), as well as nicotine and flavorants. Device characteristics are known to affect the HPHC emissions of ENDS devices [30]. One important device characteristic is the ability to limit coil temperature based on changes to the resistance of the coil during heating. The JUUL system includes tightly controlled temperature regulation of the coil, with the goal of minimizing potential heat degradation by-products and HPHCs in the resulting aerosols [31]. Unlike prior work [31,32], the targeted analysis described in this paper combined with the non-targeted analysis described in our companion paper provide a more complete analysis of aerosol constituents in the JUUL device [33].

The JUUL system is a pre-filled, closed-system, cartridge-based ENDS product that consists of two main components: a JUUL device and a pre-filled JUULpod. The primary e-liquid ingredients in a JUULpod are nicotine, propylene glycol (PG), vegetable glycerin (VG), benzoic acid, and flavorants. Although no single study has comprehensively characterized the aerosol chemistry of the JUUL system, it has been reported to produce lower levels of carbonyls, free radicals, and CO than mainstream cigarette smoke and other tested ENDS products [32]. The JUUL device itself is designed to operate below combustion temperatures [34]. Talih and colleagues have reported reduced carbonyl emissions in the JUUL system and attributed the reduced carbonyl levels, versus other ENDS products, to the lower coil temperature of the JUUL system [31,35]. The primary objective of this research was to quantify an expanded set of aerosol constituents generated from the four US products (Virginia Tobacco 5% [VT5], Virginia Tobacco 3% [VT3], Menthol 5% [Me5], and Menthol 3% [Me3]). The secondary objective of this research was to compare the measured aerosol constituent levels of the four JUUL products to the reported literature values from the mainstream cigarette smoke of the 3R4F reference cigarette and the aerosol from the IQOS Tobacco Heating System.

2. Materials and Methods

2.1. Test Items

JUULpods used in testing were commercial products stored in their original commercially marketed packaging and tested within two months of pod filling. Aerosol constituents chosen for targeted analysis were based on the FDA Final Guidance on PM-

TAs for ENDS (Group I) and the FDA Draft Guidance on PMTAs for ENDS (Group II) (Table 1) [36,37]. Many of the aerosol constituents listed in Table 1 are also classified by the FDA as HPHCs [1,38]. The Virginia Tobacco and Menthol JUULpods used in this study contained 3.0% and 5.0% nicotine on a mass-to-mass basis and 35 and 59 mg/mL on a mass-to-volume basis.

Table 1. Aerosol Constituents Included in the Study.

Aerosol Constituent Names		
Group I		Group II
1-Butanol	Glycerol	1-Aminonaphthalene
β -Nicotyrine	Glycidol *	1,3-Butadiene
Acetaldehyde	Isoamyl Acetate	2-Aminonaphthalene
Acetyl Propionyl	Isobutyl Acetate	4-Aminobiphenyl
Acrolein	Isobutyraldehyde	Ammonia
Acrylonitrile	Lead	Anabasine
Benzene	Menthol	Anatabine
Benzoic Acid	Methyl Acetate	Benzo(a)pyrene
Benzyl Acetate	n-Butyraldehyde	Carbon Monoxide
Cadmium	Nickel	Gold
Chromium	Nicotine	Isoprene
Copper	Nicotine-N-Oxide	Myosmine
Cotinine	NNK	Nornicotine
Crotonaldehyde	NNN	
Diacetyl	Propionic Acid	
Diethylene Glycol	Propylene Glycol	
Ethyl Acetate	Propylene Oxide	
Ethyl Acetoacetate	Toluene	
Ethylene Glycol	Water	
Formaldehyde		
Furfural		

*: Method unavailable at the time of the study.

2.2. Generation and Collection of Aerosol

Group 1 aerosol constituent analysis was conducted by Labstat International ULC (Labstat; Kitchener, Ontario, Canada). Group II aerosol constituent analysis were performed by Enthalpy Analytical LLC (800 Capitola Drive, Suite 1, Durham, NC, 27713 and 1470 East Parham Road, Richmond, VA, 23228). Both Labstat International ULC and Enthalpy Analytical were International Organization for Standardization (ISO) 17025 accredited at the time of this study. All analytical methods were validated for the analysis of ENDS aerosol according to ICH guidance Q2 (R1) with the exception of gold and carbon monoxide [39]. Method validations included an assessment of accuracy, precision, repeatability, intermediate precision, specificity, detection limit, quantitation limit, linearity, and recovery from the trapping systems. All method validations were reviewed by an independent accreditation body as part of the ISO 17025 accreditation process. Carbon monoxide was determined following ISO 8454 and gold was determined by ICP-MS and method performance was verified for accuracy, detection limit, quantitation limit, and linearity [40]. A summary of the analytical methods used in this study are presented in Supplemental Table S1.

JUUL devices used in the testing were commercial products. Prior to aerosol collection, the JUULpod was attached to a fully charged device. Devices were replaced every 50 puffs during aerosol collection of Group I aerosol constituents. All aerosol collections were performed on linear puffing machines and the JUUL System was inserted into a custom pad holder containing a glass fiber filter pad to trap non-volatile compounds during aerosol collection. Depending on the test method, an impinger containing a trapping solvent may have been used in conjunction with, or instead of the glass fiber pad

(Supplemental Table S1). The JUUL device was oriented at a 45° angle to gravity with the battery end downward.

For Group I aerosol constituents, a total of ten replicate measurements were performed from each of three lots of each JUUL flavor/concentration ($n = 30$) for each puffing regimen. Data for Group II aerosol constituents was based upon the FDA Draft Guidance on PMTAs for ENDS and utilized 10 replicate measurements of one batch of each of the four JUUL products. Aerosol samples were collected under two puffing regimes: non-intense (NI) and intense. NI puffing collection for Group I analytes was conducted consistent with ISO 20768 (55 mL puff volume over 3 s with one puff every 30 s). For Group II analytes, which were collected prior to the existence of ISO 20768, a 70 mL puff volume was used as opposed to 55 mL [41]. Currently, there is no standardized topography for intense ENDS puffing conditions. The intense puffing regime employed here used a 110 mL puff volume over a 6 s puff duration (maximum possible with the JUUL device) with one puff every 30 s.

At present, there is no standardized method for the collection of ENDS product aerosols. The yield of aerosol mass and selected aerosol constituents has been shown to vary across the life of a device with aerosol mass decreasing and analyte levels increasing as the e-liquid is depleted [42,43]. To determine the impact of total puff count on aerosol mass and aerosol constituent yield, Group I aerosol constituents were analyzed over three 50-puff collections: one at the beginning (first 50 puffs), one in the middle (45–50% of the total device mass loss (DML)), and one at the end (85–90% of total DML). For Group II aerosol constituents, only the beginning 50-puff segment was analyzed.

2.3. Measurement of Aerosol Constituents

All contract research organization (CRO) aerosol constituent measurement methods were validated and included in their scope of accreditation when the analyses were performed. Methods are summarized in Supplemental Table S1. Air blank samples were collected and analyzed together with the JUULpod aerosol samples for each method.

2.4. Estimated Values

The majority of the JUUL systems aerosol constituents were below the limit of detection (BLOD) or below limit of quantification (BLOQ). To facilitate comparison, when a constituent in JUUL system aerosol was BLOD, its level was computed as half of reported LOD; when the constituent was BLOQ, the level was considered as the average of reported LOD and LOQ [12]. A potential limitation of using estimated values are instances where an estimated value is larger than a quantified value, as it is not possible to determine if the difference is a reduction or an increase.

2.5. Background Subtraction

To mitigate the impact of environmental background during aerosol collection, which can lead to false-positives and/or overestimation of results, laboratory background control (air blank) measurements were performed [12]. Blank background subtraction was applied to aerosol sample datasets when reporting results for ammonia, chromium, formaldehyde, and lead. The background subtraction approach was applied separately to the respective puff segment group (i.e., puffs 1–50 segment analyte measurements were compared against puffs 1–50 segment blank values). When evaluating specific puff segment collections, no numerical imputed values were applied when a not different from blank (NDFB) measurement was determined. In cases where results for the air blank average value were below the level of detection (BLOD) or below the limit of quantitation (BLOQ), no action was taken. In cases where results for the air blank average value and the analyte average value are above the LOQ, and the average air blank value was greater than or equal to the average analyte value, the analyte value was reported as NDFB. In cases where results for the air blank value were non-zero, a statistical analysis was performed using Student's *t*-test (unpaired, nonparametric, 2-tailed) to establish if the sample and the blank results were significantly different ($p < 0.05$). If there was no statistically significant

difference, the analyte result was reported as NDFB. If the background and sample results were statistically different, the difference between the sample mean and the background mean was computed (sample mean minus blank mean).

2.6. Comparators Testing and Value Sources

The primary comparator used in this work was the 3R4F Kentucky Reference Cigarette (University of Kentucky, USA). IQOS 2.2 Regular and IQOS 2.2 Menthol (mIQOS) heated tobacco products were used as secondary comparators.

Aerosol constituent values for mainstream 3R4F reference cigarette smoke were obtained from peer-reviewed literature for both ISO Non-Intense [44] and ISO Intense [45] smoking regimes. Values for the majority of mainstream smoke constituents were taken from Jaccard et al., 2019 [46]; Acetyl propionyl and diacetyl from Moldoveneau et al., 2017 [47]; chromium, lead and nickel from Pappas et al., 2014 [48]. IQOS and mIQOS aerosol constituent values were obtained from the literature and public sources [49,50] for ISO Intense smoking regime. ISO Non-Intense values were not available for IQOS aerosols. Source data, by constituent, is given in Supplemental Tables S4–S15.

