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1 **Title Page**

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4 **Comparison of the chemical composition of aerosols from heated tobacco products,**
5 **electronic cigarettes and tobacco cigarettes and their toxic impacts on the human**
6 **bronchial epithelial BEAS-2B cells**
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8

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64

65

Abstract

66

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

83

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

85

Abbreviations

86

87 ALI: air-liquid interface

88 e-cig: electronic cigarette

89 GSH: reduced glutathione

90 GSSG: oxidized glutathione

91 HTP: heated tobacco products

92 Mb18W: Modbox e-cig model set at 18 W

93 Mb30W: Modbox e-cig model set at 30 W

94 PAHs: polycyclic aromatic hydrocarbons

95 **1. Introduction**

96

97 The tobacco epidemic causes 8 million deaths each year worldwide. Responsible for almost
98 30 % of cancer-related deaths (especially 90 % of lung cancers) and being the major risk
99 factor for chronic obstructive pulmonary diseases, smoking is the main single cause of
100 preventable deaths in the world (WHO, 2019). Out of the more than 7,000 chemicals present
101 in tobacco smoke, at least 250 are known to be harmful and about 70 can cause cancer:
102 benzene, formaldehyde, acetaldehyde, acrylamide, nitrosamines, arsenic, cadmium... There
103 is no safe level of tobacco use and quitting smoking significantly reduces the risk of
104 developing smoking-related diseases. Although the health benefits are greater for people
105 who stop at earlier ages, benefits exist at any age (Babb et al., 2017). However, smoking
106 cessation is a difficult and challenging task because of the addictive power of nicotine, which
107 is naturally found in tobacco (Benowitz, 2010). Nicotine replacement therapy (mostly
108 available as transdermal patch, nasal spray, inhaler, gum and sublingual tablets) can help to
109 increase chances of sustainable smoking cessation (Stead et al., 2012). However, none of
110 these devices mimics the feeling of satisfaction that occurs with the rapid and abundant
111 delivery of nicotine during smoking. New alternatives to regular cigarettes, such electronic
112 cigarettes (e-cigs) and heated tobacco products (HTP), also called heat-not-burn tobaccos,
113 have emerged on the market over the past decade. The e-cigs and emergent tobacco
114 products are generally perceived as low-risk substitutes for cigarette and have quickly gained
115 popularity, well before sufficient scientific evidence would allow to determine their potential
116 detrimental effects on users.

117

118 E-cigs comprise a battery-powered heating element that is designed to vaporize a solution
119 (“e-liquid”) made of propylene glycol and/or glycerine and frequently flavouring and
120 nicotine. The vapour is then inhaled by the user. Concerns raised about the levels of some
121 harmful constituents found in e-cig aerosols, such as carbonyl compounds (including
122 formaldehyde, acetaldehyde and acrolein) (Beauval et al., 2017, 2019; Belushkin et al., 2020)
123 and polycyclic aromatic hydrocarbons (PAHs) (including benzo[a]pyrene) (Beauval et al.,
124 2017; Belushkin et al., 2020), which may be produced by thermal decomposition of e-liquid
125 components. The presence of several trace metals was also reported in e-cig emissions,
126 likely released by cartomizer components (Beauval et al., 2017; Zhao et al., 2019). In parallel,

127 some studies investigated the effects of e-cig vapour exposure on human bronchial epithelial
128 cells cultured at air-liquid interface, and found that e-vapours did not lead to significant
129 cytotoxicity, but induced oxidative stress and/or increased the secretion of pro-
130 inflammatory mediators (Anthérieu et al., 2017; Iskandar et al., 2016; Scheffler et al., 2015).
131 In addition, transcriptomic modifications induced by e-cig vapour exposure were
132 demonstrated to be lesser than those induced by tobacco smoke (Anthérieu et al., 2017;
133 Iskandar et al., 2016). Evolution in e-cig devices (especially the development of new e-cig
134 generation with **high power, sub-Ohm devices**) may increase the amounts of harmful and
135 potentially-harmful compounds in emissions (Beauval et al., 2019; Belushkin et al., 2020;
136 Farsalinos et al., 2018c; Zhao et al., 2019). Consequently, further research is needed to
137 better understand the impacts of e-cig model and power output on cellular toxicity.

138
139 Although e-cig could help to quit smoking (Kalkhoran et al., 2019; Nelson et al., 2015), some
140 vapers could be unsatisfied because e-cig devices do not use real tobacco ingredients and
141 lack the so-called “throat-hit” or authentic tobacco tastes that cigarettes offer. These
142 elements may encourage some people to go back to conventional cigarettes (Staal et al.,
143 2018). Therefore, tobacco companies developed new tobacco products to keep meeting the
144 changing needs of their consumers. They launched HTP that taste more like conventional
145 cigarettes while still delivering nicotine to consumers. In particular, Philip Morris
146 International created the IQOS device: disposable tobacco sticks soaked in propylene glycol
147 (called heatsticks) are inserted in a holder in the IQOS device and heated with an electric
148 blade (Smith et al., 2016). These new devices are marketed by Philip Morris International as
149 products that do not combust tobacco, as cigarettes do, but heat it to a lower temperature
150 (less than 350 °C) with the aim to avoid the combustion-related production of harmful
151 compounds. To support the health claims of IQOS, Philip Morris International published
152 several peer-reviewed papers (Malinska et al., 2018; Schaller et al., 2016a, 2016b; Sewer et
153 al., 2016; Smith et al., 2016; van der Toorn et al., 2015; Wong et al., 2016). They showed a
154 reduction of most of the harmful constituents found in tobacco smoke (carbonyl
155 compounds, PAHs, nitrosamines...), as well as a reduction of cytotoxicity and genotoxicity in
156 comparison to cigarette smoke exposure (Schaller et al., 2016a). Today, most data about
157 HTP are published by HTP or tobacco industries themselves and toxicity assessment is

158 limited to a comparison with cigarette smoke. However, it is crucial for smokers to know the
159 toxicity of HTP compared to conventional cigarette and e-cigs.

160

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

172

173

174 2. Material and methods

175

176 2.1 Tobacco products and e-cig

177 The HTP used in this study was the iQOS model manufactured by Philip Morris (Neuchâtel,
178 Switzerland), with iQOS heatsticks (amber box from Philip Morris) purchased in a local
179 tobacco shop. IQOS was cleaned regularly after each 20th heatstick, as recommended by the
180 manufacturer. Two models of e-cig from a French manufacturer (NHOSS[®] brand) were used
181 in these experiments. The first one was the second generation “Lounge” model, equipped
182 with a 2.8 Ω nichrome coil and 4.6 W power supply. The coil heating was triggered by air
183 suction. The second one was the third generation “ModBox” model, used with the “Air Tank”
184 clearomiser equipped with a 0.5 Ω kanthal coil and with a partially closed air flow. Heating
185 was pre-activated 1 s prior the puff, as recommended by the manufacturer and accordingly
186 with the CORESTA standard puffing method CRM81 (CORESTA, 2015). Modbox model was
187 tested at two power settings, 18 W and 30 W. These settings correspond to the lower and
188 upper range power supplies recommended by the manufacturer for the coils used. One e-
189 liquid was used, “blond tobacco” flavoured (NHOSS[®] brand) and labelled as follows:

190 propylene glycol < 65 %; glycerol < 35 %; food flavourings; nicotine 16 mg/mL. 3R4F research
191 cigarettes were purchased from the University of Kentucky (Lexington, KY, USA).

