Impact of more intense smoking parameters and flavor variety on toxicant levels in emissions of a Heated Tobacco Product.

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Abstract

Introduction: IQOS HEETS are promoted as reduced risk alternatives to cigarettes. Although some studies have investigated the chemical composition of HEETS emissions, little is known on whether toxicant levels in such emissions are affected by different puffing parameters and flavor varieties. This has important implications when assessing actual human exposure, since IQOS users develop a specific and personalized puffing behavior and may use different HEETS variants.

Methods: This study measured the levels of nicotine, Total Particulate Matter (TPM), carbonyl compounds and tobacco-Specific Nitrosamines (TSNAs) in the emissions of nine differently flavored HEETS and two cigarettes (1R6F and Marlboro Red, MR). Emissions from Yellow HEETS, 1R6F and MR were collected using the World Health Organization Intense (WHOI) smoking regime and four more intense smoking regimes.

Results: Yellow HEETS aerosol contained lower levels of toxicants compared to 1R6F and MR smoke. More intense smoking regimes increased carbonyls release in cigarette smoke, whereas only higher puff frequency led to lower levels of toxicants in Yellow HEETS aerosol. Some HEETS varieties exhibited higher levels of formaldehyde and TSNAs in their aerosol compared to Yellow HEETS.

Conclusions: Puff frequency was identified as the only smoking parameter that significantly lowered the release of almost all toxicants in Yellow HEETS, whereas a combination of higher puff volume and puff duration led to increased levels of some carbonyls. Differences in toxicants levels between various commercially-available HEETS have important implications when assessing their health impact, as their consumption might induce different toxicant exposure and health effects.

Implications:

HEETS release about half as much nicotine and substantially lower levels of toxicants compared to cigarettes. Literature data showed that puffing intensity is increased in cigarette smokers switching to HEETS, maybe in reaction to these lower nicotine levels. Our results show a differential impact of increased puff frequency, puff duration and puff volume in the release of toxicants from HEETS. Thus, industry-independent studies on puff topography are critical to make choices for the most relevant puffing regime for HTP regulation. Regulators should consider evaluating the health impact of multiple HEETS varieties, as the tobacco filler composition significantly affects the release of certain toxicants.

Keywords

IQOS HEETS, cigarettes, smoking regime, nicotine, TPM, carbonyls, tobacco-specific nitrosamines, puff frequency.

1. Introduction

Tobacco consumption is a leading cause of morbidity and mortality worldwide. It is estimated that each year about 8.7 million people lose their life due to tobacco-related diseases. This includes 1.3 million non-smokers exposed to side stream emissions [1]. Due to this alarming scenario, global political efforts to reduce the public health burden of tobacco use have been successfully implemented. Thanks to such measures, global tobacco consumption among people aged 15 or older declined from 33.3% in 2000 to 24.9% in 2015, and it is expected to fall to 22% by 2025 [2].

In response to anti-cigarette policies and the consequent decrease in cigarette use, tobacco industries designed and launched novel tobacco products, such as heated tobacco products (HTPs) [3]. An HTP can consist of a pen-like heating device that heats a tobacco-containing stick at lower temperatures than regular cigarettes [4]. The heating process generates an aerosol containing nicotine and other potentially harmful chemicals [5-7]. The various commercially available HTPs mainly differ due to brand-specific designs and the composition of the tobacco stick [8]. For instance, the HTP device marketed by Japan Tobacco International (Ploom TECH+) uses a heating mechanism that vaporizes a liquid solution containing nicotine and flavors that is then passed through a capsule containing tobacco. Contrarily, the HTP from British American Tobacco (glo) and from Philip Morris International (PMI; IQOS) use a heating system to heat specifically-designed tobacco sticks [4]. The IQOS device, with its specific tobacco sticks (HEETS) was launched in 2014, and it is nowadays leader in the global HTP market [8]. The claims of reduced toxicity represent the most important reason for the worldwide diffusion of IQOS. In fact, PMI promotes IQOS as "reduced risk product" and "cleaner and smoke-free alternative" to cigarettes [9]. Such risk-reduction claims can attract both (young) non-smokers and smokers that are willing to quit, as they perceive IQOS as less harmful than cigarettes [9,10]. Noteworthy, a 2020 survey showed that susceptibility to try IQOS in England, Canada and USA was higher than cigarettes, albeit lower than e-cigarettes [9].

