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Comparison of the chemical composition of aerosols from heated tobacco products, electronic cigarettes and tobacco cigarettes and their toxic impacts on the human bronchial epithelial BEAS-2B cells

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Title Page

Comparison of the chemical composition of aerosols from heated tobacco products, electronic cigarettes and tobacco cigarettes and their toxic impacts on the human bronchial epithelial BEAS-2B cells

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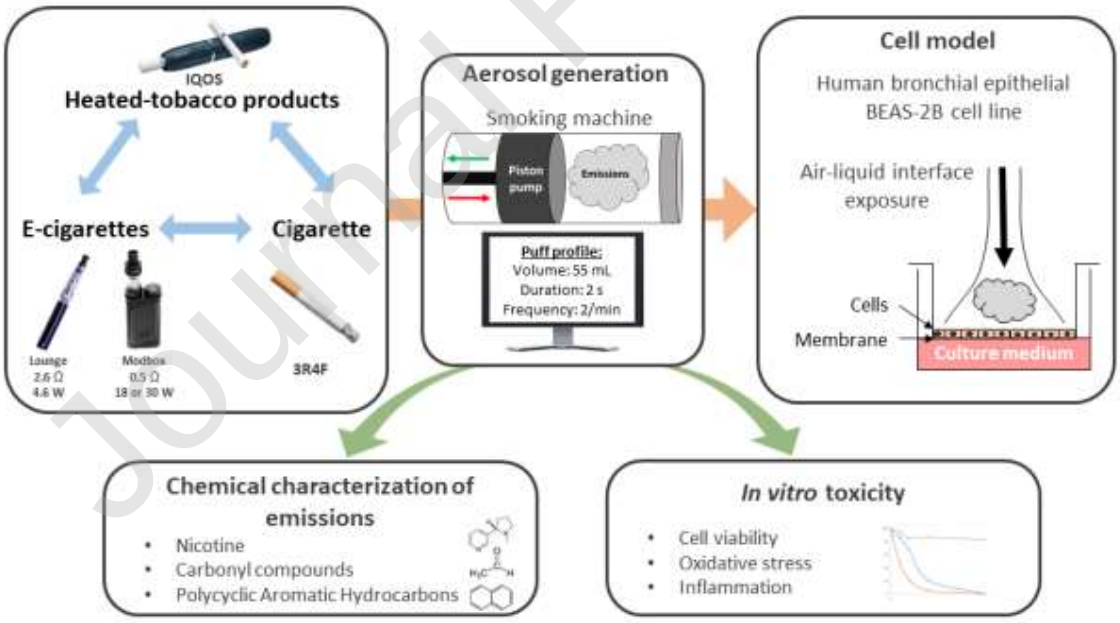
Graphical abstract



Highlights

- Comparison of heated tobacco product (HTP), e-cig and cigarette toxicity is lacking
- HTP emits lesser carbonyls and PAHs than cigarette but more than e-cig
- Cytotoxicity of HTP is lower than that of cigarette but stronger than that of e-cig
- Increasing e-cig power impacts toxic compound levels and related oxidative stress
- All devices have the potential to induce oxidative stress and inflammatory response

Abstract

The electronic cigarettes (e-cigs) and more recently the heated tobacco products (HTP) provide alternatives for smokers as they are generally perceived to be less harmful than conventional cigarettes. However, it is crucial to compare the health risks of these different emergent devices, in order to determine which product should be preferred to substitute cigarette. The present study aimed to compare the composition of emissions from HTP, e-cigs and conventional cigarettes, regarding selected harmful or potentially harmful compounds, and their toxic impacts on the human bronchial epithelial BEAS-2B cells. The HTP emitted less polycyclic aromatic hydrocarbons and carbonyls than the conventional cigarette. However, amounts of these compounds in HTP aerosols were still higher than in e-cig vapours. Concordantly, HTP aerosol showed reduced cytotoxicity compared to cigarette smoke but higher than e-cig vapours. HTP and e-cig had the potential to increase oxidative stress and inflammatory response, in a manner similar to that of cigarette smoke, but after more intensive exposures. In addition, increasing e-cig power impacted levels of certain toxic compounds and related oxidative stress. This study provides important data necessary for risk assessment by demonstrating that HTP might be less harmful than tobacco cigarette but considerably more harmful than e-cig.

Abbreviations

ALI: air-liquid interface

e-cig: electronic cigarette

GSH: reduced glutathione

GSSG: oxidized glutathione

HTP: heated tobacco products

Mb18W: Modbox e-cig model set at 18 W

Mb30W: Modbox e-cig model set at 30 W

PAHs: polycyclic aromatic hydrocarbons

Keywords: heat-not-burn tobacco; e-cigarette; lung; PAHs; carbonyls; toxicity

1. Introduction

The tobacco epidemic causes 8 million deaths each year worldwide. Responsible for almost 30 % of cancer-related deaths (especially 90 % of lung cancers) and being the major risk factor for chronic obstructive pulmonary diseases, smoking is the main single cause of preventable deaths in the world (WHO, 2019). Out of the more than 7,000 chemicals present in tobacco smoke, at least 250 are known to be harmful and about 70 can cause cancer: benzene, formaldehyde, acetaldehyde, acrylamide, nitrosamines, arsenic, cadmium… There is no safe level of tobacco use and quitting smoking significantly reduces the risk of developing smoking-related diseases. Although the health benefits are greater for people who stop at earlier ages, benefits exist at any age (Babb et al., 2017). However, smoking cessation is a difficult and challenging task because of the addictive power of nicotine, which is naturally found in tobacco (Benowitz, 2010). Nicotine replacement therapy (mostly available as transdermal patch, nasal spray, inhaler, gum and sublingual tablets) can help to increase chances of sustainable smoking cessation (Stead et al., 2012). However, none of these devices mimics the feeling of satisfaction that occurs with the rapid and abundant delivery of nicotine during smoking. New alternatives to regular cigarettes, such electronic cigarettes (e-cigs) and heated tobacco products (HTP), also called heat-not-burn tobaccos, have emerged on the market over the past decade. The e-cigs and emergent tobacco products are generally perceived as low-risk substitutes for cigarette and have quickly gained popularity, well before sufficient scientific evidence would allow to determine their potential detrimental effects on users.

E-cigs comprise a battery-powered heating element that is designed to vaporize a solution ("e-liquid") made of propylene glycol and/or glycerine and frequently flavouring and nicotine. The vapour is then inhaled by the user. Concerns raised about the levels of some harmful

constituents found in e-cig aerosols, such as carbonyl compounds (including formaldehyde, acetaldehyde and acrolein) (Beauval et al., 2017, 2019; Belushkin et al., 2020) and polycyclic aromatic hydrocarbons (PAHs) (including benzo[a]pyrene) (Beauval et al., 2017; Belushkin et al., 2020), which may be produced by thermal decomposition of e-liquid components. The presence of several trace metals was also reported in e-cig emissions, likely released by cartomizer components (Beauval et al., 2017; Zhao et al., 2019). In parallel, some studies investigated the effects of e-cig vapour exposure on human bronchial epithelial cells cultured at air-liquid interface, and found that e-vapours did not lead to significant cytotoxicity, but induced oxidative stress and/or increased the secretion of pro-inflammatory mediators (Anthérieu et al., 2017; Iskandar et al., 2016; Scheffler et al., 2015). In addition, transcriptomic modifications induced by e-cig vapour exposure were demonstrated to be lesser than those induced by tobacco smoke (Anthérieu et al., 2017; Iskandar et al., 2016). Evolution in e-cig devices (especially the development of new e-cig generation with high power, sub-Ohm devices) may increase the amounts of harmful and potentially-harmful compounds in emissions (Beauval et al., 2019; Belushkin et al., 2020; Farsalinos et al., 2018c; Zhao et al., 2019). Consequently, further research is needed to better understand the impacts of e-cig model and power output on cellular toxicity.

Although e-cig could help to quit smoking (Kalkhoran et al., 2019; Nelson et al., 2015), some vapers could be unsatisfied because e-cig devices do not use real tobacco ingredients and lack the so-called “throat-hit” or authentic tobacco tastes that cigarettes offer. These elements may encourage some people to go back to conventional cigarettes (Staal et al., 2018). Therefore, tobacco companies developed new tobacco products to keep meeting the changing needs of their consumers. They launched HTP that taste more like conventional cigarettes while still delivering nicotine to consumers. In particular, Philip Morris International created the IQOS device: disposable tobacco sticks soaked in propylene glycol (called heatsticks) are inserted in a holder in the IQOS device and heated with an electric blade (Smith et al., 2016). These new devices are marketed by Philip Morris International as products that do not combust tobacco, as cigarettes do, but heat it to a lower temperature (less than 350 °C) with the aim to avoid the combustion-related production of harmful compounds. To support the health claims of IQOS, Philip Morris International published several peer-reviewed papers (Malinska et al., 2018; Schaller et al., 2016a, 2016b; Sewer et al., 2016; Smith et al.,

2016; van der Toorn et al., 2015; Wong et al., 2016). They showed a reduction of most of the harmful constituents found in tobacco smoke (carbonyl compounds, PAHs, nitrosamines…), as well as a reduction of cytotoxicity and genotoxicity in comparison to cigarette smoke exposure (Schaller et al., 2016a). Today, most data about HTP are published by HTP or tobacco industries themselves and toxicity assessment is limited to a comparison with cigarette smoke. However, it is crucial for smokers to know the toxicity of HTP compared to conventional cigarette and e-cigs.

The aim of this study is to compare the chemical composition and the toxicological effects of aerosols from HTP (iQOS model), conventional cigarette smoke (3R4F) and vapours from one nicotine-containing e-liquid vaporized by different e-cig models or conditions (a second generation model with 2.6 Ohms coil and 4.6 W-fixed power and a third generation "Modbox" model with 0.5 Ohms coil and set at 18 W (Mb18W) or 30 W (Mb30W) power). Chemical characterization analyses focused on nicotine, carbonyl compounds and PAHs. The toxicity of the aerosols was evaluated in the air-liquid interface-cultured BEAS-2B cell line, which is the most commonly used human bronchial epithelial cell line for respiratory toxicological studies. Cellular effects were assessed by measuring cytotoxicity, oxidative stress and inflammatory response, which are key mechanisms leading to chronic respiratory diseases.

2. Material and methods

2.1 Tobacco products and e-cig

The HTP used in this study was the iQOS model manufactured by Philip Morris (Neuchâtel, Switzerland), with iQOS heatsticks (amber box from Philip Morris) purchased in a local tobacco shop. IQOS was cleaned regularly after each 20th heatstick, as recommended by the manufacturer. Two models of e-cig from a French manufacturer (NHOSS® brand) were used in these experiments. The first one was the second generation "Lounge" model, equipped with a 2.8 Ω nichrome coil and 4.6 W power supply. The coil heating was triggered by air suction. The second one was the third generation "ModBox" model, used with the "Air Tank" clearomiser equipped with a 0.5 Ω kanthal coil and with a partially closed air flow. Heating was pre-activated 1 s prior the puff, as recommended by the manufacturer and accordingly

with the CORESTA standard puffing method CRM81 (CORESTA, 2015). Modbox model was tested at two power settings, 18 W and 30 W. These settings correspond to the lower and upper range power supplies recommended by the manufacturer for the coils used. One e-liquid was used, "blond tobacco" flavoured (NHOSS® brand) and labelled as follows: propylene glycol < 65 %; glycerol < 35 %; food flavourings; nicotine 16 mg/mL. 3R4F research cigarettes were purchased from the University of Kentucky (Lexington, KY, USA).