2.7. Data Processing for Comparison between Test Systems

Comparison of aerosol constituent values from ENDS products to the values in mainstream cigarette smoke is a non-trivial task. Cigarettes may be consumed in 10–15 puffs when smoked with an intense puffing regime [12]. In contrast, a VT5 JUULpod puffed under our intense regimen lasts for slightly more than 300 puffs. Dividing collection values by the number of puffs provides a means to compare the products on a per puff basis, but this may not be representative of real-world usage, as nicotine product users are known to modify their topographies in order to titrate their nicotine intake to desired levels [51–53]. With this in mind, in addition to per puff, aerosol constituent values were normalized to nicotine content by dividing the targeted aerosol constituent value by the measured value of nicotine from the same study. This provides aerosol constituent intake on a per nicotine basis. In general, the highest reported JUUL aerosol constituent levels across the three puff blocks (beginning, middle, and end) were used as the basis for comparison to 3R4F and IQOS. The highest aerosol constituent level across the three puff blocks is designated in bold text in Table 2. When values were BLOD for all three puff blocks, the value from the first 50 puffs was used for comparison.

Accordingly, we normalized reported aerosol constituent values for IQOS and the 3R4F reference cigarette to nicotine as well. This allowed a more direct comparison to determine whether analytes in JUUL aerosols were reduced vs. 3R4F and/or IQOS aerosols. JUUL aerosol constituents generated using NI and intense regimes were compared directly to equivalent regimes in the comparator products.

The following equation was used to calculate the percent difference between JUUL and comparator products.

$$\% \text{ difference} = ((\text{JUUL aerosol constituent level normalized by nicotine}) / (\text{Comparator constituent level normalized by nicotine}) - 1) \times 100\%$$

The % Difference value was regarded as Not Comparable (NC) if (1) both JUUL and comparator were BLOD/BLOQ, (2) the measured JUUL value was NDFB, or (3) the measured JUUL value was BLOD or BLOQ and the comparator value was quantifiable, but lower than the method LOD/LOQ.

Table 2. Quantifiable analytes in the aerosol of VT5 (per puff).

VT5 Per Puff Values									
HPHC or Chemical	Units	LOD	LOQ	NI			Intense		
				Beginning	Middle	End	Beginning	Middle	End
Benzoic Acid	mg	9.59×10^{-5}	3.20×10^{-4}	4.14×10^{-2}	6.37×10^{-2}	7.81×10^{-2}	7.82×10^{-2}	1.37×10^{-1}	2.88×10^{-2}
Cotinine	µg	1.87×10^{-3}	6.25×10^{-3}	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$	6.57×10^{-3}	$\leq 4.06 \times 10^{-3}$
Formaldehyde	µg	5.49×10^{-3}	1.83×10^{-2}	2.72×10^{-2}	8.33×10^{-2}	7.77×10^{-2}	2.60×10^{-2}	6.03×10^{-2}	5.43×10^{-2}
Glycerol	mg	1.44×10^{-3}	4.80×10^{-3}	7.37×10^{-1}	$1.06 \times 10^{+00}$	$1.24 \times 10^{+00}$	$1.35 \times 10^{+00}$	$2.12 \times 10^{+00}$	4.53×10^{-1}
Nicotine	mg	1.35×10^{-4}	4.49×10^{-4}	5.43×10^{-2}	8.49×10^{-2}	1.05×10^{-1}	9.99×10^{-2}	1.72×10^{-1}	3.77×10^{-2}
<i>Nornicotine</i>	µg	4.76×10^{-3}	1.59×10^{-2}	2.42×10^{-2}	NT	NT	2.42×10^{-2}	NT	NT
Propylene Glycol	mg	2.40×10^{-4}	8.01×10^{-4}	2.40×10^{-1}	3.96×10^{-1}	5.32×10^{-1}	4.60×10^{-1}	8.30×10^{-1}	2.17×10^{-1}
Water	mg	3.83×10^{-3}	1.28×10^{-2}	1.11×10^{-1}	2.21×10^{-1}	2.82×10^{-1}	2.57×10^{-1}	4.31×10^{-1}	1.10×10^{-1}
VT3 Per Puff Values									
Acetaldehyde	µg	9.95×10^{-3}	3.32×10^{-2}	$\leq 2.16 \times 10^{-2}$	3.47×10^{-2}	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	4.35×10^{-2}	$\leq 2.16 \times 10^{-2}$
Benzoic Acid	mg	9.59×10^{-5}	3.20×10^{-4}	2.61×10^{-2}	4.33×10^{-2}	4.79×10^{-2}	4.23×10^{-2}	8.41×10^{-2}	3.23×10^{-2}
Formaldehyde	µg	5.49×10^{-3}	1.83×10^{-2}	2.89×10^{-2}	7.24×10^{-2}	6.17×10^{-2}	1.19×10^{-2}	8.72×10^{-2}	4.68×10^{-2}
Glycerol	mg	1.44×10^{-3}	4.80×10^{-3}	7.26×10^{-1}	$1.23 \times 10^{+00}$	$1.19 \times 10^{+00}$	$1.29 \times 10^{+00}$	$2.40 \times 10^{+00}$	9.26×10^{-1}
Nicotine	mg	1.35×10^{-4}	4.49×10^{-4}	2.93×10^{-2}	5.35×10^{-2}	5.67×10^{-2}	5.28×10^{-2}	1.05×10^{-1}	4.35×10^{-2}
<i>Nornicotine</i>	µg	4.76×10^{-3}	1.59×10^{-2}	1.66×10^{-2}	NT	NT	1.66×10^{-2}	NT	NT
Propylene Glycol	mg	2.40×10^{-4}	8.01×10^{-4}	2.31×10^{-1}	4.47×10^{-1}	5.01×10^{-1}	4.48×10^{-1}	9.19×10^{-1}	4.07×10^{-1}
Water	mg	3.83×10^{-3}	1.28×10^{-2}	1.75×10^{-1}	2.75×10^{-1}	2.37×10^{-1}	3.00×10^{-1}	4.57×10^{-1}	2.49×10^{-1}
Me5 Per Puff Values									
β-Nicotyrine	µg	3.81×10^{-3}	1.27×10^{-2}	$\leq 1.90 \times 10^{-3}$	$\leq 8.25 \times 10^{-3}$	$\leq 8.25 \times 10^{-3}$	$\leq 1.90 \times 10^{-3}$	1.33×10^{-2}	1.31×10^{-2}
Acetaldehyde	µg	9.95×10^{-3}	3.32×10^{-2}	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	3.57×10^{-2}
Benzoic Acid	mg	9.59×10^{-5}	3.20×10^{-4}	3.24×10^{-2}	7.30×10^{-2}	6.67×10^{-2}	5.43×10^{-2}	1.17×10^{-1}	1.16×10^{-1}
Ethylene Glycol	mg	5.05×10^{-5}	1.68×10^{-4}	$\leq 1.09 \times 10^{-4}$	$\leq 1.09 \times 10^{-4}$	$\leq 1.09 \times 10^{-4}$	$\leq 1.09 \times 10^{-4}$	$\leq 1.09 \times 10^{-4}$	1.78×10^{-4}
Formaldehyde	µg	5.49×10^{-3}	1.83×10^{-2}	1.19×10^{-2}	3.94×10^{-2}	3.44×10^{-2}	1.19×10^{-2}	5.33×10^{-2}	7.56×10^{-2}
Glycerol	mg	1.44×10^{-3}	4.80×10^{-3}	6.00×10^{-1}	$1.23 \times 10^{+00}$	$1.09 \times 10^{+00}$	9.92×10^{-1}	$1.96 \times 10^{+00}$	$1.99 \times 10^{+00}$
Menthol	mg	2.44×10^{-4}	8.14×10^{-4}	7.31×10^{-3}	1.81×10^{-2}	1.67×10^{-2}	1.20×10^{-2}	2.85×10^{-2}	2.90×10^{-2}
Nicotine	mg	1.35×10^{-4}	4.49×10^{-4}	4.03×10^{-2}	8.93×10^{-2}	8.24×10^{-2}	6.92×10^{-2}	1.56×10^{-1}	1.55×10^{-1}
<i>Nornicotine</i>	µg	4.76×10^{-3}	1.59×10^{-2}	1.99×10^{-5}	NT	NT	1.99×10^{-5}	NT	NT
Propylene Glycol	mg	2.40×10^{-4}	8.01×10^{-4}	1.78×10^{-1}	4.16×10^{-1}	4.24×10^{-1}	3.50×10^{-1}	7.58×10^{-1}	7.83×10^{-1}
Water	mg	3.83×10^{-3}	1.28×10^{-2}	1.79×10^{-1}	2.52×10^{-1}	2.46×10^{-1}	2.42×10^{-1}	3.60×10^{-1}	3.84×10^{-1}
Me3 Per Puff Values									
Acetaldehyde	µg	9.95×10^{-3}	3.32×10^{-2}	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	$\leq 2.16 \times 10^{-2}$	3.56×10^{-2}
Benzoic Acid	mg	9.59×10^{-5}	3.20×10^{-4}	2.07×10^{-2}	4.10×10^{-2}	4.08×10^{-2}	3.76×10^{-2}	7.24×10^{-2}	8.57×10^{-2}
Cotinine	µg	1.87×10^{-3}	6.25×10^{-3}	9.37×10^{-4}	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$	$\leq 4.06 \times 10^{-3}$
Ethylene Glycol	mg	5.05×10^{-5}	1.68×10^{-4}	$\leq 1.09 \times 10^{-4}$	$\leq 1.09 \times 10^{-4}$	$\leq 1.09 \times 10^{-4}$	2.20×10^{-4}	1.09×10^{-4}	1.09×10^{-4}
Formaldehyde	µg	5.49×10^{-3}	1.83×10^{-2}	NDFB	1.85×10^{-2}	2.19×10^{-2}	NDFB	3.78×10^{-2}	4.47×10^{-2}
Glycerol	mg	1.44×10^{-3}	4.80×10^{-3}	6.78×10^{-1}	$1.22 \times 10^{+00}$	$1.16 \times 10^{+00}$	$1.16 \times 10^{+00}$	$2.14 \times 10^{+00}$	$2.46 \times 10^{+00}$
Menthol	mg	2.44×10^{-4}	8.14×10^{-4}	8.07×10^{-3}	1.74×10^{-2}	1.79×10^{-2}	1.32×10^{-2}	3.08×10^{-2}	3.44×10^{-2}
Nicotine	mg	1.35×10^{-4}	4.49×10^{-4}	2.55×10^{-2}	4.98×10^{-2}	5.04×10^{-2}	4.56×10^{-2}	9.38×10^{-2}	1.07×10^{-1}
Propylene Glycol	mg	2.40×10^{-4}	8.01×10^{-4}	2.03×10^{-1}	4.20×10^{-1}	4.55×10^{-1}	3.97×10^{-1}	8.20×10^{-1}	9.92×10^{-1}
Water	mg	3.83×10^{-3}	1.28×10^{-2}	1.47×10^{-1}	2.51×10^{-1}	2.38×10^{-1}	2.81×10^{-1}	3.87×10^{-1}	4.95×10^{-1}