However, despite industry statements, little is known about the chemical composition of IQOS HEETS emissions, as well as their (long-term) impact on human health. Therefore, the industry claims of reduced toxicity must be scientifically validated. Several research groups analyzed the emissions of HEETS and compared them with smoke generated from cigarettes [5,7,11-13]. In these studies, both HEETS and cigarettes were puffed through smoking/vaping machines, in which puffing parameters such as puff volume, puff duration and puff frequency can be customized. Both industry-related and independent researchers obtained emissions applying standardized smoking regimes, namely International Organization for Standardization (ISO) and World Health Organization Intense smoking of cigarettes (WHOI). ISO is characterized by a puff volume of 35ml, a puff duration of 2s and a puff frequency of 60s [14]. In the more intense WHOI regime, mainstream emissions are collected at a puff volume of 55ml, a puff duration of 2 seconds and at a puff frequency of 30 seconds [15]. When applying the same smoking regime, HEETS were found to deliver lower but still considerable levels of nicotine compared with regular cigarettes, posing substantial concerns about the addictive potential of this newly developed product [12,13]. On the other hand, significantly lower levels of aldehydes (80-95%), tobacco-specific nitrosamines (TSNAs, 90%) and volatile organic compounds (97-99%) were detected in HEETS aerosol compared to cigarette smoke [13,16]. In addition, higher levels of toxicants were detected applying WHOI compared with ISO, showing the direct correlation between the applied smoking parameters and the release of hazardous chemicals [5,12]. The correlation between toxicant release and smoking regime is crucial considering the personal puffing behavior that every smoker develops [17]. In fact, little is known about how HEETS are actually puffed by users. A PMI-funded study showed that cigarette smokers switching to HEETS drew puffs with higher puff duration (from 1.6 s at baseline to 2.1 s at day 4) and puff frequency (from 3.5 puffs/min at baseline to 5.2 puffs/min at day 4) compared to when using their favorite cigarette brand [18]. Similarly, Japanese smokers switching to HEETS were shown to take more frequent puffs than when smoking cigarettes [19]. Moreover, higher total puff volumes were measured in cigarette smokers switching to Ploom Tech+ in a 5-day industry-funded study compared to smokers that kept consuming their favorite cigarette brand (1693 mL vs 847 mL) [20]. Furthermore, the results of another industry-funded study show that HTP users take puffs with higher volumes compared with cigarette smokers (730 mL vs 682 mL) [21].

Considering such real-life smoking topography data, it is crucial to understand the impact of different smoking parameters on HEETS aerosol composition. This has critical implications when determining the actual exposure to toxicants of IQOS users, that puff HEETS with personalized and diverse smoking behaviors.

In addition to smoking topography, composition of the tobacco stick (tobacco type, presence/levels of flavorings and other additives) may influence the levels of toxicants in the emissions. To date, nine HEETS differing in flavor are commercially available in The Netherlands, and the levels of toxicants in their aerosols are unknown. Measuring the levels of toxicants in the aerosol of all the commercially available HEETS represents a first, critical step to properly assess their toxicity on human health.

Therefore, this study aims at assessing the link between more intense puffing regimes and toxicant release in IQOS Yellow HEETS. For comparison, we also measured the levels of hazardous chemicals in the smoke of two cigarettes, 1R6F and Marlboro Red (MR). Furthermore, we determined the levels of various toxicants in the emissions of nine differently flavored and commercially available HEETS.

2. Materials and Methods

2.1 Product acquisition, storage and conditioning

1R6F reference cigarettes were purchased from the University of Kentucky (Kentucky Tobacco Research & Development Center). Marlboro Red cigarettes (MR, Philip Morris USA Inc., Richmond, VA) were purchased online. IQOS 3 DUO (Phillip Morris Products S.A., Switzerland) and cartons of 9 IQOS HEETS (IQOS Amber, IQOS Blue, IQOS Bronze, IQOS Green, IQOS Russet, IQOS Sienna, IQOS Teak, IQOS Turquoise and IQOS Yellow selections) were purchased online in September 2020. Before smoking, all tobacco products were conditioned at 22°C and 60% relative humidity for at least 48 hours, in accordance with ISO 3402 [22]. The butt length of the assessed cigarettes was marked in accordance to ISO 4387 and World Health Organization

Tobacco Laboratory Network, Standard Operating Procedure for Intense Smoking [15,23]. According to manufacturer's instructions, the IQOS device was cleaned after puffing 20 HEETS [24].

2.2 Mainstream emissions production

Mainstream emissions from 1R6F and MR were generated on a 10-port linear smoking machine (Cerulean, Milton Keynes, United Kingdom) according to the World Health Organization standard operating procedure for intense smoking of cigarettes (WHOI) [15]. Mainstream aerosol from IQOS HEETS was obtained using a pre-defined puff count of 10 puffs per stick on a 4-port linear vaping machine (Borgwaldt, Körber Technologies GmbH, Hamburg, Germany) according to the WHO Intense protocol [15]. Emissions from both cigarettes and Yellow HEETS were generated applying WHOI and a total of four more intense smoking regimes, as indicated in **Table 1**. Such smoking regimes were chosen to assess the impact of more intense smoking parameters on toxicant release.