2.2 Aerosol generation

Aerosols from HTP, e-cig and 3R4F cigarette were generated with a Vitrocell® VC1 smoking machine (Vitrocell, Waldkirch, Germany), as described previously (Anthérieu et al., 2017; Beauval et al., 2017, 2019). All products were tested with Health Canada Intense (HCI) puff profile (55 mL puff volume, 2 s puff duration, 30 s puff period). For 3R4F cigarette, all ventilation holes were blocked using adhesive tape during the experiments to follow the recommendations of this smoking regime (WHO Tobacco Laboratory, 2012). Under these settings, one 3R4F cigarette was completely consumed after 10 puffs and one heatstick was limited to 12 puffs by the IQOS device.

2.3 Quantification of nicotine

Nicotine was collected from aerosols into two glass impingers with fritted nozzle placed in series containing 50 and 25 mL of methanol, respectively, maintained at -40 °C. Quantification of nicotine in aerosol extracts was performed as described previously (Beauval et al., 2017). Each collection was replicated four times.

2.4 Identification and quantification of carbonyl compounds

Carbonyls were collected from aerosols into two silica cartridges coated with 2,4-dinitrophenylhydrazine (DNPH) placed in series, as described previously (Beauval et al., 2019). Sep-Pak DNPH-Silica Plus Short Cartridges containing 350 or 750 mg of sorbent per cartridge (Waters, Guyancourt, France) were used for e-cig and iQOS aerosols or cigarette smoke, respectively. Each collection was replicated four times. Blank collections were performed using the smoking machine working without e-cig, HTP or conventional cigarette connected to and were taken into account for data analysis. DNPH cartridges were eluted with 3 or 6 mL of

acetonitrile for short and long cartridges, respectively. Elutions from both cartridges placed in series were pooled and then injected into a Thermo Scientific Dionex UltiMate 3000 UHPLC System with UV/VIS Detector (Thermo Scientific, Waltham, MA). The UHPLC instrument was operated at 28 C° at a constant flow rate of 0.4 mL/min, under a gradient of acetonitrile and water during 15 min. Analysis was performed using Acclaim Carbonyl C18 RSCL 150 mm x 2.1 mm x 5 µM (Thermo Fisher Scientific). Acquisition was performed at 360 nm wavelength. Instrument monitoring and data acquisition were done using Chromeleon 7.0 Data Acquisition System for LC (Thermo Scientific, Waltham, MA). The method allows the detection and quantification of nineteen compounds (Table 1) with limits of quantification (LOQs) ranging from 6 to 15 ng/mL. Acrolein was not measured in this study due to the unsuitability of using the DNPH-coated solid sorbent cartridge for its collection (Ho et al., 2011).

The number of puffs for each collection was determined in preliminary experiments in order to avoid saturation in the cartridges. Finally, for e-cig, HTP and 3R4F analyses, 20, 4 and 1 puff(s) were respectively found to be the best compromise with satisfying efficiency without saturation of the cartridge (data not shown). Moreover, concerning e-cig, we measured carbonyl emissions in the twenty last puffs of one-hour-exposure session (100-120 puffs). To compare the different aerosol samples, the levels of the carbonyls were then expressed in mass of each compounds by puff.

2.5 Identification and quantification of PAHs

PAHs were collected from aerosols simultaneously with nicotine collection in the same impingers, as described previously (Beauval et al., 2017). Each collection was replicated four times. Blank collections were performed using the smoking machine working without e-cig, HTP or conventional cigarette connected to and were taken into account for data analysis. Methanolic samples were first concentrated under a gentle nitrogen flow in a water bath at 45 °C to a final volume of 1 mL in order to subsequently extract PAHs using accelerated solvent extraction with acetonitrile. This extraction was followed by a second concentration step under a gentle nitrogen flow in a water bath at 60 °C to a final volume of 1 mL. Remaining products were then filtrated with a syringe membrane filter before injection into the chromatographic system. The analyses were performed on HPLC Alliance 2695 (Waters Corporation, MA) coupled with a multi-wavelength fluorescence detector. Chromatographic

separation was achieved on a EC 250/2 Nucleosil 100-5 C18 PAH (Macherey Nagel, Hoerdt, France). The method allows the detection and quantification of twenty-three compounds (Table 2) with LOQs ranging from 1 to 39 pg/mL.

2.6 Cell culture and cell exposure to aerosols

In vitro experiments were performed using the human bronchial epithelial BEAS-2B cell line obtained from the American Type Culture Collection (ATCC® CRL9609™). Cells were cultured in LHC-9 medium (Life Technologies, Courtabœuf, France) and seeded in CellBIND 75 cm² tissue culture flasks (Corning, Amsterdam, Netherlands) at 37 °C in a humidified incubator with 5 % CO_2 in air and 85 % relative humidity. Cell passaging was performed when cells reached 80-90 % confluence. Following subculture, cells were transferred to an air-liquid interface (ALI) system. Cells were seeded (18 000 cells/cm²) onto transwell clear culture inserts (4.67 cm²) with 0.4 µm pore size (Sigma Aldrich, Saint-Quentin Fallavier, France) pre-coated with 0.03 mg/mL type I collagen solution (Life Technologies). BEAS-2B cells were firstly maintained submerged, then ALI was established by removing medium from the apical surface, exposing only the basal surface to medium. Cells were then transferred to an exposure module (Vitrocell 6/4 CF module) and exposed to different doses (defined in puff number) of the undiluted HTP, e-cig or cigarette aerosol generated by the Vitrocell® system. Control cells were unexposed cells, which were left in the incubator. Each exposure was replicated in three independent cell cultures.

2.7 Cell viability

BEAS-2B cells were exposed to different puff number of undiluted aerosols (40, 80 and 120 puffs for e-cig; 2, 12, 40, 80 and 120 puffs for HTP and 1, 2, 4 and 10 puffs for 3R4F cigarette) and cell viability was measured 24 h after exposure *via* the Cell Titer-Glo Luminescent Cell Viability assay kit (Promega, Charbonnières, France), as described previously (Anthérieu et al., 2017). Intracellular ATP was determined as percentages related to the ATP content in control cells arbitrarily set at a value of 100 %.

2.8 Glutathione content assay

The GSH/GSSG-Glo™ Assay (Promega) was used following the manufacturer's guidelines for the determination of total GSH and oxidized glutathione (GSSG). Finally, GSSG/GSH ratios

were calculated and results are expressed as fold-change relative to the GSSG/GSH ratio in control cells arbitrarily set at a value of 1.

2.9 Gene expression analysis

The total RNA of BEAS-2B cells was extracted using the RNeasy plus mini kit (Qiagen, Courtaboeuf, France) following the manufacturer's instructions. Expression of target genes was measured by quantitative real-time PCR of corresponding reverse-transcribed mRNA. One µg of total RNA was reverse-transcribed into cDNAs using the High Capacity cDNA Reverse Transcription kit (Applied biosystems, CA, USA). qPCRs were carried out with the StepOnePlus thermocycler (Applied Biosystems), using the TaqMan Fast advanced Master Mix (Applied Biosystems) and the following TaqMan Assays: Hs99999901_s1, *18S*; Hs01054797_g1, *CYP1A1*; Hs00164383_m1 *CYP1B1*; Hs01110250_m1, *HMOX1*; Hs01045993_g1, *NQO1*. Amplification curves were read with the StepOne software V2.1 using the comparative cycle threshold method. The relative quantification of the steady-state mRNA levels was normalized against *18S* RNA. Results are expressed as fold-change relative to the levels in control cells arbitrarily set at a value of 1.

2.10 Measurement of secreted mediators of inflammation

Concentrations of ten secreted inflammation mediators were measured in the basolateral media of BEAS-2B cells: granulocyte-macrophage colony-stimulating factor (GM-CSF), growth regulated oncogene α (GRO-α), interleukin 1ß (IL-1ß), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 13 (IL-13), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1-alpha (MIP-1 α), regulated on activation, normal T cell expressed and secreted (RANTES) and interferon gamma (INF-γ). The assay has been performed based on the recommendations of the Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel kit (Merck Millipore, Molsheim, France) using the Luminex® xMAP® technology (Luminex Corp., Austin, TX). The capacity of BEAS-2B cells to secrete various mediators of inflammation had been previously tested by treating cells with lipopolysaccharide (Anthérieu et al., 2017).

2.11 Statistical analysis

Data were represented by the mean ± SD of four independent measurements. Statistical analyses were performed using the non-parametric Mann-Whitney test. Data were considered significantly different when $p < 0.05$.

3. Results and discussion

3.1 Chemical characterization of aerosols

3.1.1 Nicotine content

The potential of the HTP and e-cig to substitute smoking is expected to be at least dependent on the level of nicotine delivered in their aerosols and subsequently inhaled by the user (Farsalinos et al., 2018a). Therefore, the levels of nicotine present in the HTP aerosols were determined and compared with those in the tobacco cigarette smoke and vapours from different e-cig models used with an e-liquid containing 16 mg/mL of nicotine. Levels of nicotine delivered to the aerosols are presented in figure 1. HTP delivered about 30 % less nicotine to its aerosol (63 µg/puff) than the 3R4F cigarette (95 µg/puff) under the HCI puffing profile, as described in other studies (Belushkin et al., 2020; Farsalinos et al., 2018a; Li et al., 2019; Schaller et al., 2016a). For e-cig emissions, the second generation Lounge model provided strongly less nicotine amounts (8 µg/puff) than the two tobacco products tested, while the third generation Modbox model delivered 60 µg/puff at 18 W setting and 137 µg/puff at 30 W setting. Increasing power supply of e-cig has already been shown to increase nicotine level in vapour, mostly due to a more efficient vaporization of the e-liquid (Talih et al., 2015). In summary, the nicotine delivery is highly variable (from 8 to 137 µg of nicotine/puff) depending on the device in comparable conditions of use (under HCI puffing regime). This parameter is important, while it is expected a phenomenon of nicotine self-titration with smokers who want to switch from cigarette to nicotine-delivering alternatives, such as e-cig or HTP (Farsalinos et al., 2018c; Woodward & Tunstall-Pedoe, 1993). To compensate and obtain a similar nicotine amount from HTP or e-cig as from tobacco cigarette, they might adopt a more intense “puffing regime” and/or consume more puffs with HTP or e-cig. Consequently, the nicotine concentrations in emissions will be used to estimate a “normalized” exposure to other harmful and potentially harmful compounds.

3.1.2 Carbonyl and PAH contents

Carbonyls and PAHs are part of principal compounds released from the tobacco combustion and many of them contribute significantly to the carcinogenic activity of tobacco smoke (IARC, 2004). Indeed, the carbonyl compounds, formaldehyde and acetaldehyde, are respectively classified as carcinogenic (Group 1) and possibly carcinogenic (Group 2B) to humans by the International Agency for Research on Cancer (IARC, 2016). Among the PAHs, the benzo[a]pyrene is classified in Group 1 and the dibenzo[a,h]anthracene in Group 2A as probably carcinogenic to humans. Within the framework of tobacco harm reduction, it appears interesting to measure and compare the levels of these harmful and potentially harmful compounds in the emissions of HTP and e-cig.