Note: Values preceded with “≤” are based on estimated values. Analytes in italics were tested under a single modified puffing regimen: 70mL puff, 3 s duration, 30 s interval. Bolded values are highest of the three puff blocks.

3. Results

A total of 45/53 aerosol constituents were BLOD/BLOQ in JUUL VT5/VT3, and 43/53 aerosol constituents were BLOD/BLOQ in JUUL Me5/Me3. Quantifiable aerosol constituents (mean value > LOQ in at least one puff block in one puffing regime) in JUUL products are presented on a per puff basis in Table 2. Method LODs and LOQs for all 53 aerosol constituents are presented in Supplemental Tables S2 and S3.

The values in bold are highest of the three puff blocks. Only 10 of the 53 aerosol constituents were quantifiable in any of the JUUL systems aerosols. All of these aerosol constituents were in Group I and were collected at the beginning, middle and end of pod life (Table 2). Aerosol mass was found to increase after collection of the beginning puff block. Yields of formaldehyde and acetaldehyde were highest in the middle and end puff blocks with 50% of the highest values in the end puff block.

Aerosol constituent values for each JUUL product using both puffing regimes were then normalized to nicotine and compared to 3R4F and IQOS (Supplemental Tables S4–S15). Across all flavors and nicotine concentrations, of the aerosol constituents which could be

compared, all were reduced in JUUL aerosols compared to 3R4F mainstream cigarette smoke, excepting water and the primary ingredients PG and VG (Table 4). Water was reduced in all JUUL aerosols, except in the NI regimens of VT3 and Me3. Notably, 22/25 of the aerosol constituent reductions were based on estimated values for JUUL aerosol constituents as they were either BLOD or BLOQ in JUUL products. Only formaldehyde ($\downarrow \geq 96\%$ to $\geq 99\%$) and acetaldehyde ($\downarrow \geq 99\%$) were quantifiable (Table 3). More comprehensive results for each JUUL flavor and concentration are outlined below.

Table 3. Summary of Aerosol Constituent Comparisons between the JUUL System and 3R4F Cigarette Smoke.

Aerosol Constituents Higher in all JUUL System Aerosols vs. 3R4F Smoke	
PG (RT)	VG (RT)
Aerosol Constituents Lower in all JUUL Aerosols vs. 3R4F Smoke	
1-aminonaphthalene (CA) [$\downarrow \geq 99\%$] *	CO (RDT) [$\downarrow \geq 98\%$ to $\geq 99\%$] *
1,3-butadiene (CA, RT, RDT) [$\downarrow \geq 99\%$] *	Crotonaldehyde (CA) [$\downarrow \geq 99\%$] *
2-aminonaphthalene (CA) [$\downarrow \geq 99\%$] *	Diacetyl (RT) [$\downarrow \geq 99\%$] *
4-aminobiphenyl (CA) [$\downarrow \geq 99\%$] *	Formaldehyde (CA, RT) [$\downarrow \geq 96\%$ to $\geq 99\%$]
Acetaldehyde (CA, RT, AD) [$\downarrow \geq 99\%$]	Furfural (RT) [$\downarrow \geq 91\%$ to $\geq 97\%$] *
Acetyl propionyl (RT) [$\downarrow \geq 99\%$] *	Isoprene (CA) [$\downarrow \geq 98\%$ to $\geq 99\%$] *
Acrolein (RT, CT) [$\downarrow \geq 99\%$] *	Lead (CA, CT, RDT) [$\downarrow \geq 86\%$ to $\downarrow \geq 97\%$] *
Acrylonitrile (CA, RT) [$\downarrow \geq 99\%$] *	n-Butyraldehyde (RT) [$\downarrow \geq 99\%$] *
Ammonia (RT) [$\downarrow \geq 85\%$ to $\geq 96\%$] *	NNK (CA) [$\downarrow \geq 99\%$] *
Benzene (CA, CT, RDT) [$\downarrow \geq 99\%$] *	NNN (CA) [$\downarrow \geq 99\%$] *
Benzo(a)pyrene (CA) [$\downarrow \geq 98\%$ to $\geq 99\%$] *	Propylene oxide (CA, RT) [$\downarrow \geq 99\%$] *
Cadmium (CA, RT, RDT) [$\downarrow \geq 99\%$] *	Toluene * (RT, RDT) [$\downarrow \geq 99\%$] *

Note: Constituents with (*) were BLOD/Q in JUUL aerosols and reductions were based on estimated values. CA = Carcinogen. CT = Cardiovascular toxicant. RT = Respiratory Toxicant. AD = Addictive. RDT = Reproductive and developmental toxicant. $\downarrow$ = less than. Bolded reductions were based on quantifiable values.

With regard to IQOS, across all flavors and nicotine concentrations of JUUL products, of the aerosol constituents that were compared, only PG, VG, water ($\downarrow \geq 81\%$ to $\geq 90\%$), formaldehyde ($\downarrow \geq 80\%$ to $\geq 91\%$) and acetaldehyde ($\downarrow \geq 99\%$) were quantifiable. Twelve of the 15 aerosol constituent comparisons were based on estimated values. Seven aerosol constituents were lower in all JUUL aerosols vs. IQOS where comparisons could be made (e.g., one or more products were not compared with IQOS, but those which were compared were reduced), and cadmium, nickel, and chromium were BLOD/BLOQ/NDFB in both JUUL and IQOS products (Table 4). More comprehensive results for each JUUL flavor and concentration are outlined below.

Table 4. Summary of Constituent Comparisons between JUUL System and IQOS.

Aerosol Constituents Higher in JUUL System Aerosols vs. IQOS	
PG (RT)	VG (RT)
Aerosol Constituents Lower in all JUUL Aerosols vs. IQOS	
1-aminonaphthalene (CA) [$\downarrow \geq 66\%$ to $\geq 86\%$] *	Formaldehyde (CA, RT) [$\downarrow \geq 80\%$ to $\geq 91\%$]
Acetaldehyde (CA, RT, AD) [$\downarrow \geq 99\%$]	n-Butyraldehyde (RT) [$\downarrow \geq 99\%$] *
Acrolein (RT, CT) [$\downarrow \geq 97\%$] *	NNK (CA) [$\downarrow \geq 96\%$] *
Acrylonitrile (CA, RT) [$\downarrow \geq 61\%$ to $\geq 83\%$] *	NNN (CA) [$\downarrow \geq 99\%$] *
Benzene (CA, CT, RDT) [$\downarrow \geq 92\%$ to $\geq 96\%$] *	Propylene oxide (CA, RT) [$\downarrow \geq 72\%$ to $\geq 86\%$] *
Benzo(a)pyrene (CA) [$\downarrow \geq 77\%$ to $\geq 97\%$] *	Toluene * (RT, RDT) [$\downarrow \geq 93\%$ to $\geq 96\%$] *
Crotonaldehyde (CA) [$\downarrow \geq 97\%$] *	Water [$\downarrow \geq 81\%$ to $\geq 90\%$]
Diacetyl (RT) [$\downarrow \geq 99\%$] *	
Aerosol Constituents Lower in all JUUL Aerosols vs. IQOS where comparisons could be made	
2-Aminonaphthalene (CA) [$\downarrow \geq 75\%$ to $\geq 84\%$] *	
1,3-butadiene (CA, RT, RDT) [$\downarrow \geq 64\%$ to $\geq 80\%$] *	
Ammonia (RT) [$\downarrow \geq 83\%$ to $\geq 94\%$] *	Menthol [$\downarrow \geq 85\%$ to $\geq 91\%$] *
CO (RDT) [$\downarrow \geq 68\%$ to $\geq 80\%$] *	
Isoprene (CA) [$\downarrow \geq 98\%$ to $\geq 99\%$] *	
Lead (CA, CT, RDT) [$\downarrow \geq 86\%$ to $\geq 97\%$] *	
Aerosol Constituents BLOD/BLOQ/NDFB in both JUUL and IQOS aerosols	
Cadmium	Nickel
Chromium	

Note: Constituents with (*) were BLOD/Q in JUUL aerosols and reductions were based on estimated values. CA = Carcinogen. CT = Cardiovascular toxicant. RT = Respiratory Toxicant. AD = Addictive. RDT = Reproductive and developmental toxicant. $\downarrow$ = less than. Bolded reductions were based on quantifiable values.