2.3 Extraction procedures and determination of chemical analytes

Chemical determination of the analytes measured in this study was performed in accordance to the WHO TobLabNet standard operating procedures (SOPs) 03, 08 and 10 [25-27].

Carbon monoxide (CO) was measured with a nondispersive infrared (NDIR) analyzer integrated in the smoking machine (Borgwaldt, Körber Technologies GmbH, Hamburg, Germany). CO levels per product were calculated as described in the SOP 10, and the limit of detection (LOD) and limit of quantification (LOQ) were set accordingly (LOD: 0.43 mg/product, LOQ: 0.86 mg/product) [25].

Mainstream emissions from all the assessed tobacco products were collected on a Cambridge glass fiber filter pad (44 mm diameter). Prior and upon puffing, the Cambridge glass fiber filter pads were weighed, to calculate total particulate matter (TPM) release from each product [25]. Nicotine was extracted from the filter pad in 20 ml extraction solution (2.5 L isopropanol, 1.25 ml heptadecane solution and 3.0 ml ethanol) by shaking at 120 RPM for 20 minutes. Nicotine standard solution and working standards were prepared by mixing nicotine (Acros Organics) with extraction solution. Nicotine levels were measured by gas chromatography with flame ionization detector (Borgwaldt, Körber Technologies GmbH, Hamburg, Germany). The limit of detection and quantification for nicotine were defined according to the TobLabNet SOP adopted (SOP 10, LOD: 0.01 mg/product, LOQ: 0.02 mg/product) [25].

Four TSNAs, namely N-nitrosonornicotine (NNN), nicotine-derived nitrosamine ketone (NNK), N′-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB) were extracted from Cambridge glass fiber filter pads. After smoking, each filter pad was extracted with 20 ml aqueous ammonium acetate solution (Sigma, 100 nmol/L) by shacking at 250 RPM for 30 minutes. Prior to analysis, the extracts were filtered with 25ml x 0.45 µm GD/X nylon syringe filters (Whatman, type: Spartan 30/0.45 RC), purchased from Avantor (VWR International BV, Amsterdam, The Netherlands).

The concentrations of NNN, NNK, NAT and NAB were determined through liquid chromatography-Mass Spectrometry (LC-MS) (LC system: Shimadzu Benelux, type: Nexera X2, 's-Hertogenbosch, the Netherlands. Analytical column: Acquity UPLC BEH C18 1.7µm. Eluent A: 10 mM ammonium acetate, Eluent B: 0.1% acetic acid (Brand) in methanol (Biosolve). Flow:

0.4ml/min. MS system: AB Sciex (type: QTrap 6500, Nieuwerkerk aan den Ijssel, The Netherlands. Ion Spray Voltage: 5500). The limit of detection and quantification for TSNAs was set to the lowest point of the applied calibration curve (LOD: 0.5 ng/product, LOQ: 1 ng/product) [27].

The carbonyl compounds formaldehyde, acetaldehyde, acetone, acrolein, glyoxal and methylglyoxal were trapped on Cambridge glass fiber filter pads combined with cartridges filled with 300 mg CX-572 particles. Upon puffing, carbonyls were extracted from the filter pad and CX-572 cartridge in 10 ml carbon-disulfide/methanol solution (1/4 v/v) by shacking at 120 RPM for 30 minutes. Samples were derivatized by mixing 0.5 ml eluate with 0.2 ml 2,4-dinitrophenylhydrazine (DNPH) solution. After 10 minutes, ethanol was added to reach a final volume of 5 ml and sufficient amount of liquid was transferred in autosampler vials. Carbonyls concentrations were measured through liquid chromatography with diode array detection (LC-DAD) (Shimadzu Benelux, 's-Hertogenbosch, The Netherlands). The limit of detection and quantification for carbonyl compounds was set to the lowest point of the applied calibration curve (LOD: 0.5 µg/product, LOQ; 1 µg/product) [26].

2.4 Statistical data analysis

GraphPad Prism 8.0 software (La Jolla, USA) was applied to carry out statistical analyses and plot the data. The concentrations of nicotine and carbon monoxide were expressed as mg/product, the levels of carbonyls were expressed as µg/product and the concentrations of NNN, NNK, NAT and NAB were expressed as ng/product. Each concentration presented in tables or plotted in graphs is depicted as mean ± standard deviation (SD) of at least three independent measurements, and statistical testing of differences between levels of analytes in the assessed products was carried out with a one-way ANOVA test followed by a Tukey's post-hoc test for multiple comparisons. Differences were considered statistically significant if $P<0.05$, and indicated as *$P < 0.05$ and ** $P<0.01$.