The emission of nineteen carbonyl compounds in the aerosols of HTP, 3R4F cigarette and e-cigs was first analysed and results (expressed in mass of each compound per puff) are reported in Table 1. Fifteen compounds were quantified at levels between 2 and 15 times higher in cigarette smoke than in HTP aerosols. Hexanal was the only compound measured at a higher level in HTP emission (22.2 ng/puff) than in 3R4F tobacco smoke (10.5 ng/puff), and benzaldehyde was generated in almost equivalent amounts by HTP and cigarette. By contrast, all the carbonyl compounds were measured at very lower amounts in vapours from the different tested e-cig conditions in comparison to tobacco products, except for m-tolualdehyde and 2,5 dimethylbenzaldehyde which were only detected in aerosols from the Modbox device, at both low and high-power settings. Figure 12-A represents the sum of total carbonyl compounds measured in e-cig, HTP and 3R4F emissions. There are much less carbonyl compounds produced in one puff of HTP (35 µg/puff) than in one puff of cigarette smoke (230 µg/puff). Indeed, HTP emitted 84.7 % less carbonyl compounds than 3R4F cigarette (Figure 2–B). Levels of carbonyl compounds measured in vapours from different e-cig models were even at least 98.5 % weaker than in HTP aerosols. Comparison of data between different e-cig devices showed that Lounge and Mb18W emitted respectively 82.3 % and 51.4 % less carbonyl compounds than Mb30W (0.4 µg/puff) (Figures 2-A and 2–B).

These different tobacco products and e-cigs produced carbonyls at very different levels in emissions, probably because they generate aerosols *via* different processes and from diverse

materials (tobacco fillers or e-liquid). During smoking, carbonyls are mainly produced by the pyrolysis of carbohydrates contained in tobacco (Seeman et al., 2002) at high temperature (up to 900 °C). The aerosolization process of HTP operates at temperatures less than 350 °C with the use of heatsticks containing tobacco leaves soaked in propylene glycol. These devices are often referred to as "heat-not-burn" tobacco products with a reduction of the user's exposure to carcinogenic chemicals usually produced by the combustion of tobacco (Schaller et al., 2016a). We effectively observed a reduction of about 90 % in carbonyl emissions in accordance with data from the IQOS manufacturer (Schaller et al., 2016) and two independent studies (Farsalinos et al., 2018b; Li et al., 2019). However, several harmful carbonyls were still measured in HTP aerosol, such as acetaldehyde and formaldehyde, which are carcinogenic compounds. The presence of high levels of acetaldehyde is the mark of pyrolysis and thermogenic degradation of tobacco (Auer et al., 2017). By contrast, the main source of carbonyls in e-cig emissions is the thermal degradation of glycerol and propylene glycol contained in the e-liquid (Uchiyama et al., 2020). Some carbonyls were quantifiable in the different vapours of tested e-cig, but several times far lower than in the emissions from both tested tobacco products (Table 1). In addition, there is a relation between the operating power or the e-cig model and the concentrations of carbonyl compounds detected in the e-cig aerosols. The power of e-cig, and therefore ultimately the heat generated on the evaporation coil, has been reported to affect the quantity of carbonyls formed (Geiss et al., 2016; Kosmider et al., 2014; Talih et al., 2016). Some studies reported higher carbonyl amounts in e-cig emissions (Goniewicz et al., 2014; Hutzler et al., 2014; Sleiman et al., 2016; Talih et al., 2015). However, these findings have been questioned as they could be the consequence of using unrealistic or extreme conditions (low interpuff interval or high power generating high temperatures, drypuff phenomenon) (Farsalinos et al., 2015, 2017). Indeed, e-cig can release high levels of aldehydes if the e-liquid is overheated, but the overheating generates an aversive taste that would secure such emissions to be avoided. To ensure realistic experimental conditions, two regular e-cig users tested the e-cigs used in our study for the generation of dry puffs, using the puff duration and power settings as tested with the smoking machine. The users confirmed no dry puff sensation and sufficient vapour production. In addition, the temperature of the generated aerosol was also considered as an indicator of experimental relevance and realism. The temperature of the aerosols generated from each device did not exceed 60 °C during all the collection periods (Supplemental Figure 1), following

the recommendations of the French national organisation and standardization (AFNOR, 2016). Overall, our data thus demonstrate that, at normal vaping temperatures, carbonyl content in e-cig emissions represents only a small fraction of levels inhaled by users of tobacco products.

In parallel, the emission of twenty-three PAHs in the aerosols of HTP, 3R4F cigarette and e-cigs was analysed and results expressed in ng/puff are reported in Table 2. A similar pattern than that seen for carbonyl compounds was observed for almost all emitted PAHs: the concentrations of twenty-one compounds were markedly lower in HTP emissions than in 3R4F cigarette smoke (from 2 to 676 times depending on the compound), and were even lesser in e-cig vapours. Only benzo(c)phenanthrene was reported to be higher in HTP emissions, compared to all other aerosols. The sum of total PAHs measured was calculated for each aerosol (Figure 3-A) and the reduction rate is indicated in figure 3-B: HTP (0.7 ng/puff) emitted 96.2 % less PAHs than 3R4F cigarette (19.6 ng/puff), but e-cig emitted 64.9 to 78.2 % less PAHs than HTP. Comparison of e-cig models showed no significant difference in PAH content between Mb18W and Mb30W, and about 40 % less of total PAHs in Lounge than in the Modbox model. These results support that the pyrolysis process is limited with e-cigs. The e-liquid used for vaping is generally free of tobacco ingredients which contain the PAH precursors. Moreover, the temperature required to produce an e-cig aerosol from a e-liquid is depending of the proportion in propylene glycol and glycerol. This temperature ranges from 188.6°C to 292 °C, but water and alcohol used as additives, decrease this boiling point (Duell et al., 2018). By comparison, IQOS operates at temperatures between 330°C and 349°C (Davis et al., 2019). PAH emissions released by HTP were lower than combustible cigarette but still contained harmful elements from thermal degradation that are also found in cigarette smoke (Li et al., 2019; Rodgman et al., 2000), including the carcinogenic benzo[a]pyrene.

The quantifications of carbonyl and PAH compounds were first presented in mass of analysed compounds per puff to compare devices with each other. However, users do not necessarily consume the same number of puffs when using HTP, e-cig or conventional cigarettes. They appear to self-regulate their consumption (number, frequency and volume of puffs, notably) according to their needed quantity of nicotine (Farsalinos et al., 2018c). To take into consideration this nicotine self-titration, it appears relevant to also report all the amounts of harmful and potentially harmful compounds per nicotine yield. Detailed data for carbonyl and

PAH levels, normalized by the level of emitted nicotine in aerosols, are reported in Tables S1 and S2 (supplementary materials), respectively. The comparison of total carbonyl compounds after nicotine normalization (Figures 2-C and 2-D) showed, according to previous conclusions, that the HTP emitted 76.9% less carbonyl compounds (497 ng/µg of nicotine) than the combustible cigarette (2308 ng/µg of nicotine), but at least 97.9% higher levels than the e-cig vapours (< 10 ng/µg of nicotine). However, comparing the e-cig models, Lounge emitted more carbonyl compounds than the Modbox model and no significant difference was reported between Mb18W and Mb30W. The comparison of total PAHs after nicotine normalization (Figures 3-C and 3-D) also showed substantial reduction (94.3%) in the PAH content of HTP emissions (11 pg/µg of nicotine) in comparison to cigarette smoke (207 pg/µg of nicotine). The pattern of PAH content between the different e-cig models was different after nicotine normalization: the Lounge model emitted more PAHs (15 pg/µg of nicotine) than the Modbox model (79.2-90 %) and even 27.5 % more than HTP. These results showed that the way of expressing data (emissions per puff *vs* emissions per nicotine yield) can influence their interpretation. Today, there is no standardized manner to express the amount of emitted compounds in aerosols. Indeed, data can be expressed in amount per puff (Beauval et al., 2019), per mL of puff (Beauval et al., 2017), per cigarette or per IQOS heatstick (Li et al., 2019), per mass of nicotine (Farsalinos et al., 2018b), per liquid consumption for e-cig (Beauval et al., 2017), thus hampering comparisons between studies and making interpretations difficult. In addition, it is still unclear to which extent vapor generation, collection and analysis procedures could affect results of chemical characterization. Harmonized protocols to determine the chemical composition of emissions and to express results are crucially needed to establish and compare risk profiles of each emergent tobacco products in terms of chemical composition and user exposure.

3.2 *In vitro* toxicity

The apparent reduction of some harmful constituents in HTP and e-cig emissions in comparison to tobacco cigarette cannot be directly extrapolated to a proportionate harm reduction for smokers. Today, research is needed about toxicological impacts of these products on human airway epithelial cells in comparison with tobacco cigarette. The use of undiluted aerosols is described as a more sensitive method to compare responses from aerosols produced from emergent products, such as HTP and e-cig (Bishop et al., 2019). Thus,

human bronchial epithelial BEAS-2B cells cultured at ALI were exposed to undiluted emissions from HTP, e-cig and 3R4F cigarette and effects of those emissions were evaluated on cytotoxicity, oxidative stress and inflammatory response, which are key mechanisms leading to chronic airway diseases.

3.2.1 Cell viability

Cell viability was assessed 24 h after aerosol exposure by measuring intracellular ATP content, which is directly proportional to the number of living cells. Data displayed in figure 4 (A-E) show that e-cig vapours had no significant (Modbox model) or low (Lounge model: > 75 % cell viability) cytotoxic effects up to a 120-puff-exposure. In comparison, HTP emissions caused intracellular ATP changes from 12 puffs (89 % viability) to a strong reduction of cell viability that reached 2 % after 120 puffs. Cigarette smoke demonstrated also a full dose-response curve, but its cytotoxicity appeared within fewer puffs (< 10 puffs). In order to better compare the different devices, the effective dose of aerosol which results in a 50 % reduction of cell viability (ED50, expressed here in puff number) was calculated. Although the e-cig vapours did not induce sufficient cell mortality to calculate an ED50 (whatever the tested e-cig power or model), ED50 was 45 puffs for HTP aerosol and 2 puffs for 3R4F cigarette smoke. Bishop *et al.* have exposed a 3D-reconstituted human airway epithelium to undiluted cigarette smoke and e-cig aerosol (Bishop et al., 2019). They fund ED50 equal to 4 puffs and 60 puffs under HCI regime for cigarette and e-cig exposures, respectively. However, they voluntarily used extreme conditions for e-cig exposure with an airflow vent closed to achieve a worst case for carbonyl production and, consequently, higher cytotoxicity. In a previous study using diluted aerosols (Anthérieu et al., 2017), we have tested the Lounge model with different e-liquids (with or without nicotine, flavoured or unflavoured) and demonstrated that none of the aerosols induced cytotoxicity in BEAS-B cell line up to an exposure of 576 puffs. Today, few *in vitro* studies have compared the relative cytotoxicity of HTP aerosols with both cigarette smoke and e-cig vapours, and most of these assays have been performed using submersed cultures exposed to aerosol extracts (Ito et al., 2019; Munakata et al., 2018; Sohal et al., 2019). ALI exposures provide a more pertinent approach to perform toxicological studies related to inhalation of emerging e-cigs or novel tobacco products (Johnson et al., 2009). Our present results are in agreement with a study performed in ALI-cultured human bronchial epithelial H292 cells, demonstrating that HTP show reduced cytotoxicity relative to combustible

cigarette, but higher cytotoxicity than e-cig (Leigh et al., 2018). There is no standardized approach to compare the *in vitro* toxicity between emergent tobacco products, e-cigs and tobacco cigarette. Some authors used the same exposure time or the same puff number between the different aerosols while some used comparable nicotine exposure (Wang et al., 2019). Therefore, we also reported cell viability as a function of emitted nicotine (Figure 4-F). The results were equivalent with or without nicotine normalization: a higher cytotoxicity for tobacco cigarette (ED50 = 0.2 mg of nicotine) than for HTP (ED50 = 2.8 mg of nicotine) and low cytotoxicity for e-cig. These differences in cytotoxic effects are probably attributable to lower concentrations of potentially harmful chemicals in HTP and especially e-cig aerosols. Based on these cytotoxicity data, sub-toxic (> 75 % cell viability) or toxic doses were chosen for the further analyses that assessed oxidative stress and inflammation: 40 and 120 puffs for e-cig; 2, 12 and 40 puffs for HTP; 1 and 2 puffs for 3R4F cigarette.