3.1. Virginia Tobacco

Table 5 and Supplemental Table S16 outline the comparisons of VT5 and VT3 aerosol constituents to 3R4F mainstream cigarette smoke and IQOS aerosol constituents.

Table 5. Comparison of Aerosol Constituents in VT5 aerosol generated using the intense puffing regime vs. comparator products.

Aerosol Constituent	Aerosol Constituent Normalized by Nicotine (mg/mg)					% Difference		
	VT5		3R4F		IQOS	vs. 3R4F		vs. IQOS
	NI	Intense	NI	Intense	Intense	NI	Intense	Intense
1-Aminonaphthalene (CA)	$\leq 1.96 \times 10^{-08} a$	$\leq 1.96 \times 10^{-08} a$	2.02×10^{-05}	1.46×10^{-05}	5.83×10^{-08}	$\downarrow \geq 99.90$	$\downarrow \geq 99.87$	$\downarrow \geq 66.39$
1-Butanol	$\leq 5.52 \times 10^{-04} a$	$\leq 3.00 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
1,3-Butadiene (CA)	$\leq 6.83 \times 10^{-05} a$	$\leq 6.83 \times 10^{-05} a$	5.40×10^{-02}	5.03×10^{-02}	2.23×10^{-04}	$\downarrow \geq 99.87$	$\downarrow \geq 99.86$	$\downarrow \geq 69.34$
2-Aminonaphthalene (CA)	$\leq 8.53 \times 10^{-09} a$	$\leq 8.53 \times 10^{-09} a$	1.25×10^{-05}	9.10×10^{-06}	3.48×10^{-08}	$\downarrow \geq 99.93$	$\downarrow \geq 99.91$	$\downarrow \geq 75.53$
4-Aminobiphenyl (CA)	$\leq 1.48 \times 10^{-08} a$	$\leq 1.48 \times 10^{-08} a$	2.19×10^{-06}	1.96×10^{-06}	6.98×10^{-09}	$\downarrow \geq 99.32$	$\downarrow \geq 99.24$	NC
Acetaldehyde (CA)	$\leq 3.97 \times 10^{-04} b$	$\leq 2.16 \times 10^{-04} b$	8.50×10^{-01}	9.24×10^{-01}	1.66×10^{-01}	$\downarrow \geq 99.95$	$\downarrow \geq 99.98$	$\downarrow \geq 99.87$
Acetyl Propionyl	$\leq 3.23 \times 10^{-05} a$	$\leq 1.76 \times 10^{-05} a$	4.21×10^{-02}	4.62×10^{-02}	N/A	$\downarrow \geq 99.92$	$\downarrow \geq 99.96$	N/A
Acrolein	$\leq 1.92 \times 10^{-04} a,b$	$\leq 1.17 \times 10^{-04} a,b$	7.58×10^{-02}	9.05×10^{-02}	8.56×10^{-03}	$\downarrow \geq 99.75$	$\downarrow \geq 99.87$	$\downarrow \geq 98.64$
Acrylonitrile (CA)	$\leq 5.89 \times 10^{-05} a$	$\leq 3.20 \times 10^{-05} a$	7.45×10^{-03}	1.03×10^{-02}	1.95×10^{-04}	$\downarrow \geq 99.21$	$\downarrow \geq 99.69$	$\downarrow \geq 83.61$
Ammonia	NDFB	NDFB	1.37×10^{-02}	1.71×10^{-02}	1.08×10^{-02}	NC	NC	NC
Anabasine	$\leq 2.98 \times 10^{-04} a$	$\leq 2.98 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Anatabine	$\leq 5.70 \times 10^{-05} a$	$\leq 5.70 \times 10^{-05} a$	N/A	N/A	N/A	N/A	N/A	N/A
Benzene (CA)	$\leq 3.14 \times 10^{-05} a$	$\leq 1.71 \times 10^{-05} a$	4.79×10^{-02}	4.46×10^{-02}	4.92×10^{-04}	$\downarrow \geq 99.93$	$\downarrow \geq 99.96$	$\downarrow \geq 96.53$

Table 5. Cont.

Aerosol Constituent	Aerosol Constituent Normalized by Nicotine (mg/mg)					% Difference		
	VT5		3R4F		IQOS	vs. 3R4F		vs. IQOS
	NI	Intense	NI	Intense	Intense	NI	Intense	Intense
<i>Benzo(a)pyrene (CA)</i>	$\leq 1.27 \times 10^{-07} a$	$\leq 1.27 \times 10^{-07} a$	8.97×10^{-06}	7.59×10^{-06}	5.71×10^{-07}	$\downarrow \geq 98.58$	$\downarrow \geq 98.32$	$\downarrow \geq 77.70$
Benzoic Acid	7.45×10^{-01}	7.94×10^{-01}	N/A	N/A	N/A	N/A	N/A	N/A
Benzyl Acetate	$\leq 5.52 \times 10^{-04} a$	$\leq 3.00 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
β -Nicotyrine	$\leq 9.72 \times 10^{-05} a,b$	$\leq 4.79 \times 10^{-05} a,b$	N/A	N/A	N/A	N/A	N/A	N/A
Cadmium (CA)	$\leq 9.66 \times 10^{-08} a$	$\leq 5.26 \times 10^{-08} a$	3.49×10^{-05}	4.68×10^{-05}	$\leq 2.65 \times 10^{-07}$	$\downarrow \geq 99.72$	$\downarrow \geq 99.89$	NC
Carbon Monoxide	$\leq 1.26 \times 10^{-01} a$	$\leq 1.26 \times 10^{-01} a$	$1.42 \times 10^{+01}$	$1.53 \times 10^{+01}$	4.02×10^{-01}	$\downarrow \geq 99.12$	$\downarrow \geq 99.18$	$\downarrow \geq 68.75$
Chromium	NDFB	NDFB	$\leq 1.25 \times 10^{-06}$	$\leq 6.84 \times 10^{-06}$	$\leq 4.17 \times 10^{-07}$	NC	NC	NC
Copper	$\leq 2.71 \times 10^{-06} a$	$\leq 6.57 \times 10^{-07} a$	N/A	N/A	N/A	N/A	N/A	N/A
Cotinine	$\leq 7.48 \times 10^{-05} a$	3.81×10^{-05}	N/A	N/A	N/A	N/A	N/A	N/A
Crotonaldehyde (CA)	$\leq 5.73 \times 10^{-05} a$	$\leq 3.12 \times 10^{-05} a$	1.41×10^{-02}	2.99×10^{-02}	3.14×10^{-03}	$\downarrow \geq 99.59$	$\downarrow \geq 99.90$	$\downarrow \geq 99.01$
Diacetyl	$\leq 1.60 \times 10^{-05} a$	$\leq 8.71 \times 10^{-06} a$	1.64×10^{-01}	1.69×10^{-01}	3.58×10^{-02}	$\downarrow \geq 99.99$	$\downarrow \geq 99.99$	$\downarrow \geq 99.98$
Diethylene Glycol	$\leq 2.21 \times 10^{-03} a$	$\leq 1.20 \times 10^{-03} a$	N/A	N/A	N/A	N/A	N/A	N/A
Ethyl Acetate	$\leq 5.52 \times 10^{-04} a$	$\leq 3.45 \times 10^{-03} a,b$	N/A	N/A	N/A	N/A	N/A	N/A
Ethyl Acetoacetate	$\leq 4.42 \times 10^{-05} a$	$\leq 2.40 \times 10^{-05} a$	N/A	N/A	N/A	N/A	N/A	N/A
Ethylene Glycol	$\leq 2.01 \times 10^{-03} a$	$\leq 1.09 \times 10^{-03} b$	N/A	N/A	N/A	N/A	N/A	N/A
Formaldehyde (CA)	9.82×10^{-04}	3.50×10^{-04}	3.52×10^{-02}	4.38×10^{-02}	4.19×10^{-03}	$\downarrow 97.21$	$\downarrow 99.20$	$\downarrow 91.64$
Furfural	$\leq 7.73 \times 10^{-04} a$	$\leq 4.20 \times 10^{-04} a$	1.84×10^{-02}	1.49×10^{-02}	2.41×10^{-02}	$\downarrow \geq 95.80$	$\downarrow \geq 97.18$	$\downarrow \geq 98.26$
Glycerol	$1.19 \times 10^{+01}$	$1.23 \times 10^{+01}$	$3.85 \times 10^{+00}$	$1.36 \times 10^{+00}$	$3.51 \times 10^{+00}$	$2.09 \times 10^{+02}$	$8.08 \times 10^{+02}$	$2.51 \times 10^{+02}$
Glycidol (CA)	NR	NR	N/A	8.84×10^{-04}	4.43×10^{-03}	NC	NC	NC
Gold	$\leq 1.52 \times 10^{-07} a$	$\leq 1.52 \times 10^{-07} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isoamyl Acetate	$\leq 8.83 \times 10^{-04} a$	$\leq 4.80 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isobutyl Acetate	$\leq 5.52 \times 10^{-04} a$	$\leq 3.00 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isobutyraldehyde	$\leq 1.52 \times 10^{-05} a$	$\leq 8.27 \times 10^{-06} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isoprene (CA)	$\leq 9.72 \times 10^{-05} a$	$\leq 9.72 \times 10^{-05} a$	4.10×10^{-01}	4.02×10^{-01}	1.78×10^{-03}	$\downarrow \geq 99.98$	$\downarrow \geq 99.98$	$\downarrow \geq 94.54$
Lead	$\leq 4.34 \times 10^{-07} a$	$\leq 4.55 \times 10^{-07} b$	1.31×10^{-05}	1.79×10^{-05}	1.73×10^{-06}	$\downarrow \geq 96.69$	$\downarrow \geq 97.46$	$\downarrow \geq 73.65$
Menthol	$\leq 2.25 \times 10^{-03} a$	$\leq 1.22 \times 10^{-03} a$	$\leq 1.32 \times 10^{-05}$	$\leq 4.76 \times 10^{-06}$	3.42×10^{-04}	NC	NC	NC
Methyl Acetate	$\leq 6.62 \times 10^{-04} a$	$\leq 3.60 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Myosmine	$\leq 4.62 \times 10^{-04} a$	$\leq 4.62 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
n-Butyraldehyde	$\leq 3.23 \times 10^{-05} a$	$\leq 1.76 \times 10^{-05} a$	4.05×10^{-02}	4.67×10^{-02}	1.98×10^{-02}	$\downarrow \geq 99.92$	$\downarrow \geq 99.96$	$\downarrow \geq 99.91$
Nickel	$\leq 8.37 \times 10^{-06} a$	$\leq 1.05 \times 10^{-06} a$	$\leq 5.41 \times 10^{-07}$	$\leq 7.41 \times 10^{-06}$	$\leq 4.17 \times 10^{-07}$	NC	NC	NC
Nicotine	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	N/A	N/A	N/A
Nicotine-N-Oxide	$\leq 1.60 \times 10^{-04} a$	$\leq 8.73 \times 10^{-05} a$	N/A	N/A	N/A	N/A	N/A	N/A
NNK (CA)	$\leq 1.39 \times 10^{-07} a$	$\leq 7.54 \times 10^{-08} a$	1.61×10^{-04}	1.48×10^{-04}	5.08×10^{-06}	$\downarrow \geq 99.91$	$\downarrow \geq 99.95$	$\downarrow \geq 98.52$
NNN (CA)	$\leq 9.06 \times 10^{-08} a$	$\leq 4.93 \times 10^{-08} a$	1.87×10^{-04}	1.69×10^{-04}	1.30×10^{-05}	$\downarrow \geq 99.95$	$\downarrow \geq 99.97$	$\downarrow \geq 99.62$
Nornicotine	$5.78 \times 10^{-04} a$	$5.78 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Propionic Acid	$\leq 3.31 \times 10^{-04} a$	$\leq 1.80 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Propylene Glycol	$5.07 \times 10^{+00}$	$4.82 \times 10^{+00}$	4.21×10^{-03}	1.36×10^{-02}	1.36×10^{-01}	$1.20 \times 10^{+05}$	$3.52 \times 10^{+04}$	$3.45 \times 10^{+03}$
Propylene Oxide (CA)	$\leq 2.87 \times 10^{-05} a$	$\leq 1.56 \times 10^{-05} a$	4.23×10^{-01}	5.03×10^{-01}	1.12×10^{-04}	$\downarrow \geq 99.99$	$\downarrow \geq 100.00$	$\downarrow \geq 86.07$
Toluene	$\leq 1.13 \times 10^{-04} a$	$\leq 6.12 \times 10^{-05} a$	7.32×10^{-02}	7.69×10^{-02}	1.96×10^{-03}	$\downarrow \geq 99.85$	$\downarrow \geq 99.92$	$\downarrow \geq 96.88$
Water	$2.69 \times 10^{+00}$	$2.50 \times 10^{+00}$	$4.21 \times 10^{+00}$	$8.36 \times 10^{+00}$	$2.77 \times 10^{+01}$	$\downarrow 36.14$	$\downarrow 70.11$	$\downarrow 90.96$
						Average ^c $\downarrow 99.31$	Average ^c $\downarrow 99.53$	Average ^c $\downarrow 88.72$