192

193 2.2 Aerosol generation

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

202

203 2.3 Quantification of nicotine

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

208

209 2.4 Identification and quantification of carbonyl compounds

210 Carbonyls were collected from aerosols into two silica cartridges coated with 2,4-
211 dinitrophenylhydrazine (DNPH) placed in series, as described previously (Beauval et al.,
212 2019). Sep-Pak DNPH-Silica Plus Short Cartridges containing 350 or 750 mg of sorbent per
213 cartridge (Waters, Guyancourt, France) were used for e-cig and IQOS aerosols or cigarette
214 smoke, respectively. Each collection was replicated four times. Blank collections were
215 performed using the smoking machine working without e-cig, HTP or conventional cigarette
216 connected to and were taken into account for data analysis. DNPH cartridges were eluted with
217 3 or 6 mL of acetonitrile for short and long cartridges, respectively. Elutions from both
218 cartridges placed in series were pooled and then injected into a Thermo Scientific Dionex
219 UltiMate 3000 UHPLC System with UV/VIS Detector (Thermo Scientific, Waltham, MA). The
220 UHPLC instrument was operated at 28 C° at a constant flow rate of 0.4 mL/min, under a

221 gradient of acetonitrile and water during 15 min. Analysis was performed using Acclaim
222 Carbonyl C18 RSCL 150 mm x 2.1 mm x 5 μ M (Thermo Fisher Scientific). Acquisition was
223 performed at 360 nm wavelength. Instrument monitoring and data acquisition were done
224 using Chromeleon 7.0 Data Acquisition System for LC (Thermo Scientific, Waltham, MA). The
225 method allows the detection and quantification of nineteen compounds (Table 1) with limits
226 of quantification (LOQs) ranging from 6 to 15 ng/mL. Acrolein was not measured in this study
227 due to the unsuitability of using the DNPH-coated solid sorbent cartridge for its collection
228 (Ho et al., 2011).

229

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

237

238 2.5 Identification and quantification of PAHs

239 PAHs were collected from aerosols simultaneously with nicotine collection in the same
240 impingers, as described previously (Beauval et al., 2017). Each collection was replicated four
241 times. Blank collections were performed using the smoking machine working without e-cig,
242 HTP or conventional cigarette connected to and were taken into account for data analysis.
243 Methanolic samples were first concentrated under a gentle nitrogen flow in a water bath at
244 45 °C to a final volume of 1 mL in order to subsequently extract PAHs using accelerated
245 solvent extraction with acetonitrile. This extraction was followed by a second concentration
246 step under a gentle nitrogen flow in a water bath at 60 °C to a final volume of 1 mL.
247 Remaining products were then filtrated with a syringe membrane filter before injection into
248 the chromatographic system. The analyses were performed on HPLC Alliance 2695 (Waters
249 Corporation, MA) coupled with a multi-wavelength fluorescence detector. Chromatographic
250 separation was achieved on a EC 250/2 Nucleosil 100-5 C18 PAH (Macherey Nagel, Hoerd, France).
251 The method allows the detection and quantification of twenty-three compounds
252 (Table 2) with LOQs ranging from 1 to 39 pg/mL.

253

254 2.6 Cell culture and cell exposure to aerosols

255 *In vitro* experiments were performed using the human bronchial epithelial BEAS-2B cell line
256 obtained from the American Type Culture Collection (ATCC® CRL9609™). Cells were cultured
257 in LHC-9 medium (Life Technologies, Courtabœuf, France) and seeded in CellBIND 75 cm²
258 tissue culture flasks (Corning, Amsterdam, Netherlands) at 37 °C in a humidified incubator
259 with 5 % CO₂ in air and 85 % relative humidity. Cell passaging was performed when cells
260 reached 80-90 % confluence. Following subculture, cells were transferred to an air-liquid
261 interface (ALI) system. Cells were seeded (18 000 cells/cm²) onto transwell clear culture
262 inserts (4.67 cm²) with 0.4 µm pore size (Sigma Aldrich, Saint-Quentin Fallavier, France) pre-
263 coated with 0.03 mg/mL type I collagen solution (Life Technologies). BEAS-2B cells were
264 firstly maintained submerged, then ALI was established by removing medium from the apical
265 surface, exposing only the basal surface to medium. Cells were then transferred to an
266 exposure module (Vitrocell 6/4 CF module) and exposed to different doses (defined in puff
267 number) of the undiluted HTP, e-cig or cigarette aerosol generated by the Vitrocell® system.
268 Control cells were unexposed cells, which were left in the incubator. Each exposure was
269 replicated in three independent cell cultures.

270

271 2.7 Cell viability

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

278

279 2.8 Glutathione content assay

280 The GSH/GSSG-Glo™ Assay (Promega) was used following the manufacturer's guidelines for
281 the determination of total GSH and oxidized glutathione (GSSG). Finally, GSSG/GSH ratios
282 were calculated and results are expressed as fold-change relative to the GSSG/GSH ratio in
283 control cells arbitrarily set at a value of 1.

284

285 2.9 Gene expression analysis

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

298

299 2.10 Measurement of secreted mediators of inflammation

300 Concentrations of ten secreted inflammation mediators were measured in the basolateral
301 media of BEAS-2B cells: granulocyte-macrophage colony-stimulating factor (GM-CSF),
302 growth regulated oncogene α (GRO- α), interleukin 1β (IL- 1β), interleukin 6 (IL-6), interleukin
303 8 (IL-8), interleukin 13 (IL-13), monocyte chemoattractant protein 1 (MCP-1), macrophage
304 inflammatory protein 1-alpha (MIP-1 α), regulated on activation, normal T cell expressed and
305 secreted (RANTES) and interferon gamma (INF- γ). The assay has been performed based on
306 the recommendations of the Milliplex MAP Human Cytokine/Chemokine Magnetic Bead
307 Panel kit (Merck Millipore, Molsheim, France) using the Luminex[®] xMAP[®] technology
308 (Luminex Corp., Austin, TX). The capacity of BEAS-2B cells to secrete various mediators of
309 inflammation had been previously tested by treating cells with lipopolysaccharide (Anthérieu
310 et al., 2017).