3.2.2 Oxidative stress

The generation of oxidative stress was first assessed by measuring the intracellular content of oxidized (GSSG) and reduced (GSH) glutathione. GSH is considered to be one of the most important scavengers of reactive oxygen species (ROS), and the ratio GSSG/GSH may be used as a marker of oxidative stress. We have previously described that the generation of oxidative stress was transient and no change in glutathione levels was found in BEAS-2B cells 3 h after the end of cigarette smoke exposure (Anthérieu et al., 2017). Consequently, we have measured GSH and GSSG contents immediately (0 h) after cell exposures (Figure 5). In these experimental conditions, HTP induced a significant increase of GSSG/GSH ratio in comparison to control cells (2.7 and 4.5 fold-changes after 12 and 40 puffs, respectively). 3R4F cigarette induced also an increase of the GSSG/GSH ratio but already after only 1 puff (2.7 fold-change) and a 7.8 fold-change was observed after an exposure to 2 puffs. For e-cig exposures, anti-oxidative response was evidenced only after a longer exposure of 120 puffs with Mb30W (2.9 fold-change), although no significant change in the GSSG/GSH ratio was observed with Mb18W and Lounge.

The cellular defense mechanisms against toxic substances also include transcriptomic regulations of genes involved in detoxification processes and the anti-oxidative response. Thus, the expression of selected genes involved in xenobiotic metabolism (cytochrome P450 1A1, *CYP1A1*; cytochrome P450 1B1, *CYP1B1*) and the anti-oxidative response (heme

oxygenase 1, *HMOX1*; NADPH Quinone Dehydrogenase 1, *NQO1*) was quantified 4 or 24 h after exposure to 120 puffs of e-cig vapours, 12 puffs of HTP aerosol or 1 puff of 3R4F cigarette smoke (Figure 6). For all the products and both time points, an increase of *CYP1A1* and *CYP1B1* expression was observed, with a higher induction for *CYP1A1* than for *CYP1B1*. PAHs contained in cigarette smoke are known to induce expression of *CYP1A1/1B1 via* the Aryl Hydrocarbon Receptor (AhR) pathway and, subsequently, to affect the metabolism of tobacco carcinogens (Sacks et al., 2011). The CYP1A1/1B1 expression is also induced in the lungs of smokers (Kim et al., 2004). E-cig aerosols can also induce *CYP1A1* and *CYP1B1* and enhance the metabolism of some PAHs (e.g. benzo[a]pyrene) to genotoxic products by activating AhR (Sun et al., 2019). Furthermore, the expression of the downstream target genes of the nuclear erythroid 2-related factor 2 (Nrf2), *NQO1* and *HMOX1*, was up-regulated 4 h after exposure to the Lounge model vapours and at both time points for all other emissions. The up-regulation of NQO1 and HMOX1 levels 4 h after exposure increased with the e-cig power, reaching a 3.3 or 95-fold-change, respectively, for Mb30W. Some smoke compounds, including carbonyls, PAHs, quinones, naphthoquinones and benzenediols, were identified as activators of the Nrf2/antioxidant response element (ARE) pathway and *HMOX1* induction in response to oxidative stress (Chan et al., 2013; Sekine et al., 2016; Zhang et al., 2019). Some of these different components of cigarette smoke were also found in aerosols of HTP or e-cig, which can explain the up-regulation of *NQO1* and *HMOX1* in BEAS-2B cells whatever the products. Almost all of these transcriptomic modulations were higher after 4 h exposure than after 24 h and correspond to early adaptive mechanisms set up by BEAS-2B cells in response to a cellular stress after aerosol exposure. It is important to note that these transcriptomic mechanisms were globally similar for all the devices tested, demonstrating that all products have the potential to induce detoxification and an anti-oxidative response. However, these molecular and cellular responses were observed for highly different exposure levels (from 120 puffs for e-cig, 12 puffs for HTP and only 1 puff for tobacco cigarette). In addition, an exposure of 120 puffs in one hour is representative of an intense exposure session, compared to data from topography studies with e-cig users (Jones et al., 2020; Lee et al., 2018).

These results are in accordance with other studies demonstrating that the generation of ROS is observed in HTP emissions after a more intensive use than with cigarette smoke (Munakata et al., 2018). ROS would be mainly generated by exposure to chemicals derived from

combustion processes (Kopa & Pawliczak, 2020) and, therefore, can be directly linked to the relative amounts of carbonyls and PAHs measured in HTP and cigarette smoke emissions (Figures 2 and 3). The increase in the amount of carbonyl compounds produced when Modbox was used under high power setting could explain, at least in part, the greater induction of ROS production that would contribute to alter the oxidative/antioxidative balance. Indeed, higher power leads to higher filament temperature, which enhances the e-liquid vaporization process, pyrolysis and chain reactions with the production of hydroxide and superoxide free radicals (Haddad et al., 2019; Son et al., 2019; Zhao et al., 2018). However, Son *et al.* reported that the dose of free radicals per puff associated with e-cig vaping was 10-1000 times lower than the reported dose generated by cigarette smoking (Son et al., 2019).

3.2.3 Inflammatory response

The inflammatory response of BEAS-2B cells was assessed by measuring the secretion of ten cytokines and chemokines 24 h after exposure to defined subtoxic doses of HTP, 3R4F cigarette smoke or e-cig emissions. Only four mediators were found in quantifiable levels (IL-6, IL-8, GROα, and MCP-1) after exposure. GM-CSF, IL-13, IL-1ß, MIP-1α, RANTES and TNF-α were not detected in cell culture medium of BEAS-2B cells exposed to aerosols, despite the capacity of these cells to secrete these mediators after treatment with lipopolysaccharide (Anthérieu et al., 2017). The results for the four detected mediators were expressed in fold-change relative to control cells (Table 3). A significant increase of IL-6 secretion was observed in the culture medium of cells exposed to Mb18W (for 120 puffs) and Mb30W (for both 40 and 120 puffs) aerosols, while no significant change was evidenced for IL-8 secretion. Increase of IL-6 and/or IL-8 had already been described in different cell models after e-cig exposures (Merecz-Sadowska et al., 2020), and more remarkably in the plasma of e-cig users (Singh et al., 2019), demonstrating that e-cig vapors could induce a pro-inflammatory response. For HTP, a biphasic response was observed for IL-6 and IL-8 with a greater increase in secretion after 12 puffs (3 and 3.3 fold-change, respectively) than after 40 puffs (1.3 fold-change). A comparable biphasic response in IL-8 secretion was previously described in BEAS-2B cells exposed to HTP aerosol or tobacco smoke and a similar trend was also observed for GM-CSF (Munakata et al., 2018), which was undetectable in our study. These differences in results could be explained by the different protocols of cell exposure. Indeed, Munataka *et al.*

exposed submerged BEAS-2B cells to aerosol extracts, while ALI-cultured cells were exposed directly to aerosols in our study. Finally, the secretion of MCP-1 and GRO-α was significantly decreased for the longer exposures to HTP aerosol, 3R4F cigarette smoke and Mb30W vapour (Table 3). A down-regulation of GRO-α and MCP-1 was also demonstrated after exposure to cigarette smoke in human endothelial cells (Allam et al., 2013). GRO-α plays a significant role in the chemotaxis of neutrophils to the site of inflammation and MCP-1 is a potent chemoattractant for monocytes and macrophages. This recruitment and subsequent activation of monocytes into the inflamed tissues play a central role in determining the outcomes of the immune responses of the tissues. Thus, alteration of GRO-α and MCP-1 secretion after aerosol exposure could affect the tissue immune and protective responses.

These different modulations in inflammatory mediators can be explained partially by the carbonyl and PAH levels measured in the different emissions. Indeed, some carbonyls found in aerosols or smoke (such as acrolein, acetaldehyde and formaldehyde) are known strong irritants that may cause inflammation (Lino-dos-Santos-Franco et al., 2011; Shields et al., 2017). The benzo[a]pyrene is also known as an inducer of the secretion of pro-inflammatory cytokines (Chen et al., 2012). Other constituents of aerosols or smoke could play a major role in the inflammatory response. Metals are capable of causing inflammatory cytokine induction (Lerner et al., 2015). In addition, propylene glycol and glycerol produce a hygroscopic/hyperosmolar aerosol which could deposit on the surface of lung cells and trigger local inflammation (Chaumont et al., 2019).

Carbonyls and PAHs represent only a part of the complex mixture constituting the cigarette smoke or the HTP and e-cig aerosols. Therefore, these pollutants take part in the cellular response but all the changes observed in BEAS-2B cells (cytotoxicity, oxidative stress, inflammation) cannot be explained solely by PAH and carbonyl emissions. A multitude of other harmful compounds comprising other volatile organic compounds, metals, nitrosamines *etc.* (Li et al., 2019; Schaller et al., 2016; Zhao et al., 2019) should take into account to better characterize the toxic profile of these novel tobacco products and e-cigs in comparison to tobacco cigarette.

4. Conclusion

Within the framework of tobacco harm reductions, in which smokers ideally should be able to freely choose from a variety of alternatives for smoking, emerging tobacco products (such as HTP) and e-cig seem to have potential of a promising new offering. However, it is fundamental for smokers to know and compare the health risks of these different emergent devices in order to determine which product should be preferred for smoking cessation. Our study provides comparative data on both chemical composition of HTP, e-cig and tobacco cigarette emissions and their toxicological impacts on human bronchial epithelial cells. We first report that HTP deliver slightly less nicotine and emit much lower amounts of carbonyl and PAH compounds than tobacco cigarette. However, HTP emissions still contain carcinogenic compounds (*e.g.* formaldehyde, acetaldehyde and benzo[a]pyrene) and the amounts of carbonyls and PAHs in HTP aerosols are higher than in e-cig vapours. In accordance with the levels of toxic compounds in each aerosol, HTP aerosol exhibit reduced cytotoxicity compared to cigarette smoke but higher than e-cig vapours. HTP and e-cig have the potential to increase oxidative stress and inflammatory response, in a manner very similar to that of cigarette smoke, but only after a more intensive exposure. In addition, our data support that e-cig use at higher power settings emit higher carbonyl and PAH compounds and, consequently, generate more oxidative stress. Finally, this study contributes to a better understanding of HTP and e-cig emission properties and their related toxicological impacts and provides important data needed for risk assessment purposes, by demonstrating that HTP might be less harmful than tobacco cigarette but considerably more harmful than e-cig. Further long-term studies in animal models should be conducted to confirm these *in vitro* findings and to allow the assessment of chronic exposures to emergent tobacco products. In addition to the toxic impacts of these products, comparison of their addictiveness is another key element to take into account in the tobacco harm-reduction strategy.

Credit Author Statement

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6. Declaration of interest

The authors declare that they have no conflict of interest with tobacco, HTP or e-cig industries.

References

AFNOR. (2016). *Association Française de Normalisation. Norme XP D90-300-3 Cigarettes électroniques et e-liquides*.