BLOD = Below limit of quantitation; BLOQ = Below limit of quantitation; NDFB = Not different from background; NC = Not comparable; NR = Not reported; $\downarrow$ = less than. Note: Values preceded with “ $\geq$ ” are based on estimated values. Values in bold were quantifiable. CA = carcinogen. Analytes in italics were tested under a single modified puffing regimen: 70 mL puff, 3 s duration, 30 s interval. ^a analyte was BLOD for all three 50 puff blocks. ^b analyte was BLOQ for all three 50 puff blocks. ^{a,b} analyte was either BLOD or BLOQ for all three 50 puff blocks. ^c excluding PG, VG, and water.

Although not all aerosol constituents examined in this targeted analysis were reported in the literature for the mainstream smoke of the 3R4F reference cigarette, of the compounds

unavailable for comparison, only benzoic acid, cotinine (VT5-intense), and nornicotine were detectable in VT aerosols. Of the remaining targeted aerosol constituents reported for 3R4F, comparisons were not made between VT and 3R4F for chromium, nickel, glycidol, lead (VT3-intense), menthol, and ammonia (VT5). Chromium was not detected, nickel was BLOD/BLOQ in both products, ammonia and lead (VT3-intense) were not different from background (NDFB), and glycidol data was not reported since an appropriate test method was not available at the time of this study. As expected, PG and VG were higher in VT vs. 3R4F as they are primary ingredients in JUUL pods. As so few aerosol constituents in VT5 and VT3 aerosols were above LOD/LOQ, estimated values (as outlined in the methods) were used to provide a comparison to quantified aerosol constituents in 3R4F smoke. These aerosol constituent values and % Differences are preceded by a “≤” symbol in Table 5 and Supplementary Table S16. Every aerosol constituent in 3R4F mainstream cigarette smoke included here for comparison was quantifiable, except for chromium, nickel, and menthol. Correspondingly, every aerosol constituent in VT that could be compared to reported values for the 3R4F mainstream cigarette smoke (excluding PG, VG, and water) was reduced. Reductions ranged from ≥92.22% (furfural; VT3, NI) to 99.99% (propylene oxide; VT5, intense). Notably, quantifiable levels of formaldehyde were reduced from between 96.16% (VT3-NI) and 99.20% (VT5-intense) and acetaldehyde was reduced by ≥99% (VT5 and VT3, both regimes). Differences in reductions from 3R4F between the aerosol generated using intense and NI puffing regimens were within 3%, excepting water. Average reductions for aerosol constituents (excluding PG, VG and water) from VT5 and VT3 were ≥98.59% (Table 5 and Supplementary Table S16).

Similar to the data for 3R4F mainstream cigarette smoke, not all of the 53 aerosol constituents included in this analysis were reported in the literature for IQOS tobacco flavor heat sticks. Excluding benzoic acid, cotinine (VT5), and nornicotine, none of the chemicals unavailable for comparison were detectable in VT aerosol. Twenty-seven out of thirty-one aerosol constituents reported for the IQOS tobacco heat stick were found at detectable levels. Only 5 of these aerosol constituents were detectable in VT5 and 6 in VT3 (formaldehyde, acetaldehyde [VT3], glycerol, nicotine, propylene glycol, and water). Comparisons were not made for 4-aminobiphenyl (uncertain comparison), ammonia (VT5; NDFB), cadmium (all products BLOD/BLOQ), chromium (all products BLOD/BLOQ), glycidol (not recorded), nickel (all products BLOD/BLOQ), and lead (VT3; NDFB). Similar to the comparison with 3R4F mainstream cigarette smoke, to provide a comparison to aerosol constituents in IQOS, estimated values based on LOD and LOQ were employed for the remainder of JUUL VT5 aerosol constituents, where IQOS aerosol constituent levels were available and quantifiable. All VT5 aerosol constituents which were compared to IQOS were reduced, save glycerol and propylene glycol. Aerosol constituent reductions ranged from ≥66.39% (VT5) for 1-aminonaphthalene to ≥99.98% (VT5) for diacetyl. Average aerosol constituent reductions (excluding PG, VG, and water) for VT5 and VT3 were ≥89.12% (Table 5 and Supplementary Table S16).

3.2. Menthol

The aerosol constituent levels of Me5 and Me3 as compared to 3R4F and IQOS is outlined in Table 6 and Supplementary Table S17. Two of the 53 aerosol constituents (carbon monoxide and gold) were not tested in Menthol 3.0% under either puffing regimen. Me5 contained 10/53 and 8/53 quantifiable aerosol constituents under the intense and NI regimes, respectively, while Me3 contained 8/53 and 7/53. Estimated aerosol constituents are indicated by “≤”. Of the aerosol constituents not reported for the mainstream smoke from the 3R4F reference cigarette, only benzoic acid and nornicotine (Me5) were detected.

Table 6. Comparison of Aerosol Constituents in Me5 aerosol Generated Using the Intense Puffing Regime vs. Comparator Products.