3. Results

3.1 Aerosol from Yellow HEETS contains significantly lower levels of several toxicants compared with cigarette smoke

In order to chemically characterize Yellow HEETS emissions and to compare them with cigarettes smoke, the concentration of several toxicants was assessed in Yellow HEETS, 1R6F and Marlboro Red mainstream emissions. All emissions were retrieved applying the WHO Intense smoking regime. Yellow HEETS aerosol contained around 55% less nicotine and 25% less total particulate matter (TPM) than cigarette smoke, whereas carbon monoxide levels were found to be below the limit of quantification. Furthermore, the levels of several carbonyl compounds (formaldehyde, acetaldehyde, acetone, acrolein, glyoxal and methylglyoxal) were 63-89% lower in Yellow HEETS aerosol compared with 1R6F and MR smoke. Finally, Yellow HEETS aerosol contained 95-98% less TSNAs than 1R6F and MR (**Table 2**).

3.2 More intense smoking parameters differentially affect toxicant release in cigarette and Yellow HEETS emissions

To unravel the effect of more intense smoking parameters on toxicant delivery, 1R6F, MR and Yellow HEETS emissions were collected applying four more intense smoking regimes, in which puff volume and/or puff duration and/or puff frequency were systematically changed (**Table 1**). **Figure 1** reports the percentage of variation in toxicant levels detected in 1R6F, MR and Yellow HEETS emissions applying the more intense smoking regimes in comparison with WHOI. The levels retrieved with WHOI were set at 100% (horizontal line in **Figure 1**), to visualize the differences when applying more intense smoking parameters.

Interestingly, nicotine and TPM release was almost not affected, whereas all four more intense smoking topographies substantially impacted carbonyls and TSNAs release in the smoke of 1R6F and MR. More specifically, the release of carbonyl compounds was always significantly increased (up to 400% in some instances) in cigarette smoke applying more intense smoking topographies, with the exception of higher puff duration in MR (**Figure 1A-D**). On the other hand, the impact of more intense smoking parameters on TSNAs levels in cigarette smoke was lower, and their release was only increased in MR emissions when a combination of higher puff volume and puff duration was applied (**Figure 1D**).

In general, the more intense smoking parameters had a smaller impact on toxicants release in Yellow HEETS aerosol compared to cigarette smoke. In fact, an increased puff volume did not significantly impact toxicant release in Yellow HEETS aerosol, as shown in **Figure 1A**. In addition, only formaldehyde levels were substantially increased in Yellow HEETS emissions when a higher puff duration was applied (**Figure 1B**) On the other hand, an increased puff frequency led to a significant reduction in the levels of most carbonyls and all TSNAs in Yellow HEETS aerosol. (**Figure 1C**). Finally, a combination of higher puff volume and puff duration induced higher formaldehyde and methylglyoxal levels in Yellow HEETS emissions, as depicted in **Figure 1D.**

3.3 Toxicant levels differ between different HEETS flavors

Emissions from nine commercially available HEETS differing in flavor were retrieved applying WHOI, and the levels of toxicants in their emissions were compared. Nicotine and TPM concentrations were not significantly different in the aerosol of the assessed products (**Figure 2A, Supplementary Figure 1**). On the other hand, Yellow and Bronze HEETS aerosol contained the lowest TSNAs levels, and various other HEETS emitted substantially higher concentrations of NNN, NNK and NAT (**Figure 2B**). In all variants, NAT was the most abundant TSNA. With regard to the carbonyl compounds, acetaldehyde was by far the most abundant compared to the other investigated carbonyls. In general, no differences were observed in the levels of carbonyls released by the different HEETS, although three HEETS variants (Amber, Bronze and Green) emitted higher formaldehyde levels than Yellow HEETS (**Figure 2C** and **D**).

Altogether, some differently flavored HEETS emitted higher TSNAs and formaldehyde levels than Yellow HEETS, while no significant differences were measured for nicotine, TPM and for the other assessed carbonyls.

4. Discussion

In this study, we first compared the levels of known hazardous compounds in the emissions of one HEET variant (IQOS Yellow HEETS) and two cigarettes (1R6F and Marlboro Red). We detected significantly lower levels of nicotine, TPM, carbonyl compounds and TSNAs in the aerosol of Yellow HEETS compared with 1R6F and Marlboro Red smoke. Such results are very much in line with the body of evidence available in literature that describes lower levels of harmful and potentially harmful compounds (HPHCs) in IQOS HEETS emissions compared with cigarette smoke [5,12,28,29]. The IQOS device heats the tobacco material at lower temperature than regular cigarettes, resulting in a reduced thermal decomposition and reduced combustion of the tobacco [30].