Allam, E., Delacruz, K., Ghoneima, A., Sun, J., & Windsor, L. J. (2013). Effects of tobacco on cytokine expression from human endothelial cells. *Oral Diseases*, *19*(7), 660–665. https://doi.org/10.1111/odi.12050

Anthérieu, S., Garat, A., Beauval, N., Soyez, M., Allorge, D., Garçon, G., & Lo-Guidice, J.-M. (2017). Comparison of cellular and transcriptomic effects between electronic cigarette vapor and cigarette smoke in human bronchial epithelial cells. *Toxicology in Vitro : An International Journal Published in Association with BIBRA*, *45*(Pt 3), 417–425. https://doi.org/10.1016/j.tiv.2016.12.015

Auer, R., Concha-Lozano, N., Jacot-Sadowski, I., Cornuz, J., & Berthet, A. (2017). Heat-Not-Burn Tobacco Cigarettes: Smoke by Any Other Name. *JAMA Internal Medicine*, *177*(7), 1050–1052. https://doi.org/10.1001/jamainternmed.2017.1419

Babb, S., Malarcher, A., Schauer, G., Asman, K., & Jamal, A. (2017). Quitting Smoking Among Adults — United States, 2000–2015. *MMWR. Morbidity and Mortality Weekly Report*, *65*(52), 1457–1464. https://doi.org/10.15585/mmwr.mm6552a1

Beauval, N., Antherieu, S., Soyez, M., Gengler, N., Grova, N., Howsam, M., Hardy, E. M., Fischer, M., Appenzeller, B. M. R., Goossens, J. F., Allorge, D., Garçon, G., Lo-Guidice, J. M., & Garat, A. (2017). Chemical evaluation of electronic cigarettes: Multicomponent analysis of liquid refills and their corresponding aerosols. *Journal of Analytical Toxicology*, *41*(8), 670–678. https://doi.org/10.1093/jat/bkx054

Beauval, N., Verrièle, M., Garat, A., Fronval, I., Dusautoir, R., Anthérieu, S., Garçon, G., Lo-Guidice, J.-M., Allorge, D., & Locoge, N. (2019). Influence of puffing conditions on the carbonyl composition of e-cigarette aerosols. *International Journal of Hygiene and Environmental Health*, *222*(1), 136–146. https://doi.org/10.1016/j.ijheh.2018.08.015

Belushkin, M., Tafin Djoko, D., Esposito, M., Korneliou, A., Jeannet, C., Lazzerini, M., & Jaccard, G. (2020). Selected Harmful and Potentially Harmful Constituents Levels in Commercial e-Cigarettes. *Chemical Research in Toxicology*, *33*(2), 657–668. https://doi.org/10.1021/acs.chemrestox.9b00470

Benowitz, N. L. (2010). Nicotine addiction. In *New England Journal of Medicine* (Vol. 362, Issue 24, p. 2295). Massachussetts Medical Society. https://doi.org/10.1056/NEJMra0809890

Bishop, E., Haswell, L., Adamson, J., Costigan, S., Thorne, D., & Gaca, M. (2019). An approach to testing undiluted e-cigarette aerosol in vitro using 3D reconstituted human airway epithelium. *Toxicology in Vitro : An International Journal Published in Association with BIBRA*, *54*, 391–401. https://doi.org/10.1016/j.tiv.2018.01.010

Chan, J. K. W., Charrier, J. G., Kodani, S. D., Vogel, C. F., Kado, S. Y., Anderson, D. S., Anastasio, C., & Van Winkle, L. S. (2013). Combustion-derived flame generated ultrafine soot generates reactive oxygen species and activates Nrf2 antioxidants differently in neonatal and adult rat lungs. *Particle and Fibre Toxicology*, *10*, 34. https://doi.org/10.1186/1743-8977-10-34

Chaumont, M., van de Borne, P., Bernard, A., Van Muylem, A., Deprez, G., Ullmo, J., Starczewska, E., Briki, R., de Hemptinne, Q., Zaher, W., & Debbas, N. (2019). Fourth

generation e-cigarette vaping induces transient lung inflammation and gas exchange disturbances: Results from two randomized clinical trials. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *316*(5), L705–L719. https://doi.org/10.1152/ajplung.00492.2018

Chen, W., Xu, X., Bai, L., Padilla, M. T., Gott, K. M., Leng, S., Tellez, C. S., Wilder, J. A., Belinsky, S. A., Scott, B. R., & Lin, Y. (2012). *Low-dose gamma-irradiation inhibits IL-6 secretion from human lung fibroblasts that promotes bronchial epithelial cell transformation by cigarette-smoke carcinogen*. https://doi.org/10.1093/carcin/bgs159

CORESTA. (2015). *E-cigarette Task Force Technical Report, 2014 Electronic Cigarette Aerosol Parameters Study.*

Davis, B., Williams, M., & Talbot, P. (2019). iQOS: evidence of pyrolysis and release of a toxicant from plastic. *Tobacco Control*, *28*(1), 34–41. https://doi.org/10.1136/tobaccocontrol-2017-054104

Duell, A. K., Pankow, J. F., Gillette, S. M., & Peyton, D. H. (2018). Boiling points of the propylene glycol + glycerol system at 1 atmosphere pressure: 188.6–292 °C without and with added water or nicotine. *Chemical Engineering Communications*, *205*(12), 1691–1700. https://doi.org/10.1080/00986445.2018.1468758

Farsalinos, K. E., Voudris, V., & Poulas, K. (2015). E-cigarettes generate high levels of aldehydes only in "dry puff" conditions. *Addiction*, *110*(8), 1352–1356. https://doi.org/10.1111/add.12942

Farsalinos, K. E., Voudris, V., Spyrou, A., & Poulas, K. (2017). E-cigarettes emit very high formaldehyde levels only in conditions that are aversive to users: A replication study under verified realistic use conditions. *Food and Chemical Toxicology*, *109*, 90–94. https://doi.org/10.1016/j.fct.2017.08.044

Farsalinos, K. E., Yannovits, N., Sarri, T., Voudris, V., & Poulas, K. (2018a). Nicotine Delivery to the Aerosol of a Heat-Not-Burn Tobacco Product: Comparison With a Tobacco Cigarette and E-Cigarettes. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *20*(8), 1004–1009. https://doi.org/10.1093/ntr/ntx138

Farsalinos, K. E., Yannovits, N., Sarri, T., Voudris, V., Poulas, K., & Leischow, S. J. (2018b). Carbonyl emissions from a novel heated tobacco product (IQOS): comparison with an e-cigarette and a tobacco cigarette. *Addiction*, *113*(11), 2099–2106.

https://doi.org/10.1111/add.14365

Farsalinos, K. E., Yannovits, N., Sarri, T., Voudris, V., Poulas, K., & Leischow, S. J. (2018c). Carbonyl emissions from a novel heated tobacco product (IQOS): comparison with an e-cigarette and a tobacco cigarette. *Addiction*, *113*(11), 2099–2106. https://doi.org/10.1111/add.14365

Farsalinos, K., Poulas, K., & Voudris, V. (2018). Changes in Puffing Topography and Nicotine Consumption Depending on the Power Setting of Electronic Cigarettes. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *20*(8), 993–997. https://doi.org/10.1093/ntr/ntx219

Geiss, O., Bianchi, I., & Barrero-Moreno, J. (2016). Correlation of volatile carbonyl yields emitted by e-cigarettes with the temperature of the heating coil and the perceived sensorial quality of the generated vapours. *International Journal of Hygiene and Environmental Health*, *219*(3), 268–277. https://doi.org/10.1016/j.ijheh.2016.01.004

Goniewicz, M. L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C., Jacob, P., & Benowitz, N. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tobacco Control*, *23*(2), 133–139. https://doi.org/10.1136/tobaccocontrol-2012-050859

Haddad, C., Salman, R., El-Hellani, A., Talih, S., Shihadeh, A., & Saliba, N. A. (2019). Reactive Oxygen Species Emissions from Supra- and Sub-Ohm Electronic Cigarettes. *Journal of Analytical Toxicology*, *43*(1), 45–50. https://doi.org/10.1093/jat/bky065

Ho, S. S. H., Ho, K. F., Liu, W. D., Lee, S. C., Dai, W. T., Cao, J. J., & Ip, H. S. S. (2011). Unsuitability of using the DNPH-coated solid sorbent cartridge for determination of airborne unsaturated carbonyls. *Atmospheric Environment*, *45*(1), 261–265. https://doi.org/10.1016/j.atmosenv.2010.09.042

Hutzler, C., Paschke, M., Kruschinski, S., Henkler, F., Hahn, J., & Luch, A. (2014). Chemical hazards present in liquids and vapors of electronic cigarettes. *Archives of Toxicology*, *88*(7), 1295–1308. https://doi.org/10.1007/s00204-014-1294-7

IARC. (2004). *Tobacco smoking - IARC Monographs*.

Iskandar, A. R., Gonzalez-Suarez, I., Majeed, S., Marescotti, D., Sewer, A., Xiang, Y., Leroy, P., Guedj, E., Mathis, C., Schaller, J.-P., Vanscheeuwijck, P., Frentzel, S., Martin, F., Ivanov, N. V, Peitsch, M. C., & Hoeng, J. (2016). A framework for in vitro systems toxicology assessment of e-liquids. *Toxicology Mechanisms and Methods*, *26*(6), 389–413.

https://doi.org/10.3109/15376516.2016.1170251

Ito, S., Taylor, M., Mori, S., Thorne, D., Nishino, T., Breheny, D., Gaça, M., Yoshino, K., & Proctor, C. (2019). An inter-laboratory in vitro assessment of cigarettes and next generation nicotine delivery products. *Toxicology Letters*, *315*, 14–22. https://doi.org/10.1016/j.toxlet.2019.08.004

Johnson, M. D., Schilz, J., Djordjevic, M. V, Rice, J. R., & Shields, P. G. (2009). Evaluation of in vitro assays for assessing the toxicity of cigarette smoke and smokeless tobacco. *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *18*(12), 3263–3304. https://doi.org/10.1158/1055-9965.EPI-09-0965

Jones, J., Slayford, S., Gray, A., Brick, K., Prasad, K., & Proctor, C. (2020). A cross-category puffing topography, mouth level exposure and consumption study among Italian users of tobacco and nicotine products. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-019-55410-5

Kalkhoran, S., Chang, Y., & Rigotti, N. A. (2019). E-cigarettes and Smoking Cessation in Smokers With Chronic Conditions. *American Journal of Preventive Medicine*, *57*(6), 786–791. https://doi.org/10.1016/j.amepre.2019.08.017

Kim, J. H., Sherman, M. E., Curriero, F. C., Guengerich, F. P., Strickland, P. T., & Sutter, T. R. (2004). Expression of cytochromes P450 1A1 and 1B1 in human lung from smokers, non-smokers, and ex-smokers. *Toxicology and Applied Pharmacology*, *199*(3), 210–219. https://doi.org/10.1016/j.taap.2003.11.015

Kopa, P. N., & Pawliczak, R. (2020). IQOS - a heat-not-burn (HnB) tobacco product - chemical composition and possible impact on oxidative stress and inflammatory response. A systematic review. *Toxicology Mechanisms and Methods*, *30*(2), 81–87. https://doi.org/10.1080/15376516.2019.1669245

Kosmider, L., Sobczak, A., Fik, M., Knysak, J., Zaciera, M., Kurek, J., & Goniewicz, M. L. (2014). Carbonyl compounds in electronic cigarette vapors: Effects of nicotine solvent and battery output voltage. *Nicotine and Tobacco Research*, *16*(10), 1319–1326. https://doi.org/10.1093/ntr/ntu078