311

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313 2.11 Statistical analysis

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

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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 HCl 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 HCl 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

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

357
358 The emission of nineteen carbonyl compounds in the aerosols of HTP, 3R4F cigarette and e-
359 cigs was first analysed and results (expressed in mass of each compound per puff) are
360 reported in Table 1. Fifteen compounds were quantified at levels between 2 and 15 times
361 higher in cigarette smoke than in HTP aerosols. Hexanal was the only compound measured
362 at a higher level in HTP emission (22.2 ng/puff) than in 3R4F tobacco smoke (10.5 ng/puff),
363 and benzaldehyde was generated in almost equivalent amounts by HTP and cigarette. By
364 contrast, all the carbonyl compounds were measured at very lower amounts in vapours from
365 the different tested e-cig conditions in comparison to tobacco products, except for m-
366 tolualdehyde and 2,5 dimethylbenzaldehyde which were only detected in aerosols from the
367 Modbox device, at both low and high-power settings. Figure 2-A represents the sum of total
368 carbonyl compounds measured in e-cig, HTP and 3R4F emissions. There are much less
369 carbonyl compounds produced in one puff of HTP (35 µg/puff) than in one puff of cigarette
370 smoke (230 µg/puff). Indeed, HTP emitted 84.7 % less carbonyl compounds than 3R4F
371 cigarette (Figure 2-B). Levels of carbonyl compounds measured in vapours from different e-
372 cig models were even at least 98.5 % weaker than in HTP aerosols. Comparison of data
373 between different e-cig devices showed that Lounge and Mb18W emitted respectively 82.3
374 % and 51.4 % less carbonyl compounds than Mb30W (0.4 µg/puff) (Figures 2-A and 2-B).

375
376 These different tobacco products and e-cigs produced carbonyls at very different levels in
377 emissions, probably because they generate aerosols *via* different processes and from diverse
378 materials (tobacco fillers or e-liquid). During smoking, carbonyls are mainly produced by the
379 pyrolysis of carbohydrates contained in tobacco (Seeman et al., 2002) at high temperature

380 (up to 900 °C). The aerosolization process of HTP operates at temperatures less than 350 °C
381 with the use of heatsticks containing tobacco leaves soaked in propylene glycol. These
382 devices are often referred to as “heat-not-burn” tobacco products with a reduction of the
383 user’s exposure to carcinogenic chemicals usually produced by the combustion of tobacco
384 (Schaller et al., 2016a). We effectively observed a reduction of about 90 % in carbonyl
385 emissions in accordance with data from the IQOS manufacturer (Schaller et al., 2016) and
386 two independent studies (Farsalinos et al., 2018b; Li et al., 2019). However, several harmful
387 carbonyls were still measured in HTP aerosol, such as acetaldehyde and formaldehyde,
388 which are carcinogenic compounds. The presence of high levels of acetaldehyde is the mark
389 of pyrolysis and thermogenic degradation of tobacco (Auer et al., 2017). By contrast, the
390 main source of carbonyls in e-cig emissions is the thermal degradation of glycerol and
391 propylene glycol contained in the e-liquid (Uchiyama et al., 2020). Some carbonyls were
392 quantifiable in the different vapours of tested e-cig, but several times far lower than in the
393 emissions from both tested tobacco products (Table 1). **In addition, there is a relation**
394 **between the operating power or the e-cig model and the concentrations of carbonyl**
395 **compounds detected in the e-cig aerosols. The power of e-cig, and therefore ultimately the**
396 **heat generated on the evaporation coil, has been reported to affect the quantity of**
397 **carbonyls formed (Geiss et al., 2016; Kosmider et al., 2014; Talih et al., 2016).** Some studies
398 reported higher carbonyl amounts in e-cig emissions (Goniewicz et al., 2014; Hutzler et al.,
399 2014; Sleiman et al., 2016; Talih et al., 2015). However, these findings have been questioned
400 as they could be the consequence of using unrealistic or extreme conditions (low interpuff
401 interval or high power generating high temperatures, drypuff phenomenon) (Farsalinos et
402 al., 2015, 2017). Indeed, e-cig can release high levels of aldehydes if the e-liquid is
403 overheated, but the overheating generates an aversive taste that would secure such
404 emissions to be avoided. To ensure realistic experimental conditions, two regular e-cig users
405 tested the e-cigs used in our study for the generation of dry puffs, using the puff duration
406 and power settings as tested with the smoking machine. The users confirmed no dry puff
407 sensation and sufficient vapour production. In addition, the temperature of the generated
408 aerosol was also considered as an indicator of experimental relevance and realism. The
409 temperature of the aerosols generated from each device did not exceed 60 °C during all the
410 collection periods (Supplemental Figure 1), following the recommendations of the French
411 national organisation and standardization (AFNOR, 2016). Overall, our data thus

412 demonstrate that, at normal vaping temperatures, carbonyl content in e-cig emissions
413 represents only a small fraction of levels inhaled by users of tobacco products.

414

415 In parallel, the emission of twenty-three PAHs in the aerosols of HTP, 3R4F cigarette and e-
416 cigs was analysed and results expressed in ng/puff are reported in Table 2. A similar pattern
417 than that seen for carbonyl compounds was observed for almost all emitted PAHs: the
418 concentrations of twenty-one compounds were markedly lower in HTP emissions than in
419 3R4F cigarette smoke (from 2 to 676 times depending on the compound), and were even
420 lesser in e-cig vapours. Only benzo(c)phenanthrene was reported to be higher in HTP
421 emissions, compared to all other aerosols. The sum of total PAHs measured was calculated
422 for each aerosol (Figure 3-A) and the reduction rate is indicated in figure 3-B: HTP (0.7
423 ng/puff) emitted 96.2 % less PAHs than 3R4F cigarette (19.6 ng/puff), but e-cig emitted 64.9
424 to 78.2 % less PAHs than HTP. Comparison of e-cig models showed no significant difference
425 in PAH content between Mb18W and Mb30W, and about 40 % less of total PAHs in Lounge
426 than in the Modbox model. These results support that the pyrolysis process is limited with e-
427 cigs. The e-liquid used for vaping is generally free of tobacco ingredients which contain the
428 PAH precursors. **Moreover, the temperature required to produce an e-cig aerosol from a e-
429 liquid is depending of the proportion in propylene glycol and glycerol. This temperature
430 ranges from 188.6°C to 292 °C, but water and alcohol used as additives, decrease this boiling
431 point (Duell et al., 2018). By comparison, IQOS operates at temperatures between 330°C and
432 349°C (Davis et al., 2019).** PAH emissions released by HTP were lower than combustible
433 cigarette but still contained harmful elements from thermal degradation that are also found
434 in cigarette smoke (Li et al., 2019; Rodgman et al., 2000), including the carcinogenic
435 benzo[a]pyrene.