HEETS emissions collected with more intense smoking parameters were shown to contain higher levels of phenols and carbonyl compounds, suggesting a correlation between puffing intensity and toxicant release in HEETS aerosol [31]. To further investigate this correlation, we collected emissions from 1R6F, MR and Yellow HEETS applying four more intense smoking regimes. In general, the release of carbonyl compounds was majorly affected by more intense smoking parameters in 1R6F and MR smoke. On the other hand, an almost analogous trend of variation was observed for nicotine, TPM and TSNAs, that were less impacted by more intense smoking regimes. Carbonyl compounds are formed when tobacco is burned/heated, whereas nicotine, TPM and TSNAs are present in the tobacco material and are transferred to the emissions [16]. Therefore, the current study shows that more intense smoking regimes can substantially increase the formation of carbonyls in cigarette smoke, but minorly affect the transfer efficiency of nicotine, TPM and TSNAs.

The applied smoking parameters always induced higher carbonyls and TSNAs levels in 1R6F and MR smoke, with the exception of higher puff duration. In fact, our data show that a 4-seconds puff duration with a 55mL puff volume induced higher carbonyls release in 1R6F smoke, but lower in MR emissions. A previous study showed that distinct brand of cigarettes can emit different levels of aldehydes, due to their tobacco composition, filter design and the applied smoking regime [32].

Regarding Yellow HEETS, formaldehyde levels were increased when higher puff duration was applied, whereas both formaldehyde and methylglyoxal levels were increased when a combination of higher puff volume and puff duration was applied. Higher formaldehyde levels were previously detected in IQOS HEETS emissions retrieved with a more intense puffing regime (90 mL puff volume and 3 seconds puff duration) than WHOI [33]. Similarly, Ardati *et al* identified puff duration and puffing flow rate as critical parameters affecting toxicants release in IQOS HEETS aerosol [31]. In fact, HEETS puffed with more intense smoking parameters (110 mL puff volume and 4 seconds puff duration) emitted significantly higher formaldehyde, acetaldehyde, acetone and acrolein levels than HEETS puffed with WHOI [31]. Furthermore, the correlation between more intense puffing parameters and increased carbonyl release was previously described in the emissions of several commercially available electronic cigarettes [34].

In our study, the only smoking parameter that influenced both carbonyls and TSNAs release in Yellow HEETS aerosol was puff frequency. Specifically, the concentration of both carbonyl compounds and TSNAs was significantly reduced in Yellow HEETS emissions when puff frequency was increased (from a 30s to a 15s puff interval). On the other hand, the release of all assessed carbonyl compounds was significantly increased in 1R6F and MR smoke when higher puff frequency was applied. The distinct impact of puff frequency on toxicant levels in cigarettes and Yellow HEETS emissions mainly depends on their different product design. Cigarettes are smoked until the cigarette butt is reached, thus with higher puff frequency more puffs per cigarette are taken (**Supplementary Material 1**). On the other hand, with higher puff frequency more puffs per time unit are taken from an operating IQOS, resulting in lower temperatures reached by the tobacco material in the HEET (**Supplementary Material 2**). Hence, the HEET will be puffed faster and at a lower temperatures, and this is the reason for the lower toxicant levels detected in Yellow HEETS aerosol when applying higher puff frequency [30].

With regard to the chemical composition of the aerosol of different HEETS, we did not observe any significant difference in nicotine and TPM release from the assessed HEETS. Such finding is in line with the results of previous studies that reported similar nicotine levels in the emissions of differently flavored HEETS [5,7,35]. However, we detected slightly lower levels of nicotine in HEETS emissions compared with previous studies, likely due to the different amount of puffs (10 in our study, 12 in previous investigations) taken per HEET [5,12].

On the other hand, two HEETS variants (namely HEETS Yellow and HEETS Bronze) emitted significantly lower TSNAs levels than the other assessed HEETS. In addition, formaldehyde levels were substantially higher in HEETS Amber, Bronze and Green emissions compared with Yellow HEETS. Such differences in toxicant release among the assessed HEETS can depend on the different tobacco blends present in the tobacco filler or different class/levels of (flavoring) additives. The materials and the composition of the different IQOS HEETS were notified by the manufacturer to the Dutch regulator via the European Union Common Entry Gate (EU-CEG), following the obligation by the EU Tobacco Products Directive [36]. IQOS HEETS consist of tobacco, humectants compounds (glycerol, water, cellulose, guar gum and propylene glycol) and natural and artificial flavorings [36]. The filler of the assessed HEETS contains around 200 mg of tobacco, divided into 3 different blends (Virginia, Burley and Oriental), present in similar ratios in the assessed products [36]. On the other hand, the different HEETS varieties contain variable levels of propylene glycol, filtration material and papers and wrappers, that might be responsible for the detected differences in TSNAs release [36]. Furthermore, it has previously been shown that the tobacco filler of HEETS contains TSNAs [16]. Although we did not measure the concentrations of TSNAs in the tobacco material of the studied products, the assessed HEETS emitted variable TSNAs levels, suggesting a correlation between the composition of the tobacco filler and TSNAs release. This has important implications when assessing the health burden of HEETS, since we now show that distinct flavor varieties emit different levels of carcinogens, thus their consumption might induce different health effects.