Lee, Y. O., Nonnemaker, J. M., Bradfield, B., Hensel, E. C., & Robinson, R. J. (2018). Examining Daily Electronic Cigarette Puff Topography Among Established and Nonestablished Cigarette Smokers in their Natural Environment. *Nicotine & Tobacco Research*, *20*(10),

1283–1288. https://doi.org/10.1093/NTR/NTX222

Leigh, N. J., Tran, P. L., O'Connor, R. J., & Goniewicz, M. L. (2018). Cytotoxic effects of heated tobacco products (HTP) on human bronchial epithelial cells. *Tobacco Control*, *27*(Suppl 1), s26–s29. https://doi.org/10.1136/tobaccocontrol-2018-054317

Lerner, C. A., Sundar, I. K., Watson, R. M., Elder, A., Jones, R., Done, D., Kurtzman, R., Ossip, D. J., Robinson, R., McIntosh, S., & Rahman, I. (2015). Environmental health hazards of e-cigarettes and their components: Oxidants and copper in e-cigarette aerosols. *Environmental Pollution (Barking, Essex : 1987)*, *198*, 100–107. https://doi.org/10.1016/j.envpol.2014.12.033

Li, X., Luo, Y., Jiang, X., Zhang, H., Zhu, F., Hu, S., Hou, H., Hu, Q., & Pang, Y. (2019). Chemical Analysis and Simulated Pyrolysis of Tobacco Heating System 2.2 Compared to Conventional Cigarettes. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *21*(1), 111–118. https://doi.org/10.1093/ntr/nty005

Lino-dos-Santos-Franco, A., Correa-Costa, M., Dos Santos Durão, A. C. C., Ligeiro de Oliveira, A. P., Breithaupt-Faloppa, A. C., Bertoni, J. de A., Oliveira-Filho, R. M., Câmara, N. O. S., Marcourakis, T., & Tavares-de-Lima, W. (2011). Formaldehyde induces lung inflammation by an oxidant and antioxidant enzymes mediated mechanism in the lung tissue. *Toxicology Letters*, *207*(3), 278–285. https://doi.org/10.1016/j.toxlet.2011.09.026

Malinska, D., Szymański, J., Patalas-Krawczyk, P., Michalska, B., Wojtala, A., Prill, M., Partyka, M., Drabik, K., Walczak, J., Sewer, A., Johne, S., Luettich, K., Peitsch, M. C., Hoeng, J., Duszyński, J., Szczepanowska, J., van der Toorn, M., & Wieckowski, M. R. (2018). Assessment of mitochondrial function following short- and long-term exposure of human bronchial epithelial cells to total particulate matter from a candidate modified-risk tobacco product and reference cigarettes. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *115*, 1–12. https://doi.org/10.1016/j.fct.2018.02.013

Merecz-Sadowska, A., Sitarek, P., Zielinska-Blizniewska, H., Malinowska, K., Zajdel, K., Zakonnik, L., & Zajdel, R. (2020). A summary of in vitro and in vivo studies evaluating the impact of E-Cigarette exposure on living organisms and the environment. In *International Journal of Molecular Sciences* (Vol. 21, Issue 2). MDPI AG. https://doi.org/10.3390/ijms21020652

Munakata, S., Ishimori, K., Kitamura, N., Ishikawa, S., Takanami, Y., & Ito, S. (2018). Oxidative

stress responses in human bronchial epithelial cells exposed to cigarette smoke and vapor from tobacco- and nicotine-containing products. *Regulatory Toxicology and Pharmacology : RTP*, *99*, 122–128. https://doi.org/10.1016/j.yrtph.2018.09.009

Nelson, V. A., Goniewicz, M. L., Beard, E., Brown, J., Sheals, K., West, R., & Shahab, L. (2015). Comparison of the characteristics of long-term users of electronic cigarettes versus nicotine replacement therapy: A cross-sectional survey of English ex-smokers and current smokers. *Drug and Alcohol Dependence*, *153*, 300–305. https://doi.org/10.1016/j.drugalcdep.2015.05.005

Rodgman, A., Smith, C. J., & Perfetti, T. A. (2000). The composition of cigarette smoke: a retrospective, with emphasis on polycyclic components. *Human & Experimental Toxicology*, *19*(10), 573–595. https://doi.org/10.1191/096032700701546514

Sacks, P. G., Zhao, Z.-L., Kosinska, W., Fleisher, K. E., Gordon, T., & Guttenplan, J. B. (2011). Concentration dependent effects of tobacco particulates from different types of cigarettes on expression of drug metabolizing proteins, and benzo(a)pyrene metabolism in primary normal human oral epithelial cells. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *49*(9), 2348–2355. https://doi.org/10.1016/j.fct.2011.06.037

Schaller, J.-P., Keller, D., Poget, L., Pratte, P., Kaelin, E., McHugh, D., Cudazzo, G., Smart, D., Tricker, A. R., Gautier, L., Yerly, M., Reis Pires, R., Le Bouhellec, S., Ghosh, D., Hofer, I., Garcia, E., Vanscheeuwijck, P., & Maeder, S. (2016). Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. *Regulatory Toxicology and Pharmacology : RTP*, *81 Suppl 2*, S27–S47. https://doi.org/10.1016/j.yrtph.2016.10.001

Schaller, J.-P., Pijnenburg, J. P. M., Ajithkumar, A., & Tricker, A. R. (2016). Evaluation of the Tobacco Heating System 2.2. Part 3: Influence of the tobacco blend on the formation of harmful and potentially harmful constituents of the Tobacco Heating System 2.2 aerosol. *Regulatory Toxicology and Pharmacology : RTP*, *81 Suppl 2*, S48–S58. https://doi.org/10.1016/j.yrtph.2016.10.016

Scheffler, S., Dieken, H., Krischenowski, O., Förster, C., Branscheid, D., & Aufderheide, M. (2015). Evaluation of E-cigarette liquid vapor and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells. *International Journal of Environmental Research and Public Health*, *12*(4), 3915–3925.

https://doi.org/10.3390/ijerph120403915

Seeman, J. I., Dixon, M., & Haussmann, H. J. (2002). Acetaldehyde in mainstream tobacco smoke: Formation and occurrence in smoke and bioavailability in the smoker. In *Chemical Research in Toxicology* (Vol. 15, Issue 11, pp. 1331–1350). https://doi.org/10.1021/tx020069f

Sekine, T., Hirata, T., Mine, T., & Fukano, Y. (2016). Activation of transcription factors in human bronchial epithelial cells exposed to aqueous extracts of mainstream cigarette smoke in vitro. *Toxicology Mechanisms and Methods*, *26*(1), 22–31. https://doi.org/10.3109/15376516.2015.1123788

Sewer, A., Kogel, U., Talikka, M., Wong, E. T., Martin, F., Xiang, Y., Guedj, E., Ivanov, N. V., Hoeng, J., & Peitsch, M. C. (2016). Evaluation of the Tobacco Heating System 2.2 (THS2.2). Part 5: microRNA expression from a 90-day rat inhalation study indicates that exposure to THS2.2 aerosol causes reduced effects on lung tissue compared with cigarette smoke. *Regulatory Toxicology and Pharmacology*, *81*, S82–S92. https://doi.org/10.1016/j.yrtph.2016.11.018

Shields, P. G., Berman, M., Brasky, T. M., Freudenheim, J. L., Mathe, E., McElroy, J. P., Song, M. A., & Wewers, M. D. (2017). A review of pulmonary toxicity of electronic cigarettes in the context of smoking: A focus on inflammation. In *Cancer Epidemiology Biomarkers and Prevention* (Vol. 26, Issue 8, pp. 1175–1191). American Association for Cancer Research Inc. https://doi.org/10.1158/1055-9965.EPI-17-0358

Singh, K. P., Lawyer, G., Muthumalage, T., Maremanda, K. P., Khan, N. A., McDonough, S. R., Ye, D., McIntosh, S., & Rahman, I. (2019). Systemic biomarkers in electronic cigarette users: implications for noninvasive assessment of vaping-associated pulmonary injuries. *ERJ Open Research*, *5*(4), 00182–02019. https://doi.org/10.1183/23120541.00182-2019

Sleiman, M., Logue, J. M., Montesinos, V. N., Russell, M. L., Litter, M. I., Gundel, L. A., & Destaillats, H. (2016). Emissions from electronic cigarettes: Key parameters affecting the release of harmful chemicals. *Environmental Science and Technology*, *50*(17), 9644–9651. https://doi.org/10.1021/acs.est.6b01741

Smith, M. R., Clark, B., Lüdicke, F., Schaller, J. P., Vanscheeuwijck, P., Hoeng, J., & Peitsch, M. C. (2016). Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the system and the scientific assessment program. *Regulatory Toxicology and Pharmacology*, *81*, S17–S26. https://doi.org/10.1016/j.yrtph.2016.07.006

Sohal, S. S., Eapen, M. S., Naidu, V. G. M., & Sharma, P. (2019). IQOS exposure impairs human airway cell homeostasis: direct comparison with traditional cigarette and e-cigarette. *ERJ Open Research*, *5*(1), 00159–02018. https://doi.org/10.1183/23120541.00159-2018

Son, Y., Mishin, V., Laskin, J. D., Mainelis, G., Wackowski, O. A., Delnevo, C., Schwander, S., Khlystov, A., Samburova, V., & Meng, Q. (2019). Hydroxyl Radicals in E-Cigarette Vapor and E-Vapor Oxidative Potentials under Different Vaping Patterns. *Chemical Research in Toxicology*, *32*(6), 1087–1095. https://doi.org/10.1021/acs.chemrestox.8b00400

Staal, Y. C., van de Nobelen, S., Havermans, A., & Talhout, R. (2018). New Tobacco and Tobacco-Related Products: Early Detection of Product Development, Marketing Strategies, and Consumer Interest. *JMIR Public Health and Surveillance*, *4*(2), e55. https://doi.org/10.2196/publichealth.7359

Stead, L. F., Perera, R., Bullen, C., Mant, D., Hartmann-Boyce, J., Cahill, K., & Lancaster, T. (2012). Nicotine replacement therapy for smoking cessation. In *Cochrane Database of Systematic Reviews* (Vol. 2017, Issue 12). John Wiley and Sons Ltd. https://doi.org/10.1002/14651858.CD000146.pub4

Sun, Y.-W., Kosinska, W., & Guttenplan, J. B. (2019). E-cigarette Aerosol Condensate Enhances Metabolism of Benzo(a)pyrene to Genotoxic Products, and Induces CYP1A1 and CYP1B1, Likely by Activation of the Aryl Hydrocarbon Receptor. *International Journal of Environmental Research and Public Health*, *16*(14). https://doi.org/10.3390/ijerph16142468

Talih, S., Balhas, Z., Eissenberg, T., Salman, R., Karaoghlanian, N., El Hellani, A., Baalbaki, R., Saliba, N., & Shihadeh, A. (2015). Effects of user puff topography, device voltage, and liquid nicotine concentration on electronic cigarette nicotine yield: measurements and model predictions. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *17*(2), 150–157. https://doi.org/10.1093/ntr/ntu174

Talih, S., Balhas, Z., Salman, R., Karaoghlanian, N., & Shihadeh, A. (2016). "Direct dripping": A high-temperature, high- formaldehyde emission electronic cigarette use method. *Nicotine and Tobacco Research*, *18*(4), 453–459. https://doi.org/10.1093/ntr/ntv080

Uchiyama, S., Noguchi, M., Sato, A., Ishitsuka, M., Inaba, Y., & Kunugita, N. (2020). Determination of Thermal Decomposition Products Generated from E-Cigarettes. *Chemical Research in Toxicology*, *33*(2), 576–583. https://doi.org/10.1021/acs.chemrestox.9b00410

van der Toorn, M., Frentzel, S., De Leon, H., Goedertier, D., Peitsch, M. C., & Hoeng, J. (2015). Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *86*, 81–87. https://doi.org/10.1016/j.fct.2015.09.016

Wang, G., Liu, W., & Song, W. (2019). Toxicity assessment of electronic cigarettes. In *Inhalation Toxicology* (Vol. 31, Issue 7, pp. 259–273). Taylor and Francis Ltd. https://doi.org/10.1080/08958378.2019.1671558

WHO. (2019). *WHO report on the global tobacco epidemic*.