436

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

444 carbonyl and PAH levels, normalized by the level of emitted nicotine in aerosols, are
445 reported in Tables S1 and S2 (supplementary materials), respectively. The comparison of
446 total carbonyl compounds after nicotine normalization (Figures 2-C and 2-D) showed,
447 according to previous conclusions, that the HTP emitted 76.9% less carbonyl compounds
448 (497 ng/ μ g of nicotine) than the combustible cigarette (2308 ng/ μ g of nicotine), but at least
449 97.9% higher levels than the e-cig vapours (< 10 ng/ μ g of nicotine). However, comparing the
450 e-cig models, Lounge emitted more carbonyl compounds than the Modbox model and no
451 significant difference was reported between Mb18W and Mb30W. The comparison of total
452 PAHs after nicotine normalization (Figures 3-C and 3-D) also showed substantial reduction
453 (94.3%) in the PAH content of HTP emissions (11 pg/ μ g of nicotine) in comparison to
454 cigarette smoke (207 pg/ μ g of nicotine). The pattern of PAH content between the different
455 e-cig models was different after nicotine normalization: the Lounge model emitted more
456 PAHs (15 pg/ μ g of nicotine) than the Modbox model (79.2-90 %) and even 27.5 % more than
457 HTP. These results showed that the way of expressing data (emissions per puff vs emissions
458 per nicotine yield) can influence their interpretation. Today, there is no standardized manner
459 to express the amount of emitted compounds in aerosols. Indeed, data can be expressed in
460 amount per puff (Beauval et al., 2019), per mL of puff (Beauval et al., 2017), per cigarette or
461 per IQOS heatstick (Li et al., 2019), per mass of nicotine (Farsalinos et al., 2018b), per liquid
462 consumption for e-cig (Beauval et al., 2017), thus hampering comparisons between studies
463 and making interpretations difficult. In addition, it is still unclear to which extent vapor
464 generation, collection and analysis procedures could affect results of chemical
465 characterization. Harmonized protocols to determine the chemical composition of emissions
466 and to express results are crucially needed to establish and compare risk profiles of each
467 emergent tobacco products in terms of chemical composition and user exposure.

468

469 3.2 *In vitro* toxicity

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

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

480

481 3.2.1 Cell viability

482 Cell viability was assessed 24 h after aerosol exposure by measuring intracellular ATP
483 content, which is directly proportional to the number of living cells. Data displayed in **figure**
484 **4** (A-E) show that e-cig vapours had no significant (Modbox model) or low (Lounge model: >
485 75 % cell viability) cytotoxic effects up to a 120-puff-exposure. In comparison, HTP emissions
486 caused intracellular ATP changes from 12 puffs (89 % viability) to a strong reduction of cell
487 viability that reached 2 % after 120 puffs. Cigarette smoke demonstrated also a full dose-
488 response curve, but its cytotoxicity appeared within fewer puffs (< 10 puffs). In order to
489 better compare the different devices, the effective dose of aerosol which results in a 50 %
490 reduction of cell viability (ED50, expressed here in puff number) was calculated. Although
491 the e-cig vapours did not induce sufficient cell mortality to calculate an ED50 (whatever the
492 tested e-cig power or model), ED50 was 45 puffs for HTP aerosol and 2 puffs for 3R4F
493 cigarette smoke. Bishop *et al.* have exposed a 3D-reconstituted human airway epithelium to
494 undiluted cigarette smoke and e-cig aerosol (Bishop et al., 2019). They found ED50 equal to 4
495 puffs and 60 puffs under HCl regime for cigarette and e-cig exposures, respectively.
496 However, they voluntarily used extreme conditions for e-cig exposure with an airflow vent
497 closed to achieve a worst case for carbonyl production and, consequently, higher
498 cytotoxicity. In a previous study using diluted aerosols (Anthérieu et al., 2017), we have
499 tested the Lounge model with different e-liquids (with or without nicotine, flavoured or
500 unflavoured) and demonstrated that none of the aerosols induced cytotoxicity in BEAS-B cell
501 line up to an exposure of 576 puffs. Today, few *in vitro* studies have compared the relative
502 cytotoxicity of HTP aerosols with both cigarette smoke and e-cig vapours, and most of these
503 assays have been performed using submersed cultures exposed to aerosol extracts (Ito et al.,
504 2019; Munakata et al., 2018; Sohal et al., 2019). ALI exposures provide a more pertinent
505 approach to perform toxicological studies related to inhalation of emerging **e-cigs** or novel
506 tobacco products (Johnson et al., 2009). Our present results are in agreement with a study
507 performed in ALI-cultured human bronchial epithelial H292 cells, demonstrating that HTP

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

521

522 3.2.2 Oxidative stress

523 The generation of oxidative stress was first assessed by measuring the intracellular content
524 of oxidized (GSSG) and reduced (GSH) glutathione. GSH is considered to be one of the most
525 important scavengers of reactive oxygen species (ROS), and the ratio GSSG/GSH may be used
526 as a marker of oxidative stress. We have previously described that the generation of
527 oxidative stress was transient and no change in glutathione levels was found in BEAS-2B cells
528 3 h after the end of cigarette smoke exposure (Anthérieu et al., 2017). Consequently, we
529 have measured GSH and GSSG contents immediately (0 h) after cell exposures (Figure 5). In
530 these experimental conditions, HTP induced a significant increase of GSSG/GSH ratio in
531 comparison to control cells (2.7 and 4.5 fold-changes after 12 and 40 puffs, respectively).
532 3R4F cigarette induced also an increase of the GSSG/GSH ratio but already after only 1 puff
533 (2.7 fold-change) and a 7.8 fold-change was observed after an exposure to 2 puffs. For e-cig
534 exposures, anti-oxidative response was evidenced only after a longer exposure of 120 puffs
535 with Mb30W (2.9 fold-change), although no significant change in the GSSG/GSH ratio was
536 observed with Mb18W and Lounge.

537 The cellular defense mechanisms against toxic substances also include transcriptomic
538 regulations of genes involved in detoxification processes and the anti-oxidative response.
539 Thus, the expression of selected genes involved in xenobiotic metabolism (cytochrome P450