All the assessed IQOS HEETS delivered substantially lower levels of toxicants compared with cigarettes, and around 50% less nicotine. Therefore, one could speculate that cigarette smokers switching to HEETS might consume around two times more HEETS per day, implying that they will adapt their daily product consumption to match their desired nicotine intake [37]. Therefore, we included a supplementary table showing toxicant release from the two regular cigarettes and from

Yellow HEETS normalized for nicotine release. When normalized for nicotine, the toxicant levels in 1R6F and MR cigarettes and HEETS are more comparable than when expressed per product item (i.e. per cigarette or HEET), since HEETS release about half nicotine than 1R6F and MR (**Supplementary Table 1**). Thus, HEETS users might be exposed to higher toxicant levels than expected based on the levels per HEET, since they will adapt their behavior to achieve their desired nicotine intake level. Moreover, smokers switching to HTPs were shown to puff such products applying higher puff volume, puff duration and puff frequency, likely to satisfy their desired nicotine intake per puff [18-21]. Therefore, regulators should consider using more intense smoking regimes than WHOI when retrieving HEETS emissions in laboratory settings. Importantly, we now show that more intense puff frequency can lower the levels of some toxicants in HEETS emissions, whereas a combination of higher puff duration (4 s vs 2 s) and higher puff volume (90 ml vs 55 ml) can lead to higher levels of formaldehyde and methylglyoxal. When applying an even higher puffing volume (110 ml) and a 4 seconds puff duration, Ardati et al. also found significantly higher levels of formaldehyde, acetone and acrolein as compared to the current study [31].

Our study and previous reports clearly show a substantial reduction in the levels of certain carcinogens and toxic compounds in IQOS HEETS emissions compared with cigarettes, but this cannot directly be translated in reduced impacts on human health. In fact, IQOS HEETS aerosol contains large doses of humectants (glycerol and propylene glycol), and their health burden on human respiratory system have not been explored [38-40]. Moreover, the U.S. Food and Drug Administration identified a total of 80 compounds that are exclusively detected in IQOS HEETS emissions, or have been measured in higher levels in IQOS HEETS aerosol compared with cigarette smoke [41]. For instance, higher levels of hydroxyacetone, furfural, 5-methylfurfural and 2(5H) furanone were measured in IQOS HEETS aerosol compared to 3R4F smoke [35,42-44]. Furthermore, GC-MS headspace analysis of unused IQOS HEETS filters showed the release of formaldehyde cyanohydrin, highly toxic at very low concentration [24].

Thus, our data and previous studies clearly indicate that HEETS cannot be considered a risk-free product, and more (independent) investigations assessing the health burden of HEETS and other HTPs are needed. For instance, cigarette smoke and HEETS aerosol were shown to similarly impair heart rate, arterial stiffness and myocardial systolic and diastolic functions in humans [45,46].

Human studies are critical to compare the health burden of cigarette and HTPs, but should also aim at evaluating the smoking behaviors of IQOS users, in order to apply such real-life smoking topographies to program smoking machines in laboratory settings [31]. However, assessing the health effects of the various commercially available HTPs remains challenging, due to the variety of heating devices and tobacco sticks on the market [47]. Moreover, the majority of HTP users also smoke regular cigarettes and/or electronic cigarettes, complicating the assessments of (pulmonary) toxicity and tumor promoting potencies of HTP aerosols [48].

In conclusion, our study shows that the toxic components in tobacco smoke are also present in the emissions of IQOS HEETS, albeit in much lower levels. The emissions from regular cigarettes are more affected by more intense smoking parameters than IQOS Yellow HEETS aerosol, and several commercially available IQOS HEETS emit different levels of TSNAs.

Table 1: Overview of the smoking regimes applied to retrieve emissions from 1R6F, Marlboro Red and IQOS Yellow HEETS. Parameters differing from WHOI are presented in bold.

Smoking topography	Puff volume (mL)	Puff frequency (s)	Puff duration (s)	Flow rate (mL/s)
World Health Organization Intense (WHOI)	55	30	2	27.5
Intense regime 1	**90**	30	2	45
Intense regime 2	55	30	**4**	13.75
Intense regime 3	55	**15**	2	27.5
Intense regime 4	**90**	30	**4**	22.5

Table 2: Concentration of nicotine, total particulate matter (TPM), carbon monoxide, carbonyl compounds (formaldehyde, acetaldehyde, acetone, acrolein, glyoxal and methylglyoxal) and TSNAs (NNN, NNK, NAT and NAB) measured in the mainstream emissions of 1R6F, Marlboro Red and Yellow HEETS retrieved applying WHOI. Each value represents the mean (±SD) of three independent measurements, and statistical significance comparing the levels in Yellow HEETS emissions and in the smoke of 1R6F and MR is indicated as $^*P < 0.05$ and $^{**}P < 0.01$.