WHO Tobacco Laboratory. (2012). *WHO SOP 01Standard operating procedure for intense smoking of cigarettes, WorldHealth Organization, Geneva, Switzerland.*

Wong, E. T., Kogel, U., Veljkovic, E., Martin, F., Xiang, Y., Boue, S., Vuillaume, G., Leroy, P., Guedj, E., Rodrigo, G., Ivanov, N. V, Hoeng, J., Peitsch, M. C., & Vanscheeuwijck, P. (2016). Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarette smoke. *Regulatory Toxicology and Pharmacology : RTP*, *81 Suppl 2*, S59–S81. https://doi.org/10.1016/j.yrtph.2016.10.015

Woodward, M., & Tunstall-Pedoe, H. (1993). Self-titration of nicotine: evidence from the Scottish Heart Health Study. *Addiction*, *88*(6), 821–830. https://doi.org/10.1111/j.1360-0443.1993.tb02096.x

Zhang, S., Zhang, J., Chen, H., Wang, A., Liu, Y., Hou, H., & Hu, Q. (2019). Combined cytotoxicity of co-exposure to aldehyde mixtures on human bronchial epithelial BEAS-2B cells. *Environmental Pollution (Barking, Essex : 1987)*, *250*, 650–661. https://doi.org/10.1016/j.envpol.2019.03.118

Zhao, D., Navas-Acien, A., Ilievski, V., Slavkovich, V., Olmedo, P., Adria-Mora, B., Domingo-Relloso, A., Aherrera, A., Kleiman, N. J., Rule, A. M., & Hilpert, M. (2019). Metal concentrations in electronic cigarette aerosol: Effect of open-system and closed-system devices and power settings. *Environmental Research*, *174*, 125–134. https://doi.org/10.1016/j.envres.2019.04.003

Zhao, J., Zhang, Y., Sisler, J. D., Shaffer, J., Leonard, S. S., Morris, A. M., Qian, Y., Bello, D., & Demokritou, P. (2018). Assessment of reactive oxygen species generated by electronic

cigarettes using acellular and cellular approaches. *Journal of Hazardous Materials*, *344*, 549–557. https://doi.org/10.1016/j.jhazmat.2017.10.057

Figure 1. Nicotine levels (in µg/puff) in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F cigarette aerosols. Data represent the mean ± SD of four independent measurements. *p < 0.05.

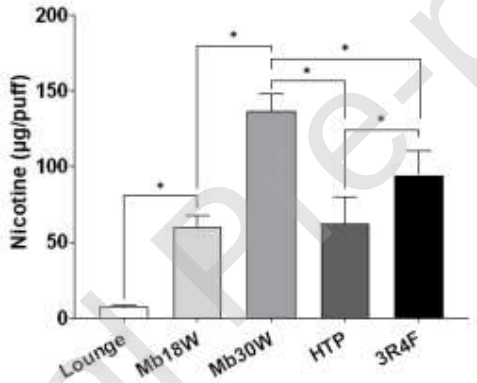

Figure 2. Total content of carbonyl compounds in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F cigarette aerosols. Data represent the mean ± SD of four independent measurements (*p < 0.05) and are expressed in µg/puff (A) or in ng/µg of nicotine (C). The corresponding reduction (%) in emissions of total carbonyl compounds (B and D, respectively) was compared to that of the 3R4F cigarette, HTP or e-cig.

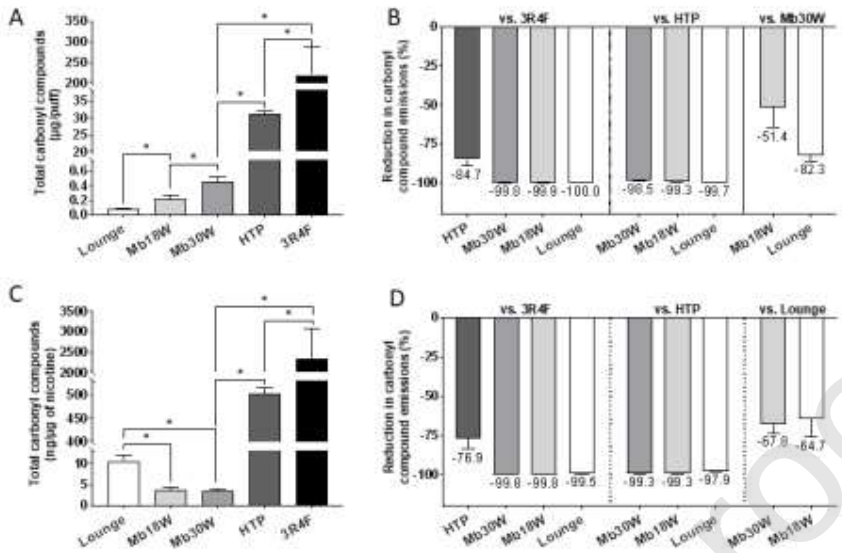

Figure 3. Total content of PAHs in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F cigarette aerosols. Data represent the mean ± SD of four independent measurements (*$p < 0.05$) and are expressed in ng/puff (A) or in pg/µg of nicotine (C). The corresponding reduction (%) in emissions of total PAHs (B and D, respectively) was compared to that of the 3R4F cigarette, HTP or e-cig.

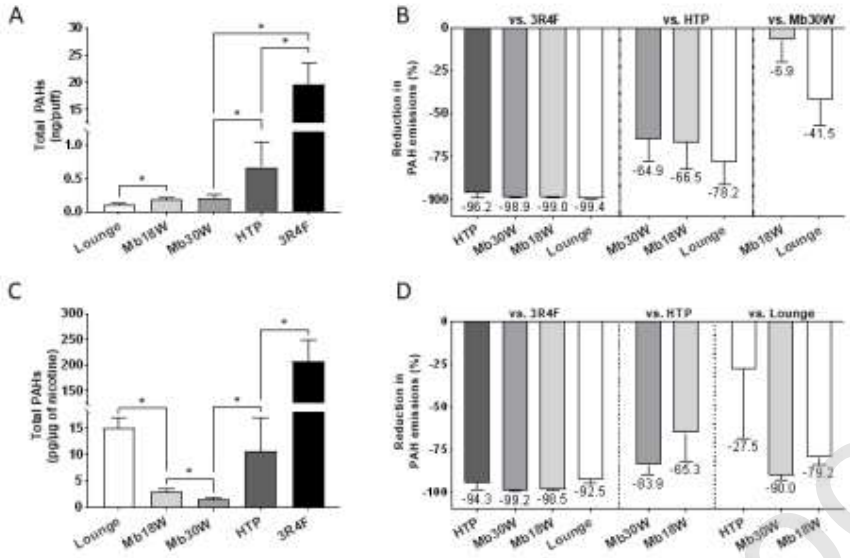

Figure 4. Cell viability after exposure of BEAS-2B cells to different puff numbers of e-cigs [Lounge (A), Mb18W (B) or Mb30W (C)], HTP (D) and 3R4F cigarette (E). The viability was assessed by measuring intracellular ATP content in cells 24 h after exposure. Results are expressed as percentages relative to the ATP content in control cells arbitrarily set at a value of 100 %. Data represent the mean ± SD of three independent culture replicates. *$p < 0.05$ compared to control cells. (F) Cell viability expressed as a function of nicotine content (mg) in aerosols from each device. Nicotine content is determined by the nicotine concentration per puff (depending of the device) multiplied by the number of puffs.

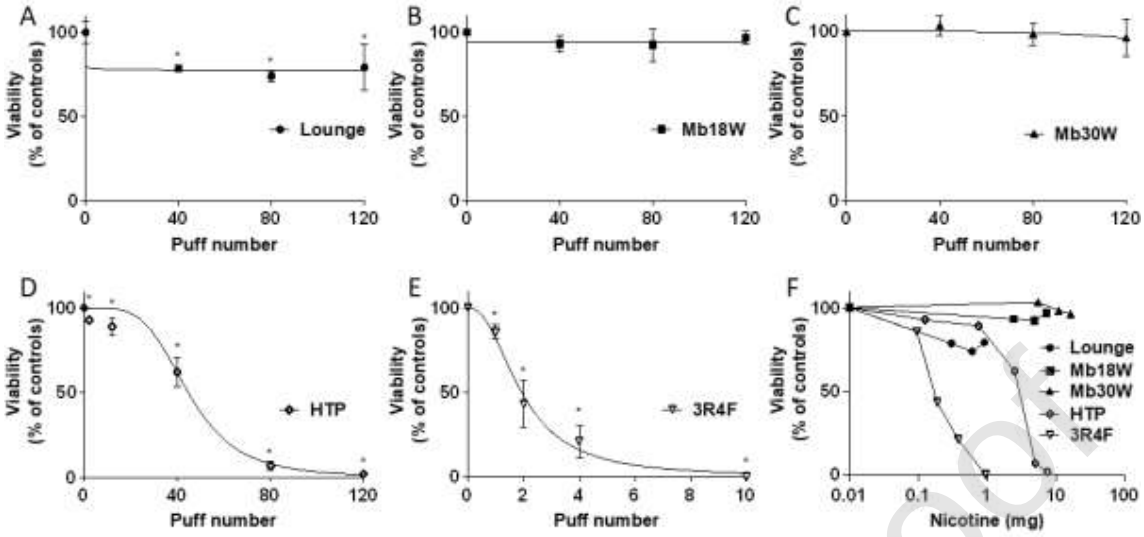

Figure 5. Glutathione ratio (GSSG/GSH) in BEAS-2B cells after exposure to e-cig [Lounge (A), Mb18W (B) or Mb30W (C)], HTP (D) and 3R4F cigarette (E) aerosols. The GSSG and GSH contents were measured immediately after cell exposure. Results are expressed as fold-change relative to the GSSG/GSH ratio in control cells arbitrarily set at a value of 1. Data represent the mean ± SD of three independent culture replicates. * $p < 0.05$ compared to control cells.