Aerosol Constituent	Aerosol Constituents Normalized by Nicotine (mg/mg)					% Difference		
	Me5		3R4F		IQOS	vs. 3R4F		vs. IQOS
	NI	Intense	NI	Intense	Intense	NI	Intense	Intense
1-Aminonaphthalene (CA)	$\leq 2.25 \times 10^{-08} a$	$\leq 2.25 \times 10^{-08} a$	2.02×10^{-05}	1.46×10^{-05}	7.11×10^{-08}	$\downarrow \geq 99.89$	$\downarrow \geq 99.85$	$\downarrow \geq 68.30$
1-Butanol	$\leq 7.45 \times 10^{-04} a$	$\leq 4.33 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
1,3-Butadiene (CA)	$\leq 7.85 \times 10^{-05} a$	$\leq 7.85 \times 10^{-05} a$	5.40×10^{-02}	5.03×10^{-02}	2.19×10^{-04}	$\downarrow \geq 99.85$	$\downarrow \geq 99.84$	$\downarrow \geq 64.17$
2-Aminonaphthalene (CA)	$\leq 9.80 \times 10^{-09} a$	$\leq 9.80 \times 10^{-09} a$	1.25×10^{-05}	9.10×10^{-06}	$\leq 2.89 \times 10^{-08}$	$\downarrow \geq 99.92$	$\downarrow \geq 99.89$	NC
4-Aminobiphenyl (CA)	$\leq 3.94 \times 10^{-09} a$	$\leq 3.94 \times 10^{-09} a$	2.19×10^{-06}	1.96×10^{-06}	$\leq 4.21 \times 10^{-08}$	$\downarrow \geq 99.82$	$\downarrow \geq 99.80$	NC
Acetaldehyde (CA)	$\leq 5.35 \times 10^{-04} a$	2.30×10^{-04}	8.50×10^{-01}	9.24×10^{-01}	1.69×10^{-01}	$\downarrow \geq 99.94$	$\downarrow 99.98$	$\downarrow 99.86$
Acetyl Propionyl	$\leq 4.36 \times 10^{-05} a$	$\leq 2.54 \times 10^{-05} a$	4.21×10^{-02}	4.62×10^{-02}	N/A	$\downarrow \geq 99.90$	$\downarrow \geq 99.95$	N/A
Acrolein	$\leq 2.44 \times 10^{-04} a, b$	$\leq 1.29 \times 10^{-04} a, b$	7.58×10^{-02}	9.05×10^{-02}	7.56×10^{-03}	$\downarrow \geq 99.68$	$\downarrow \geq 99.86$	$\downarrow \geq 98.30$
Acrylonitrile (CA)	$\leq 7.95 \times 10^{-05} a$	$\leq 4.63 \times 10^{-05} a$	7.45×10^{-03}	1.03×10^{-02}	1.82×10^{-04}	$\downarrow \geq 98.93$	$\downarrow \geq 99.55$	$\downarrow \geq 74.56$
Ammonia	NDFB	NDFB	1.37×10^{-02}	1.71×10^{-02}	1.14×10^{-02}	NC	NC	NC
Anabasine	$\leq 3.43 \times 10^{-04} a$	$\leq 3.43 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Anatabine	$\leq 2.84 \times 10^{-04} a$	$\leq 2.84 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Benzene (CA)	$\leq 4.24 \times 10^{-05} a$	$\leq 2.46 \times 10^{-05} a$	4.79×10^{-02}	4.46×10^{-02}	5.29×10^{-04}	$\downarrow \geq 99.91$	$\downarrow \geq 99.94$	$\downarrow \geq 95.34$
Benzo(a)pyrene (CA)	$\leq 1.46 \times 10^{-07} a$	$\leq 1.46 \times 10^{-07} a$	8.97×10^{-06}	7.59×10^{-06}	1.07×10^{-06}	$\downarrow \geq 98.37$	$\downarrow \geq 98.07$	$\downarrow \geq 86.29$
Benzoic Acid	8.17×10^{-01}	7.48×10^{-01}	N/A	N/A	N/A	N/A	N/A	N/A
Benzyl Acetate	$\leq 7.45 \times 10^{-04} a$	$\leq 4.33 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
β -Nicotyrine	$\leq 9.24 \times 10^{-05} a, b$	8.49×10^{-05}	N/A	N/A	N/A	N/A	N/A	N/A
Cadmium (CA)	$\leq 1.30 \times 10^{-07} a$	$\leq 7.59 \times 10^{-08} a$	3.49×10^{-05}	4.68×10^{-05}	$\leq 2.89 \times 10^{-07}$	$\downarrow \geq 99.63$	$\downarrow \geq 99.84$	NC
Carbon Monoxide	$\leq 1.44 \times 10^{-01} a$	$\leq 1.44 \times 10^{-01} a$	$1.42 \times 10^{+01}$	$1.53 \times 10^{+01}$	4.91×10^{-01}	$\downarrow \geq 98.99$	$\downarrow \geq 99.06$	$\downarrow \geq 70.57$
Chromium	NDFB	NDFB	$\leq 1.25 \times 10^{-06}$	$\leq 6.84 \times 10^{-06}$	$\leq 4.55 \times 10^{-07}$	NC	NC	NC
Copper	$\leq 1.63 \times 10^{-06} a$	$\leq 9.48 \times 10^{-07} a$	N/A	N/A	N/A	N/A	N/A	N/A
Cotinine	$\leq 1.01 \times 10^{-04} b$	$\leq 5.87 \times 10^{-05} b$	N/A	N/A	N/A	N/A	N/A	N/A
Crotonaldehyde (CA)	$\leq 7.73 \times 10^{-05} a$	$\leq 4.50 \times 10^{-05} a$	1.41×10^{-02}	2.99×10^{-02}	2.68×10^{-03}	$\downarrow \geq 99.45$	$\downarrow \geq 99.85$	$\downarrow \geq 98.32$
Diacetyl	$\leq 2.16 \times 10^{-05} a$	$\leq 1.26 \times 10^{-05} a$	1.64×10^{-01}	1.69×10^{-01}	5.42×10^{-02}	$\downarrow \geq 99.99$	$\downarrow \geq 99.99$	$\downarrow \geq 99.98$
Diethylene Glycol	$\leq 2.98 \times 10^{-03} a$	$\leq 1.73 \times 10^{-03} a$	N/A	N/A	N/A	N/A	N/A	N/A
Ethyl Acetate	$\leq 7.45 \times 10^{-04} a$	$\leq 8.32 \times 10^{-04} a, b$	N/A	N/A	N/A	N/A	N/A	N/A
Ethyl Acetoacetate	$\leq 5.96 \times 10^{-05} a$	$\leq 3.47 \times 10^{-05} a$	N/A	N/A	N/A	N/A	N/A	N/A
Ethylene Glycol	$\leq 2.71 \times 10^{-03} b$	$\leq 7.05 \times 10^{-04} b$	N/A	N/A	N/A	N/A	N/A	N/A
Formaldehyde (CA)	4.41×10^{-04}	4.88×10^{-04}	3.52×10^{-02}	4.38×10^{-02}	3.76×10^{-03}	$\downarrow 98.75$	$\downarrow 98.89$	$\downarrow 87.03$
Furfural	$\leq 1.04 \times 10^{-03} a$	$\leq 6.07 \times 10^{-04} a$	1.84×10^{-02}	1.49×10^{-02}	N/A	$\downarrow \geq 94.34$	$\downarrow \geq 95.92$	N/A
Glycerol	$1.38 \times 10^{+01}$	$1.29 \times 10^{+01}$	$3.85 \times 10^{+00}$	$1.36 \times 10^{+00}$	$3.26 \times 10^{+00}$	$2.58 \times 10^{+02}$	$8.47 \times 10^{+02}$	$2.94 \times 10^{+02}$
Glycidol (CA)	NR	NR	N/A	8.84×10^{-04}	9.24×10^{-04}	NC	NC	NC
Gold	$\leq 1.74 \times 10^{-07} a$	$\leq 1.74 \times 10^{-07} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isoamyl Acetate	$\leq 1.19 \times 10^{-03} a$	$\leq 6.94 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isobutyl Acetate	$\leq 7.45 \times 10^{-04} a$	$\leq 4.33 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Isobutylaldehyde	$\leq 2.05 \times 10^{-05} a$	$\leq 5.17 \times 10^{-05} b$	N/A	N/A	N/A	N/A	N/A	N/A
Isoprene (CA)	$\leq 1.12 \times 10^{-04} a$	$\leq 1.12 \times 10^{-04} a$	4.10×10^{-01}	4.02×10^{-01}	1.74×10^{-03}	$\downarrow \geq 99.97$	$\downarrow \geq 99.97$	$\downarrow \geq 93.59$
Lead	$\leq 5.52 \times 10^{-07} b$	$\leq 6.57 \times 10^{-07} b$	1.31×10^{-05}	1.79×10^{-05}	$\leq 2.77 \times 10^{-06}$	$\downarrow \geq 95.79$	$\downarrow \geq 96.33$	NC
Menthol	2.02×10^{-01}	1.87×10^{-01}	$\leq 1.32 \times 10^{-05}$	$\leq 4.76 \times 10^{-06}$	$2.17 \times 10^{+00}$	NC	NC	$\downarrow 91.37$
Methyl Acetate	$\leq 8.94 \times 10^{-04} a$	$\leq 5.20 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
Myosmine	$\leq 5.30 \times 10^{-04} a$	$\leq 5.30 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
n-Butylaldehyde	$\leq 4.36 \times 10^{-05} a$	$\leq 2.54 \times 10^{-05} a$	4.05×10^{-02}	4.67×10^{-02}	2.21×10^{-02}	$\downarrow \geq 99.89$	$\downarrow \geq 99.95$	$\downarrow \geq 99.89$
Nickel	$\leq 1.13 \times 10^{-05} a, b$	$\leq 6.57 \times 10^{-06} a, b$	$\leq 5.41 \times 10^{-07}$	$\leq 7.41 \times 10^{-06}$	$\leq 4.55 \times 10^{-07}$	NC	NC	NC
Nicotine	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	$1.00 \times 10^{+00}$	N/A	N/A	N/A
Nicotine-N-Oxide	$\leq 2.16 \times 10^{-04} a$	$\leq 1.26 \times 10^{-04} a$	N/A	N/A	N/A	N/A	N/A	N/A
NNK (CA)	$\leq 1.87 \times 10^{-07} a$	$\leq 1.09 \times 10^{-07} a$	1.61×10^{-04}	1.48×10^{-04}	4.88×10^{-06}	$\downarrow \geq 99.88$	$\downarrow \geq 99.93$	$\downarrow \geq 97.77$
NNN (CA)	$\leq 1.22 \times 10^{-07} a$	$\leq 7.11 \times 10^{-08} a$	1.87×10^{-04}	1.69×10^{-04}	1.13×10^{-05}	$\downarrow \geq 99.93$	$\downarrow \geq 99.96$	$\downarrow \geq 99.37$
Normicotine	$5.48 \times 10^{-05} a$	$5.48 \times 10^{-05} a$	N/A	N/A	N/A	N/A	N/A	N/A
Propionic Acid	$\leq 1.94 \times 10^{-03} b$	$\leq 1.13 \times 10^{-03} b$	N/A	N/A	N/A	N/A	N/A	N/A
Propylene Glycol	$5.14 \times 10^{+00}$	$5.05 \times 10^{+00}$	4.21×10^{-03}	1.36×10^{-02}	3.25×10^{-01}	$1.22 \times 10^{+05}$	$3.70 \times 10^{+04}$	$1.45 \times 10^{+03}$
Propylene Oxide (CA)	$\leq 3.87 \times 10^{-05} a$	$\leq 2.26 \times 10^{-05} a$	4.23×10^{-01}	5.03×10^{-01}	1.23×10^{-04}	$\downarrow \geq 99.99$	$\downarrow \geq 99.99$	$\downarrow \geq 81.69$
Toluene	$\leq 1.52 \times 10^{-04} a$	$\leq 8.84 \times 10^{-05} a$	7.32×10^{-02}	7.69×10^{-02}	1.98×10^{-03}	$\downarrow \geq 99.79$	$\downarrow \geq 99.89$	$\downarrow \geq 95.53$
Water	$2.82 \times 10^{+00}$	$2.47 \times 10^{+00}$	$4.21 \times 10^{+00}$	$8.36 \times 10^{+00}$	$2.45 \times 10^{+01}$	$\downarrow 33.00$	$\downarrow 70.40$	$\downarrow 89.92$
						Average ^c $\downarrow 99.24$	Average ^c $\downarrow 99.40$	Average ^c $\downarrow 89.00$