540 1A1, *CYP1A1*; cytochrome P450 1B1, *CYP1B1*) and the anti-oxidative response (heme
541 oxygenase 1, *HMOX1*; NADPH Quinone Dehydrogenase 1, *NQO1*) was quantified 4 or 24 h
542 after exposure to 120 puffs of e-cig vapours, 12 puffs of HTP aerosol or 1 puff of 3R4F
543 cigarette smoke (Figure 6). For all the products and both time points, an increase of *CYP1A1*
544 and *CYP1B1* expression was observed, with a higher induction for *CYP1A1* than for *CYP1B1*.
545 PAHs contained in cigarette smoke are known to induce expression of *CYP1A1/1B1* via the
546 Aryl Hydrocarbon Receptor (AhR) pathway and, subsequently, to affect the metabolism of
547 tobacco carcinogens (Sacks et al., 2011). The *CYP1A1/1B1* expression is also induced in the
548 lungs of smokers (Kim et al., 2004). E-cig aerosols can also induce *CYP1A1* and *CYP1B1* and
549 enhance the metabolism of some PAHs (e.g. benzo[a]pyrene) to genotoxic products by
550 activating AhR (Sun et al., 2019). Furthermore, the expression of the downstream target
551 genes of the nuclear erythroid 2-related factor 2 (Nrf2), *NQO1* and *HMOX1*, was up-
552 regulated 4 h after exposure to the Lounge model vapours and at both time points for all
553 other emissions. The up-regulation of *NQO1* and *HMOX1* levels 4 h after exposure increased
554 with the e-cig power, reaching a 3.3 or 95-fold-change, respectively, for Mb30W. Some
555 smoke compounds, including carbonyls, PAHs, quinones, naphthoquinones and
556 benzenediols, were identified as activators of the Nrf2/antioxidant response element (ARE)
557 pathway and *HMOX1* induction in response to oxidative stress (Chan et al., 2013; Sekine et
558 al., 2016; Zhang et al., 2019). Some of these different components of cigarette smoke were
559 also found in aerosols of HTP or e-cig, which can explain the up-regulation of *NQO1* and
560 *HMOX1* in BEAS-2B cells whatever the products. Almost all of these transcriptomic
561 modulations were higher after 4 h exposure than after 24 h and correspond to early
562 adaptive mechanisms set up by BEAS-2B cells in response to a cellular stress after aerosol
563 exposure. It is important to note that these transcriptomic mechanisms were globally similar
564 for all the devices tested, demonstrating that all products have the potential to induce
565 detoxification and an anti-oxidative response. However, these molecular and cellular
566 responses were observed for highly different exposure levels (from 120 puffs for e-cig, 12
567 puffs for HTP and only 1 puff for tobacco cigarette). In addition, an exposure of 120 puffs in
568 one hour is representative of an intense exposure session, compared to data from
569 topography studies with e-cig users (Jones et al., 2020; Lee et al., 2018).

570

571 These results are in accordance with other studies demonstrating that the generation of ROS
572 is observed in HTP emissions after a more intensive use than with cigarette smoke
573 (Munakata et al., 2018). ROS would be mainly generated by exposure to chemicals derived
574 from combustion processes (Kopa & Pawliczak, 2020) and, therefore, can be directly linked
575 to the relative amounts of carbonyls and PAHs measured in HTP and cigarette smoke
576 emissions (Figures 2 and 3). The increase in the amount of carbonyl compounds produced
577 when Modbox was used under high power setting could explain, at least in part, the greater
578 induction of ROS production that would contribute to alter the oxidative/antioxidative
579 balance. Indeed, higher power leads to higher filament temperature, which enhances the e-
580 liquid vaporization process, pyrolysis and chain reactions with the production of hydroxide
581 and superoxide free radicals (Haddad et al., 2019; Son et al., 2019; Zhao et al., 2018).
582 However, Son *et al.* reported that the dose of free radicals per puff associated with e-cig
583 vaping was 10-1000 times lower than the reported dose generated by cigarette smoking
584 (Son et al., 2019).

585

586

587 3.2.3 Inflammatory response

588 The inflammatory response of BEAS-2B cells was assessed by measuring the secretion of ten
589 cytokines and chemokines 24 h after exposure to defined subtoxic doses of HTP, 3R4F
590 cigarette smoke or e-cig emissions. Only four mediators were found in quantifiable levels (IL-
591 6, IL-8, GRO α , and MCP-1) after exposure. GM-CSF, IL-13, IL-1 β , MIP-1 α , RANTES and TNF- α
592 were not detected in cell culture medium of BEAS-2B cells exposed to aerosols, despite the
593 capacity of these cells to secrete these mediators after treatment with lipopolysaccharide
594 (Anthérieu et al., 2017). The results for the four detected mediators were expressed in fold-
595 change relative to control cells (Table 3). A significant increase of IL-6 secretion was
596 observed in the culture medium of cells exposed to Mb18W (for 120 puffs) and Mb30W (for
597 both 40 and 120 puffs) aerosols, while no significant change was evidenced for IL-8
598 secretion. Increase of IL-6 and/or IL-8 had already been described in different cell models
599 after e-cig exposures (Merecz-Sadowska et al., 2020), and more remarkably in the plasma of
600 e-cig users (Singh et al., 2019), demonstrating that e-cig vapors could induce a pro-
601 inflammatory response. For HTP, a biphasic response was observed for IL-6 and IL-8 with a
602 greater increase in secretion after 12 puffs (3 and 3.3 fold-change, respectively) than after 40

603 puffs (1.3 fold-change). A comparable biphasic response in IL-8 secretion was previously
604 described in BEAS-2B cells exposed to HTP aerosol or tobacco smoke and a similar trend was
605 also observed for GM-CSF (Munakata et al., 2018), which was undetectable in our study.
606 These differences in results could be explained by the different protocols of cell exposure.
607 Indeed, Munataka *et al.* exposed submerged BEAS-2B cells to aerosol extracts, while ALI-
608 cultured cells were exposed directly to aerosols in our study. Finally, the secretion of MCP-1
609 and GRO- α was significantly decreased for the longer exposures to HTP aerosol, 3R4F
610 cigarette smoke and Mb30W vapour (Table 3). A down-regulation of GRO- α and MCP-1 was
611 also demonstrated after exposure to cigarette smoke in human endothelial cells (Allam et
612 al., 2013). GRO- α plays a significant role in the chemotaxis of neutrophils to the site of
613 inflammation and MCP-1 is a potent chemoattractant for monocytes and macrophages. This
614 recruitment and subsequent activation of monocytes into the inflamed tissues play a central
615 role in determining the outcomes of the immune responses of the tissues. Thus, alteration of
616 GRO- α and MCP-1 secretion after aerosol exposure could affect the tissue immune and
617 protective responses.

618
619 These different modulations in inflammatory mediators can be explained partially by the
620 carbonyl and PAH levels measured in the different emissions. Indeed, some carbonyls found
621 in aerosols or smoke (such as acrolein, acetaldehyde and formaldehyde) are known strong
622 irritants that may cause inflammation (Lino-dos-Santos-Franco et al., 2011; Shields et al.,
623 2017). The benzo[a]pyrene is also known as an inducer of the secretion of pro-inflammatory
624 cytokines (Chen et al., 2012). Other constituents of aerosols or smoke could play a major role
625 in the inflammatory response. Metals are capable of causing inflammatory cytokine induction
626 (Lerner et al., 2015). In addition, propylene glycol and glycerol produce a
627 hygroscopic/hyperosmolar aerosol which could deposit on the surface of lung cells and
628 trigger local inflammation (Chaumont et al., 2019).

629
630
631 Carbonyls and PAHs represent only a part of the complex mixture constituting the cigarette
632 smoke or the HTP and e-cig aerosols. Therefore, these pollutants take part in the cellular
633 response but all the changes observed in BEAS-2B cells (cytotoxicity, oxidative stress,
634 inflammation) cannot be explained solely by PAH and carbonyl emissions. A multitude of

635 other harmful compounds comprising other volatile organic compounds, metals,
636 nitrosamines etc. (Li et al., 2019; Schaller et al., 2016; Zhao et al., 2019) should take into
637 account to better characterize the toxic profile of these novel tobacco products and e-cigs in
638 comparison to tobacco cigarette.