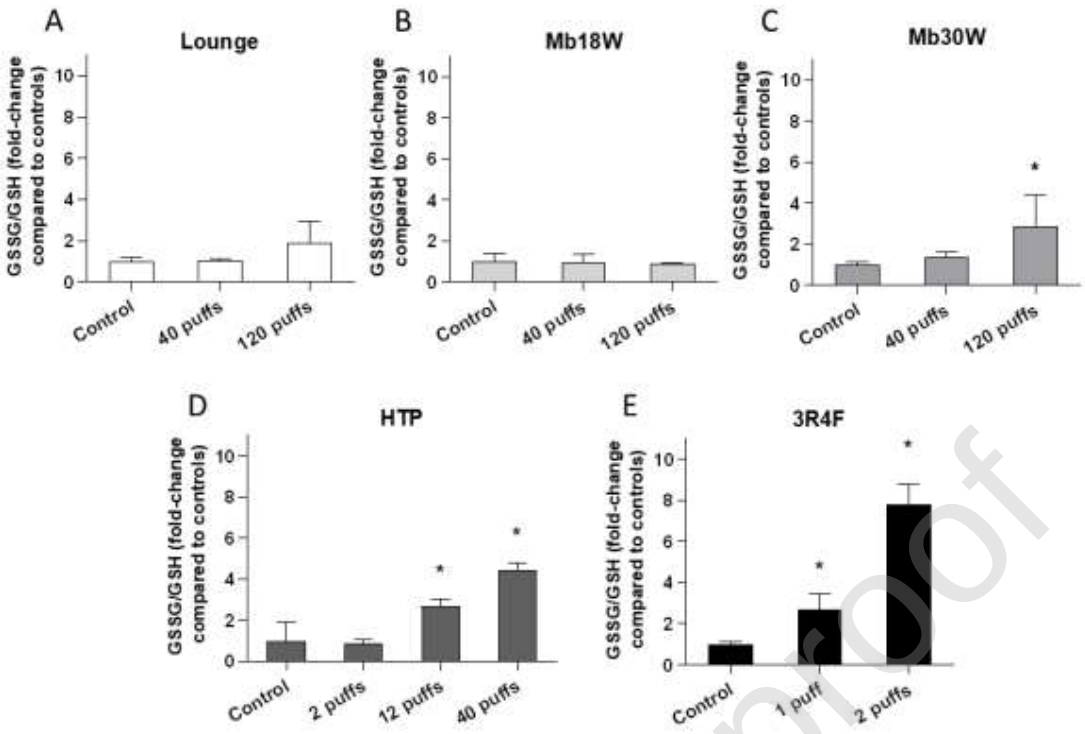

Figure 6. Expression of mRNAs encoding genes related to metabolism [*CYP1A1* (A), *CYP1B1* (B)] and oxidative stress [*HMOX1* (C)*, NQO1* (D)] in BEAS-2B cells. The gene expression was analysed 4 h or 24 h after exposure to 120 puffs of e-cig (Lounge, Mb18W or Mb30W), 12 puffs of HTP or 1 puff of 3R4F cigarette. Data represent the mean ± SD of three independent culture replicates. Results are expressed as fold-change relative to control cells, arbitrarily set at a value of 1. Data represent the mean ± SD of three independent culture replicates. * $p < 0.05$ compared to control cells.

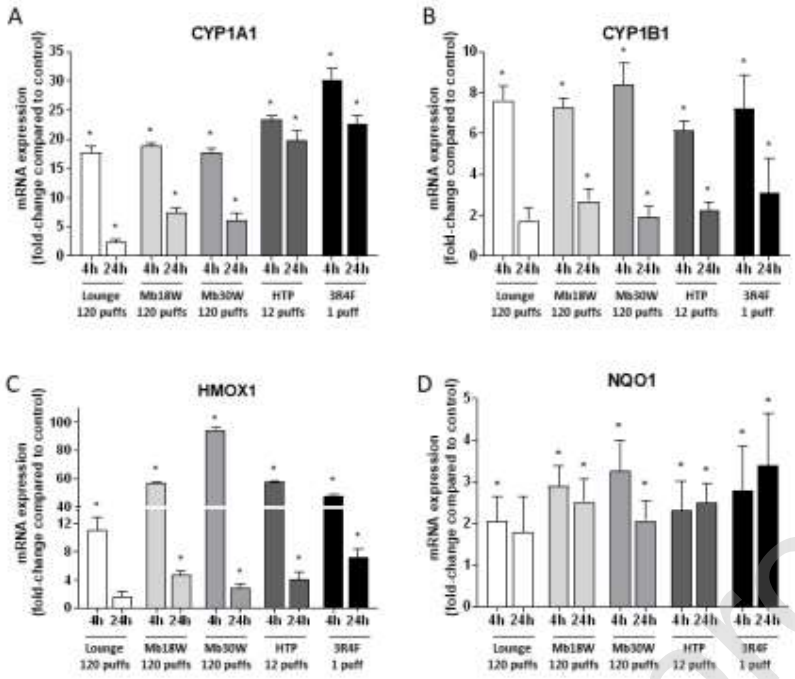

Table 1. Carbonyl concentrations (in ng/puff) in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F cigarette aerosols. Data represent the mean ± SD of four independent measurements. " ~ ": undetectable as < to LOD.

	Lounge	Mb18W	Mb30W	HTP
Formaldehyde	6.0 ± 0.7	25.8 ± 2.8	64.5 ± 23.7	156.9 ± 9
Acetaldehyde	32.9 ± 5.4	63.0 ± 10.3	160.9 ± 46.4	26687.7 ± 65
Propanone	3.9 ± 2.7	13.8 ± 3.0	28.5 ± 8.1	3132.3 ± 14
Propanal	2.1 ± 0.7	8.4 ± 2.4	23.2 ± 5.6	1400.1 ± 20
Methyl vinyl ketone	0.2 ± 0.1	6.4 ± 4.2	6.4 ± 2.1	443.1 ± 42
Crotonaldehyde	2.4 ± 0.1	16.1 ± 3.3	38.8 ± 8.1	139.9 ± 10
Methyl ethyl ketone	0.8 ± 1.6	34.7 ± 23.6	23.5 ± 9.5	625.6 ± 26
Methylpropenal	~ ± ~	~ ± ~	~ ± ~	334.8 ± 20
Butanal	0.1 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	985.9 ± 94
Benzaldehyde	0.5 ± 0.1	2.5 ± 0.3	3.2 ± 0.1	58.9 ± 2
Isopentanal	0.7 ± 0.1	7.9 ± 1.1	11.5 ± 0.6	391.3 ± 37
Pentanal	0.5 ± 1.1	1.0 ± 0.2	0.4 ± 0.1	25.2 ± 1
Glyoxal	0.6 ± 0.4	0.6 ± 0.0	0.7 ± 0.0	40.7 ± 9
o-Tolualdehyde	0.7 ± 0.1	2.9 ± 0.5	2.8 ± 0.5	6.3 ± 0
m-Tolualdehyde	~ ± ~	1.0 ± 0.6	1.1 ± 0.8	~ ±
p-Tolualdehyde	1.7 ± 0.4	0.9 ± 0.6	0.6 ± 0.7	115.0 ± 26
Methylglyoxal	25.2 ± 3.1	12.2 ± 1.1	44.1 ± 10.9	490.1 ± 69

Hexanal	0.5	±	0.1	1.5	±	0.1	1.8	±	0.1	22.1	±	1
2,5-Dimethylbenzaldehyde	~	±	~	0.6	±	0.1	0.7	±	0.1	~	±	
Total carbonyl compounds	**79**	**±**	**10**	**201**	**±**	**48**	**415**	**±**	**63**	**35056**	**±**	**8**

Table 2. PAH concentrations (in pg/puff) in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F cigarette aerosols. Data represent the mean ± SD of four independent measurements. " ~ ": undetectable as < to LOD.

	Lounge	Mb18W	Mb30W	HTP
Naphthalene	61.5 ± 9.5	75.9 ± 5.6	92.2 ± 6.2	71.2 ± 3
Acenaphthene	0.2 ± 0.1	2.6 ± 1.1	5.0 ± 1.4	12.5 ± 1
Fluorene	6.7 ± 3.3	6.7 ± 1.5	5.0 ± 1.3	26.0 ± 2
Phenanthrene	7.2 ± 0.7	25.2 ± 8.2	22.8 ± 3.5	55.9 ± 3
Anthracene	0.6 ± 0.1	1.7 ± 0.4	2.8 ± 3.7	4.7 ± 2
Fluoranthene	9.2 ± 1.4	20.1 ± 11.8	11.5 ± 11.8	131.0 ± 7
Pyrene	17.9 ± 4.3	30.9 ± 9.2	30.9 ± 10.9	153.0 ± 9
Benzo(c)phenanthrene	1.9 ± 0.6	4.5 ± 2.1	3.1 ± 4.4	10.2 ± 6
Benzo(a)anthracene	0.2 ± 0.0	2.6 ± 0.8	3.2 ± 4.0	43.8 ± 2
Chrysene	0.4 ± 0.3	1.6 ± 0.2	2.5 ± 4.0	26.3 ± 1
5-Methylchrysene	1.5 ± 0.4	1.0 ± 0.7	0.6 ± 0.4	1.7 ± 1
Benzo(e)pyrene	1.9 ± 0.2	6.1 ± 2.5	5.3 ± 3.6	22.9 ± 1
Benzo(b)fluoranthene	0.3 ± 0.1	1.2 ± 0.2	4.2 ± 7.1	18.9 ± 8
Benzo(k)fluoranthene	0.2 ± 0.1	0.5 ± 0.1	1.4 ± 2.2	18.4 ± 1
Benzo(a)pyrene	0.6 ± 0.2	0.6 ± 0.2	1.1 ± 0.3	25.6 ± 1
Dibenzo(a,l)pyrene	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0
Dibenzo(a,h)anthracene	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.8 ± 0
Benzo(g,h,i)perylene	1.5 ± 0.7	0.9 ± 0.2	4.8 ± 3.1	16.6 ± 8
Indeno(1,2,3-c,d)pyrene	0.3 ± 0.1	0.2 ± 0.0	1.5 ± 2.0	6.6 ± 5
Dibenzo(a,e)pyrene	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.5 ± 0
Anthanthrene	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	11.7 ± 6
Coronene	0.3 ± 0.1	0.5 ± 0.1	2.8 ± 1.0	5.5 ± 1
Cyclopenta(c,d)pyrene	~ ± ~	~ ± ~	~ ± ~	~ ±
Total PAHs	**113 ± 16**	**183 ± 29**	**202 ± 57**	**664 ± 3**

Table 3. Profile of inflammatory mediators (IL-6, IL-8, MCP-1 and GROα) secreted by BEAS-2B cells 24 h after exposure to the emissions of e-cigs (Lounge, Mb18W or Mb30W), HTP or 3R4F cigarette. Data represent the mean ± SD of three independent culture replicates. Results are expressed as fold-change relative to control cells, arbitrarily set at a value of 1. Data in bold are significantly different from controls ($p < 0.05$).

	IL-6	IL-8	MCP-1	GRO-α
Lounge				
40 puffs	2.1 ± 0.6	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.1
120 puffs	2.2 ± 1.0	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
Mb18W				
40 puffs	2.5 ± 1.1	1.1 ± 0.6	1.0 ± 0.1	1.0 ± 0.2
120 puffs	**2.3 ± 0.6**	1.0 ± 0.3	0.8 ± 0.1	0.8 ± 0.1

Mb30W				
40 puffs	**3.3 ± 0.4**	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.2
120 puffs	**2.4 ± 0.1**	0.6 ± 0.1	**0.6 ± 0.1**	**0.4 ± 0.1**
HTP				
2 puffs	0.4 ± 0.0	1.0 ± 0.0	**0.6 ± 0.0**	0.9 ± 0.1
12 puffs	3.0 ± 3.0	**3.3 ± 0.4**	**0.6 ± 0.1**	**0.7 ± 0.1**
40 puffs	1.3 ± 1.0	1.3 ± 1.5	**0.2 ± 0.1**	**0.2 ± 0.1**
3R4F				
1 puff	1.4 ± 0.5	1.4 ± 0.3	1.0 ± 0.1	**0.7 ± 0.1**
2 puffs	1. 5 ± 0.5	1.8 ± 0.8	**0.2 ± 0.1**	**0.2 ± 0.1**