BLOD = Below limit of quantitation; BLOQ = Below limit of quantitation; NDFB = Not different from background; NC = Not comparable; NR = Not reported; $\downarrow$ = less than. Note: Values preceded with “ $\geq$ ” are based on estimated values. Bolded values were quantifiable. CA = carcinogen. Analytes in italics were tested under a single modified puffing regimen: 70 mL puff, 3 s duration, 30 s interval. ^a analyte was BLOD for all three 50 puff blocks. ^b analyte was BLOQ for all three 50 puff blocks. ^{a,b} analyte was either BLOD or BLOQ for all three 50 puff blocks. ^c excluding PG, VG, and water.

Comparisons to 3R4F were not made for ammonia (Me5), chromium, glycidol, menthol, and nickel. Chromium was not detected, nickel was BLOD/BLOQ in the aerosols of both products, ammonia was NDFB in Me5 aerosols, and the 3R4F reference cigarette is not a mentholated product. The primary ingredients propylene glycol and glycerol were

higher in Me vs. 3R4F mainstream cigarette smoke. Estimated values were again used for aerosol constituents BLOD/BLOQ to provide comparison. Every constituent in Me aerosols which could be compared to 3R4F mainstream cigarette smoke (excluding PG, VG, and water) was reduced. Reductions ranged from $\geq 88.65\%$ (ammonia; Me3-NI) to 99.99% (propylene oxide; Me5-intense). Quantifiable levels of formaldehyde were reduced by $\geq 98.75\%$ and acetaldehyde was reduced by $\geq 99.90\%$. As with VT aerosol constituents, differences in reductions from 3R4F mainstream cigarette smoke between the intense and NI regimens were within 3%, excepting lead (Me3-based on estimated values) and water. Average reductions (excluding PG, VG, and water) for Me aerosol constituents were $\geq 97.47\%$ (Table 6 and Supplementary Table S17).

Of the aerosol constituents not reported in IQOS, benzoic acid, β -Nicotyrine (Me5-intense), ethylene glycol (Me5-intense), cotinine (Me3-intense) and nornicotine were detected in Me5. Twenty seven of 31 aerosol constituents reported for mIQOS were present at detectable levels. Seven of these aerosol constituents (acetaldehyde (intense), formaldehyde, glycerol, menthol, nicotine, propylene glycol and water) were detected in JUUL menthol products. Comparisons were not made for 1,3-butadiene (Me3; uncertain comparison), 2-aminonaphthylene (all products BLOD/BLOQ), 4-aminobiphenyl (uncertain comparison), ammonia (Me5; NDFB), cadmium (all products BLOD/BLOQ), chromium (all products BLOD/BLOQ), isoprene (Me3; uncertain comparison), lead (all products BLOD/BLOQ), nickel (all products BLOD/BLOQ) and glycidol (not recorded). All of the JUUL menthol aerosol constituents which were compared to IQOS were reduced save the primary ingredients glycerol and propylene glycol. Reductions in aerosol constituents ranged from $\geq 61.36\%$ for acrylonitrile (Me3) to $\geq 99.98\%$ (Me5) for diacetyl. Average aerosol constituent reductions (excluding PG, VG, and water) for Me aerosols were $\geq 89.00\%$ (Table 6 and Supplementary Table S17).

4. Discussion

4.1. JUUL Aerosol Characterization

The product characterization in this study was focused on 53 aerosol constituents included in draft and final FDA guidance for the tobacco industry [36,37]. Aerosol generation, collection and chemical analysis were performed by ISO 17,025 accredited CROs with validated methodology (Supplemental Table S1). Across all JUUL flavors, nicotine concentrations and puffing regimes, only 10 of the 53 aerosol constituents were measured above their limit of quantification in at least one flavor and one puffing regime. These aerosol constituents included: acetaldehyde, benzoic acid, β -nicotyrine, cotinine, formaldehyde, glycerol, nicotine, nornicotine, propylene glycol, and water. As expected, the primary e-liquid ingredients in JUUL products (i.e., nicotine, propylene glycol, glycerol, and benzoic acid) were detected in the aerosol.

Of the 10 quantifiable aerosol constituents generated from the JUUL system, acetaldehyde and formaldehyde were the only two quantifiable carbonyls. While formaldehyde was present at detectable levels in the aerosols of all four JUUL aerosols, acetaldehyde was only quantifiable in VT3, Me5 (intense), and Me3 (intense). Acetaldehyde ranged from ≤ 0.022 to $0.044 \mu\text{g}/\text{puff}$, and formaldehyde ranged from 0.022 to $0.087 \mu\text{g}/\text{puff}$. The concentrations of these carbonyl compounds in e-cigarette aerosols have been documented previously in multiple publications. Their presence is hypothesized to result mainly from the thermal degradation of the primary e-liquid ingredients PG and VG, the mechanism of which was summarized in Flora et al. [54]. This reaction is reported to correlate with coil temperature [24,55]. Conversely, the JUUL device has a regulated temperature [34]. This is likely a main contributor to the low levels of carbonyl compounds observed in JUUL aerosols. The measured values for formaldehyde and acetaldehyde, in all samples, did not significantly increase in the end puff block over the middle puff blocks. All analytes that were quantifiable in the end puff blocks were also quantifiable in the previous puff blocks.

The following nicotine-related impurities/degradants were above the limit of quantification for one or more of the JUUL system products: nornicotine (ranged from

0.017–0.024 µg/puff), cotinine (0.00094 to 0.0066 µg/puff), and β-nicotyrine (Me5-intensive, 0.013 µg/puff). The JUUL system e-liquids are formulated with USP-grade nicotine. The USP nicotine standard has acceptance criteria of not more than 0.3% for each nicotine-related compound and 0.8% for total impurities for nicotine-related compounds (i.e., anabasine, anatabine, nicotyrine, cotinine, myosmine, nicotine-N-oxide, and nornicotine) [56], so low levels of nicotine-related impurities in aerosols are expected. For all JUUL system aerosols tested, the summation of nicotine-related compounds did not exceed 0.25% of the measured nicotine concentration, which was well below the USP purity standard.

4.2. Aerosol Constituent Comparison between JUUL System and 3R4F Reference Cigarette

Across all flavors and nicotine concentrations, aerosol constituents were reduced in JUUL products relative to 3R4F mainstream cigarette smoke. Of those which could be compared, all aerosol constituents in the JUUL system (except PG, VG, and water) were present at substantially lower levels relative to the levels in 3R4F mainstream cigarette smoke. Average aerosol constituent reductions (excluding nicotine, PG, VG, and water) for all four JUULPods tested, regardless of puffing regimes, were greater than 98% lower than levels in 3R4F mainstream tobacco smoke (Table 4).