639

640

641 4. Conclusion

642

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

666 their addictiveness is another key element to take into account in the tobacco harm-reduction
667 strategy.

668

669

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673

674

675 **6. Declaration of interest**

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

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Figure legends

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987

988 **Figure 1.** Nicotine levels (in $\mu\text{g}/\text{puff}$) in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F
989 cigarette aerosols. Data represent the mean \pm SD of four independent measurements. * $p <$
990 0.05.

991

992 **Figure 2.** Total content of carbonyl compounds in e-cig (Lounge, Mb18W or Mb30W), HTP
993 and 3R4F cigarette aerosols. Data represent the mean \pm SD of four independent
994 measurements (* $p < 0.05$) and are expressed in $\mu\text{g}/\text{puff}$ (A) or in $\text{ng}/\mu\text{g}$ of nicotine (C). The
995 corresponding reduction (%) in emissions of total carbonyl compounds (B and D,
996 respectively) was compared to that of the 3R4F cigarette, HTP or e-cig.

997

998 **Figure 3.** Total content of PAHs in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F
999 cigarette aerosols. Data represent the mean \pm SD of four independent measurements (* $p <$
1000 0.05) and are expressed in ng/puff (A) or in $\text{pg}/\mu\text{g}$ of nicotine (C). The corresponding
1001 reduction (%) in emissions of total PAHs (B and D, respectively) was compared to that of the
1002 3R4F cigarette, HTP or e-cig.

1003

1004 **Figure 4.** Cell viability after exposure of BEAS-2B cells to different puff numbers of e-cigs
1005 [Lounge (A), Mb18W (B) or Mb30W (C)], HTP (D) and 3R4F cigarette (E). The viability was
1006 assessed by measuring intracellular ATP content in cells 24 h after exposure. Results are
1007 expressed as percentages relative to the ATP content in control cells arbitrarily set at a value
1008 of 100 %. Data represent the mean \pm SD of three independent culture replicates. * $p < 0.05$
1009 compared to control cells. (F) Cell viability expressed as a function of nicotine content (mg)
1010 in aerosols from each device. Nicotine content is determined by the nicotine concentration
1011 per puff (depending of the device) multiplied by the number of puffs.

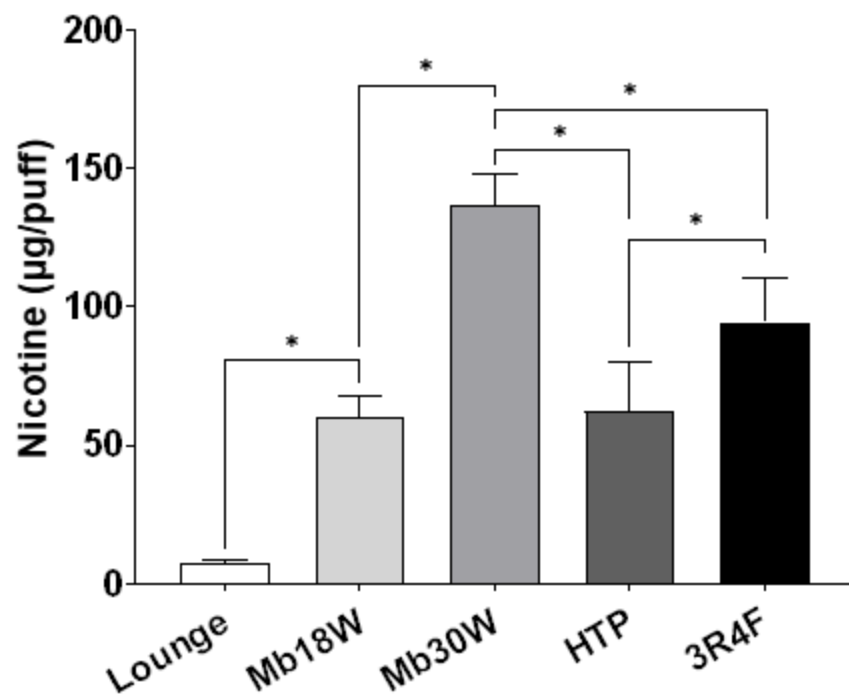
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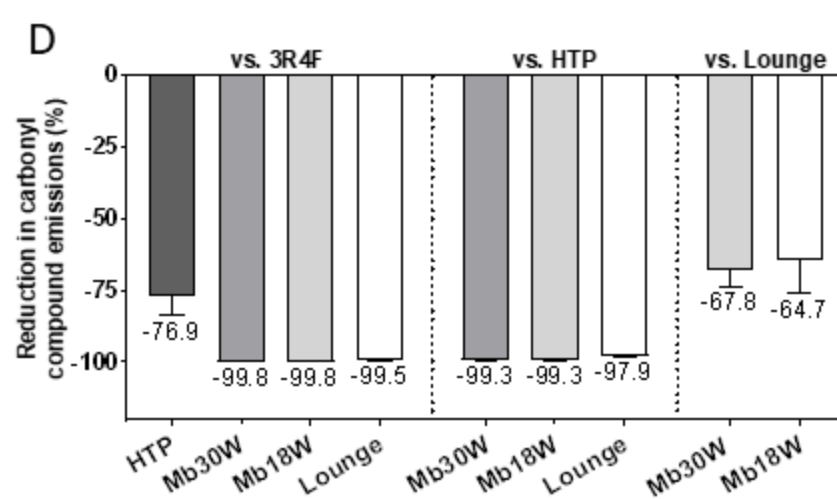
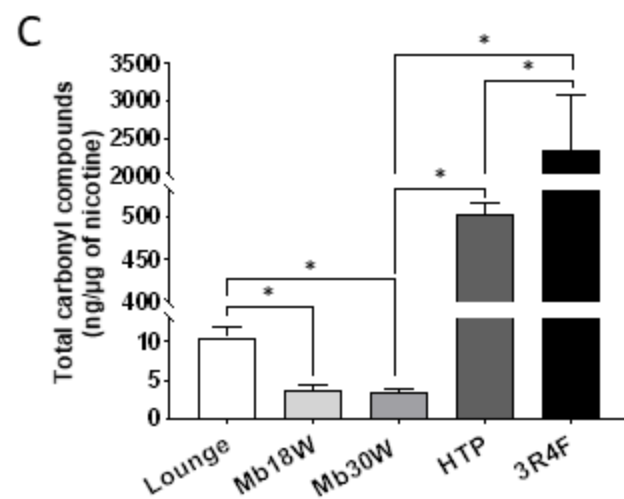
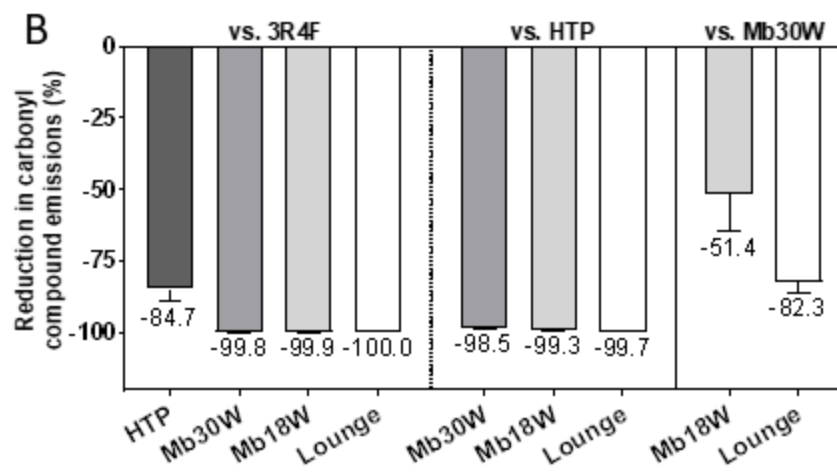
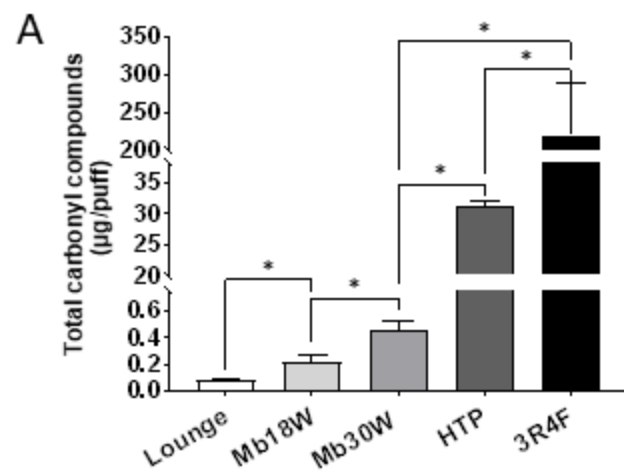
1013 **Figure 5.** Glutathione ratio (GSSG/GSH) in BEAS-2B cells after exposure to e-cig [Lounge (A),
1014 Mb18W (B) or Mb30W (C)], HTP (D) and 3R4F cigarette (E) aerosols. The GSSG and GSH
1015 contents were measured immediately after cell exposure. Results are expressed as fold-
1016 change relative to the GSSG/GSH ratio in control cells arbitrarily set at a value of 1. Data