Analyte	1R6F	Marlboro Red	Yellow HEETS	
Nicotine (mg/product)	2.6 ± 0.19	2.5 ± 0.11	1.2 ± 0.14	*
TPM (mg/product)	49.24 ± 3.18	55.94 ± 2.37	39.77 ± 2.80	*
Carbon monoxide (mg/product)	31.5 ± 0.8	30.2 ± 1.3	< LOQ	
Formaldehyde (µg/product)	29.3 ± 9.27	23.2 ± 4.20	5.4 ± 1.70	**
Acetaldehyde (µg/product)	550.5 ± 51.0	422.6 ± 31.60	156.7 ± 13.8	*
Acetone (µg/product)	259.5 ± 23.7	222.0 ± 17.50	28.6 ± 2.82	**
Acrolein (µg/product)	39.0 ± 3.07	27.1 ± 2.55	4.5 ± 2.62	**
Glyoxal (µg/product)	56.2 ± 5.71	45.7 ± 3.49	15.5 ± 2.50	**
Methylglyoxal (µg/product)	57.2 ± 9.98	53.5 ± 4.87	11.3 ± 5.4	*
NNN (ng/product)	240.8 ± 13.20	179.7 ± 7.13	3.2 ± 0.64	**
NNK (ng/product)	201.0 ± 17.10	137.0 ± 2.20	2.5 ± 0.34	**
NAT (ng/product)	256.6 ± 29.10	182.4 ± 3.69	9.4 ± 1.42	**
NAB (ng/product)	29.1 ± 4.18	21.5 ± 0.61	1.2 ± 0.10	**

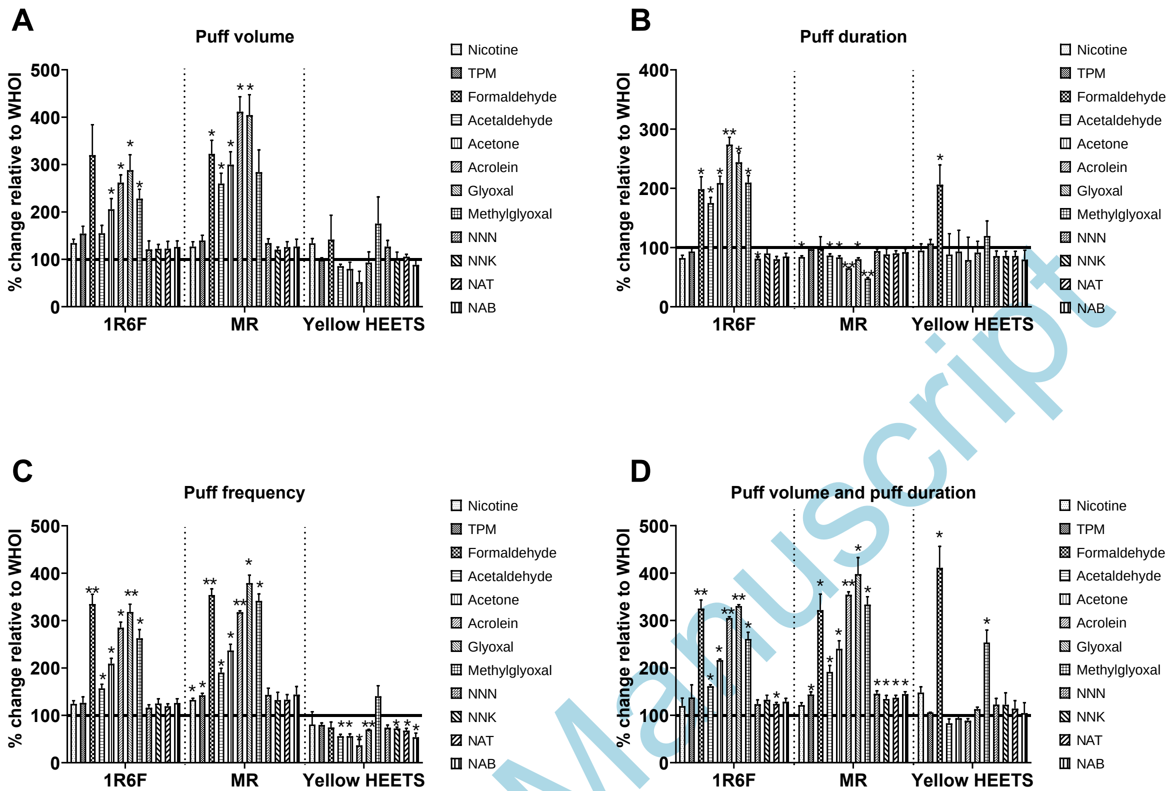

Figure 1: Percentage of variation in the levels of nicotine, TPM, carbonyl compounds and TSNAs measured in 1R6F, Marlboro Red (MR) and Yellow HEETS emissions when applying higher puff volume (from 55ml to 90ml, **A**), longer puff duration (from 2s to 4s, **B**), higher puff frequency (from 30s to 15s, **C**) or higher puff volume and higher puff duration (from 55ml to 90ml, and from 2s to 4s, **D**) in comparison with WHOI. Each bar represents the mean (±SD) of three independent measurements, and statistical significance comparing the levels detected with WHOI with the more intense smoking regimes is indicated as *P < 0.05 and ** P<0.01.