Acrolein, crotonaldehyde, diacetyl, and n-Butyraldehyde were quantifiable in 3R4F mainstream cigarette smoke and were BLOD in JUUL product aerosols. Carbonyl aerosol constituent reductions for JUUL aerosols were > 80% compared to levels in 3R4F mainstream cigarette smoke. The aromatic amines 1-amnonaphthalene, 2-aminonaphthalene, and 4-aminobiphenyl were not detected in JUUL system aerosols. Using aerosol constituent values estimated based on method LOD, these constituents are >99% lower than those in 3R4F. The volatile organic compounds 1,3-butadiene, acrylonitrile, benzene, isoprene, and toluene while quantifiable in 3R4F mainstream cigarette smoke were BLOD in JUUL aerosols. Estimated aerosol constituent values indicate a >99% reduction in the JUUL system relative to 3R4F mainstream cigarette smoke. None of the six metals tested (cadmium, chromium, copper, gold, nickel, and lead) were above LOQ in JUUL system aerosols. Cadmium and gold were BLOD, chromium was NDFB; and copper, nickel, and lead were alternately BLOD or BLOQ across flavors, nicotine concentrations, and puff blocks. Estimated aerosol constituent values indicate a >86% reduction in comparison to levels in 3R4F mainstream smoke.

The primary e-liquid ingredients PG and VG were found to be higher in the JUUL systems versus the 3R4F reference cigarette. PG and VG are common base ingredients in ENDS products [22] and are generally used as humectants in combusted cigarettes [57]. Although no comparison was made for benzoic acid, it is assumed to be higher in JUUL aerosols.

4.3. Aerosol Constituent Comparison between JUUL System and IQOS

All aerosol constituents compared in the JUUL System, excepting PG and VG, were present at lower levels relative to the yields in IQOS aerosol, resulting in > 88% average reductions (excluding PG, VG, and water) across the JUUL products (Table 4). Across all flavors and nicotine concentrations, the carbonyls (i.e., acetaldehyde, acrolein, crotonaldehyde, diacetyl, formaldehyde, and n-Butyraldehyde) were substantially lower for JUUL aerosols than for IQOS. They were all quantifiable in IQOS aerosols, while only acetaldehyde and formaldehyde were above LOQ in JUUL system aerosols.

1-Aminonaphthalene (quantifiable in IQOS aerosols) was not detectable in JUUL system aerosols. 2-Aminonaphthalene and 4-Aminobiphenyl were quantifiable in IQOS tobacco flavor aerosols and were BLOD in all JUUL system aerosols. Volatile organic compounds ([VOCs]; 1,3-butadiene, acrylonitrile, benzene, isoprene, and toluene) were quantifiable in IQOS aerosols but were BLOD in JUUL aerosols. Using estimated values indicates a >61% reduction of these VOCs in JUUL system aerosols compared to IQOS. Among the six metals targeted for analysis in JUUL system aerosols, none were quantifiable. In the available literature on IQOS aerosols, there was information on four metals. Of these,

only lead was quantifiable in IQOS tobacco flavor aerosol. Based on estimated values, lead was reduced by $\geq 86\%$ to $\geq 97\%$ in JUUL aerosols vs. IQOS.

4.4. Aerosol Constituent Comparison with Literature Values

Reilly et al. reported aerosol yields of formaldehyde, acetaldehyde, and nicotine from a 5% nicotine, tobacco flavored JUULPod and device [32]. In that study, aerosols were generated from 10 puffs using a 75 mL puff, a duration of 2.5 s, and an interpuff interval of 30 s. Four replicates were collected. This regime is similar to the NI puffing regime used in the current study. The authors reported quantifiable results for formaldehyde and nicotine while acetaldehyde was reported as BLOD. Normalized by nicotine yield, Reilly et al. reported 1.47×10^{-03} (mg/mg) of formaldehyde. Comparatively, formaldehyde is reported as 9.82×10^{-04} (mg/mg) for VT5, under NI puffing, in the current study. Collaborative study results from the CORESTA e-vapour subgroup have shown intra-laboratory differences from $\sim 40\%$ to 150% for the determination of formaldehyde in the aerosol produced using the same device. The difference between the reported values between these two studies falls within this range.

Talih et al. reported aerosol yields for a range of compounds from an unnamed US market 5% nicotine JUULPod and device [31]. Aerosol samples were collected from 15 puffs using a 66.7 mL puff, a duration of 4 s and an interpuff interval of 10 s. Although puff volume and duration are comparable to the NI regime of our study, Talih and colleagues employed a much shorter interpuff interval. We therefore make a comparison to our intense regime. They reported quantifiable results for nicotine and carbonyl compounds including formaldehyde and acetaldehyde. Normalized by nicotine yield, the authors reported 3.13×10^{-03} mg/mg and 2.98×10^{-03} of mg/m) for formaldehyde and acetaldehyde respectively. Comparatively, our values were 3.50×10^{-04} mg/mg (formaldehyde) and $\leq 2.16 \times 10^{-04}$ mg/mg (acetaldehyde) for VT5, under intense puffing. The values reported by Talih et al. are approximately 10 times higher than the current study, likely due to differences in the puffing regimen. Nevertheless, the values reported by Talih et al. represent a 92.9% and 99.9% reduction in acetaldehyde and formaldehyde, respectively, compared to the 3R4F intense data shown in Table 5. These results indicate that even when testing with a 10 s interpuff interval, JUULSystem aerosol is significantly reduced in acetaldehyde and formaldehyde compared to the mainstream smoke of a 3R4F cigarette.

4.5. Study Limitations

One of the study limitations was use of aerosol constituent levels reported in the literature. There were instances where the aerosol constituent level reported in the literature for the mainstream smoke from the 3R4F reference cigarette or IQOS were quantifiable at a level that was below the analytical method LOD or LOQ used for JUUL Product aerosol constituent measurements. In these cases, no direct comparison of aerosol constituent levels could be made. This was the case for three JUUL aerosol analytes: 4-aminobiphenyl (VT5), 1,3-Butadiene (Me3), and isoprene (Me3). Another limitation was that although originally planned, a validated fit for purpose method for the determination of glycidol was not available when this study was initiated, therefore aerosol constituent levels of glycidol are not reported. Another limitation is that targeted analysis is not comprehensive, but only quantifies pre-determined aerosol constituents. There may be additional constituents present in JUUL system aerosols that were not in the targeted list. To address this, we performed a non-targeted analysis that is described in a companion paper to this work. The values presented in this study were generated using standardized machine smoking and puffing regimes, which are appropriate for comparisons between products. The results are not intended to be representative of possible human exposure.

5. Conclusions

In this study, we measured 53 tobacco-related HPHCs and chemicals in aerosols from four JUUL products currently available on the US market and compared them to cigarette

smoke and the aerosol from a heated tobacco product. Of the 53 toxicants, only 10 were quantifiable in at least one JUUL product aerosol and puffing regime. Average reductions (excluding the primary e-liquid ingredients PG, VG, and water) for all JUUL flavors tested were reduced by more than 98% compared to the 3R4F, and 88% compared to IQOS.

The data indicated that although JUUL aerosols have detectable levels of known degradants of PG/VG (acetaldehyde and formaldehyde) and nicotine-related compounds, the vast majority of tobacco-related HPHCs were not detectable in JUUL aerosols. The low levels of HPHCs in the JUUL system aerosol are likely due to the tightly controlled temperature regulation of the JUUL system designed to reduce byproducts of combustion.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/separations8100168/s1>, Supplementary Tables S1–S17. Table S1: Supplementary Methods. Table S2: Method LOD and LOQ for Me3 analysis. Table S3: Method LOD and LOQ for VT5, VT3 and Me5 analysis. Table S4: Comparison of HPHC and Chemical Intense Aerosol Levels in Virginia Tobacco 5.0% and Smoke Levels in 3R4F Reference Cigarette. Table S5: Comparison of HPHC and Chemical Non-Intense Aerosol Levels in Virginia Tobacco 5.0% and Smoke Levels in 3R4F Reference Cigarette. Table S6: Comparison of HPHC and Chemical Non-Intense Aerosol Levels in Virginia Tobacco 5.0% and Smoke Levels in IQOS regular. Table S7: Comparison of HPHC and Chemical Intense Aerosol Levels in Virginia Tobacco 3.0% and Smoke Levels in 3R4F Reference Cigarette. Table S8: Comparison of HPHC and Chemical Non-Intense Aerosol Levels in Virginia Tobacco 3.0% and Smoke Levels in 3R4F Reference Cigarette. Table S9: Comparison of HPHC and Chemical Intense Aerosol Levels in Virginia Tobacco 3.0% and Aerosol Levels in IQOS Regular. Table S10: Comparison of HPHC and Chemical Intense Aerosol Levels in 10enthol 5.0% and Smoke Levels 3R4F Reference Cigarette. Table S11: Comparison of HPHC and Chemical Non-Intense Aerosol Levels in Menthol 5.0% and Smoke Levels in 3R4F Reference Cigarette. Table S12: Comparison of HPHC and Chemical Intense Aerosol Levels in Menthol 5.0% and Aerosol in IQOS Menthol. Table S13: Comparison of HPHC and Chemical Intense Aerosol Levels in Menthol 13.0% and Smoke Levels 3R4F Reference Cigarette. Table S14: Comparison of HPHC and Chemical Non-Intense Aerosol Levels in Menthol 3.0% and Smoke Levels in 3R4F Reference Cigarette. Table S15: Comparison of HPHC and Chemical Intense Aerosol Levels in Menthol 3.0% and Aerosol Levels in IQOS Menthol. Table S16: Comparison of HPHCs in VT3 aerosol vs. comparator products. Table S17: Comparison of HPHCs in Me3 aerosol vs. comparator products.

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