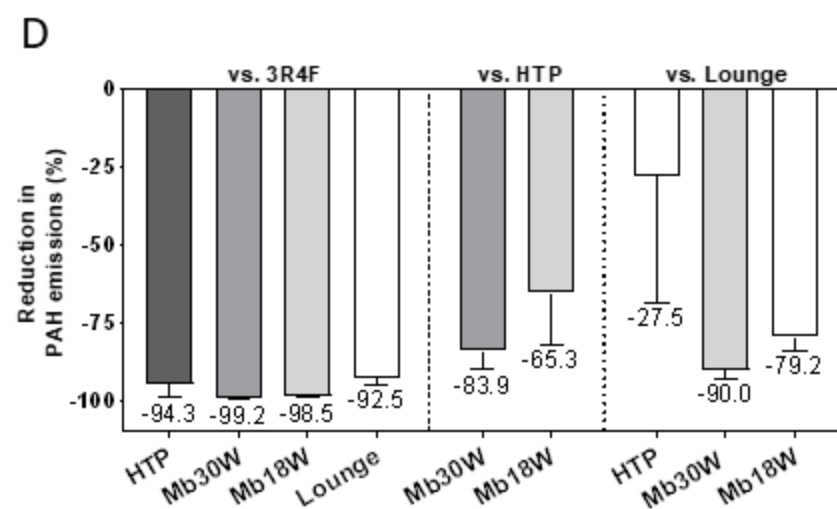
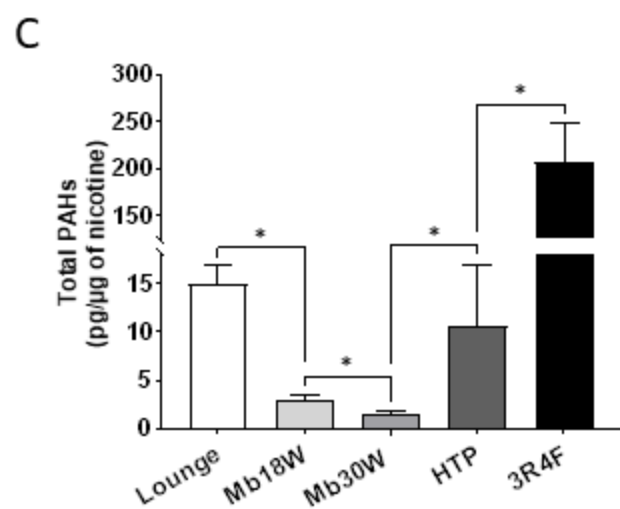
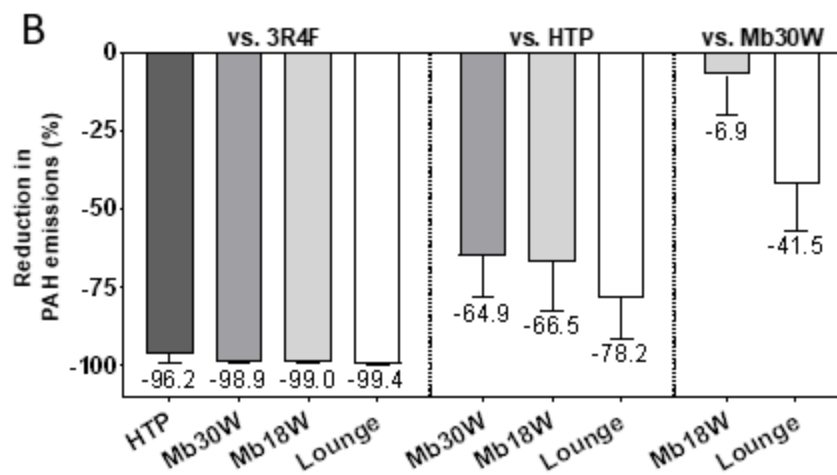
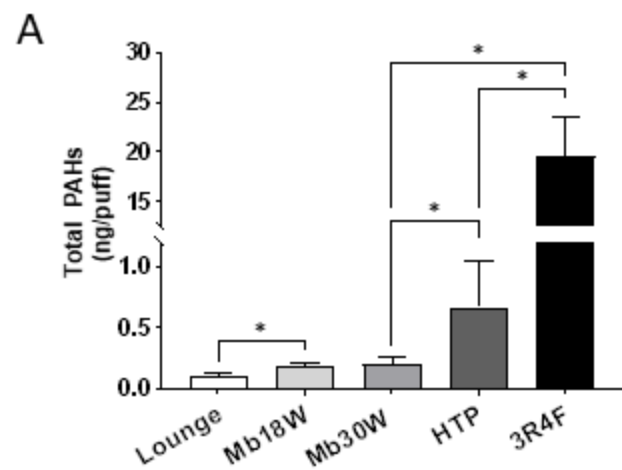
1017 represent the mean \pm SD of three independent culture replicates. * $p < 0.05$ compared to
1018 control cells.