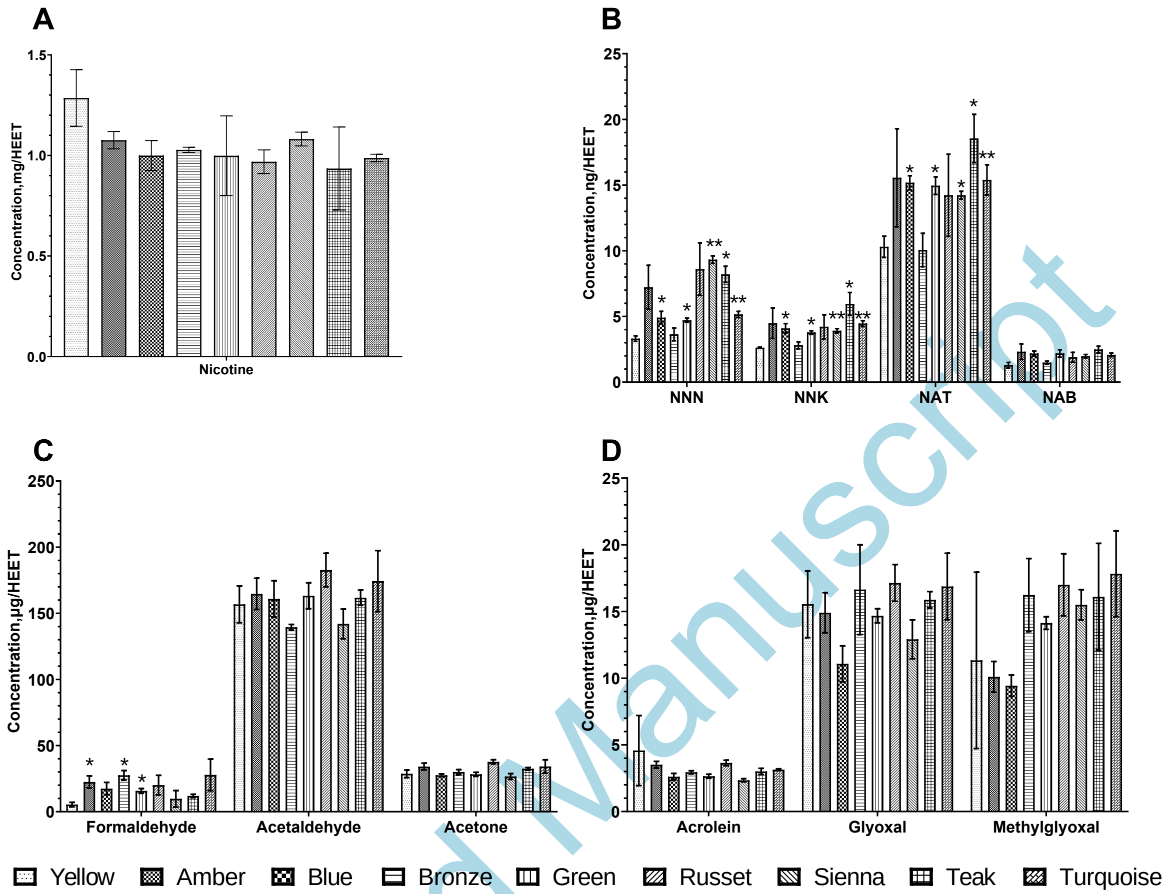

Figure 2: Concentration of nicotine (mg/HEET, **A**), tobacco-specific nitrosamines (NNN, NNK, NAT and NAB, ng/HEET, **B**) and carbonyl compounds (Formaldehyde, Acetaldehyde, Acetone, Acrolein, Glyoxal and Methylglyoxal, µg/HEET, **C** and **D**) measured in the emissions of nine commercially available HEETS, puffed applying WHOI. Each bar represent the mean (±SD) of three independent measurements, and statistical significance comparing Yellow HEETS and the other HEETS is indicated as *P < 0.05 and ** P<0.01.

5. Data availability

All the raw data used for this study are included in the supplementary table attached in the submission.

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7. Declaration of interest

The authors declare no conflict of interest

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