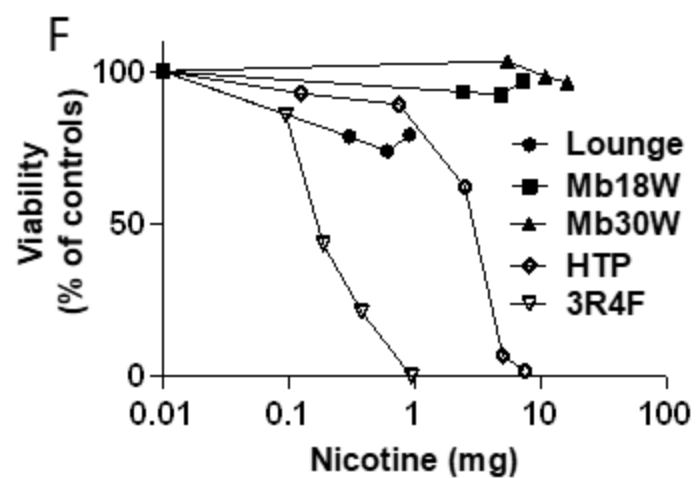
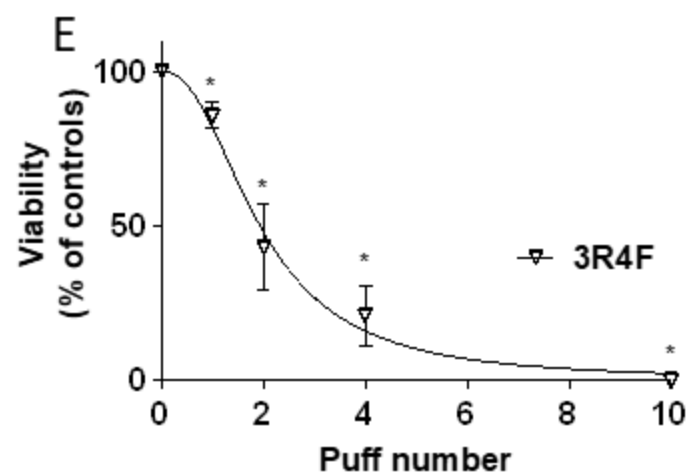
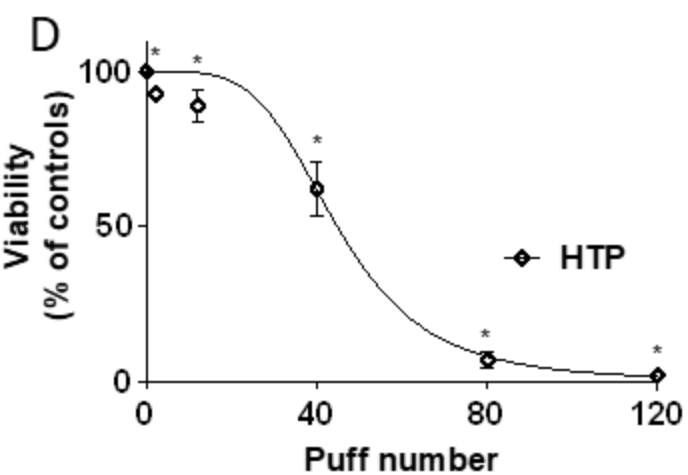
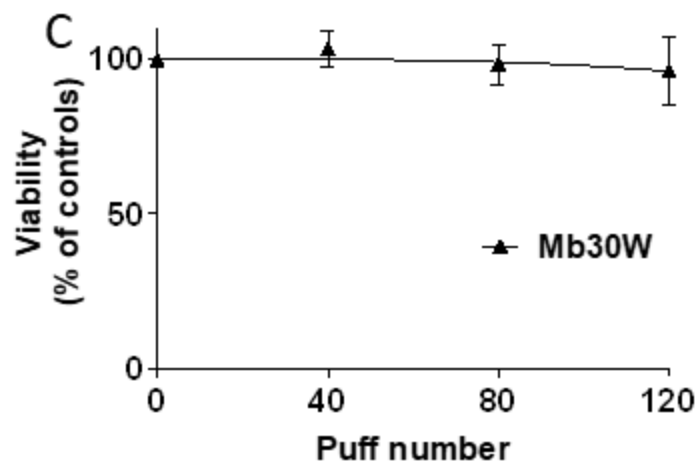
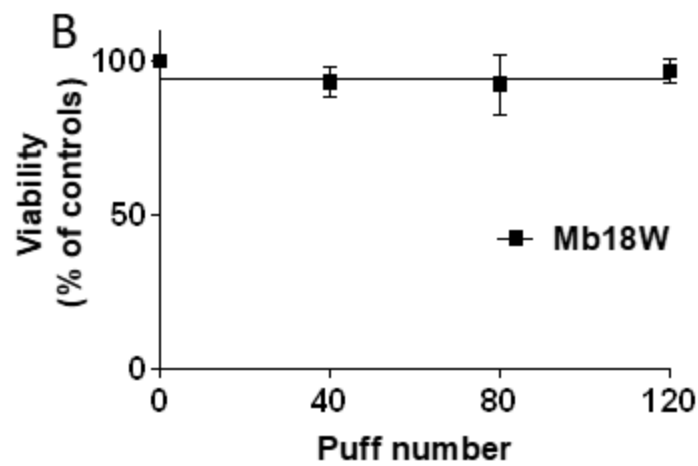
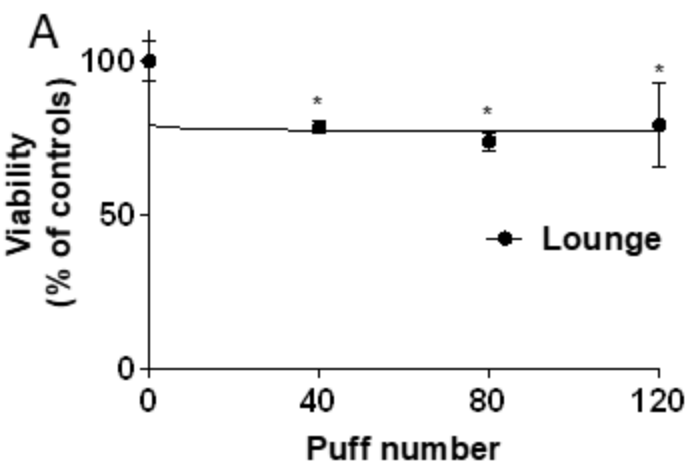
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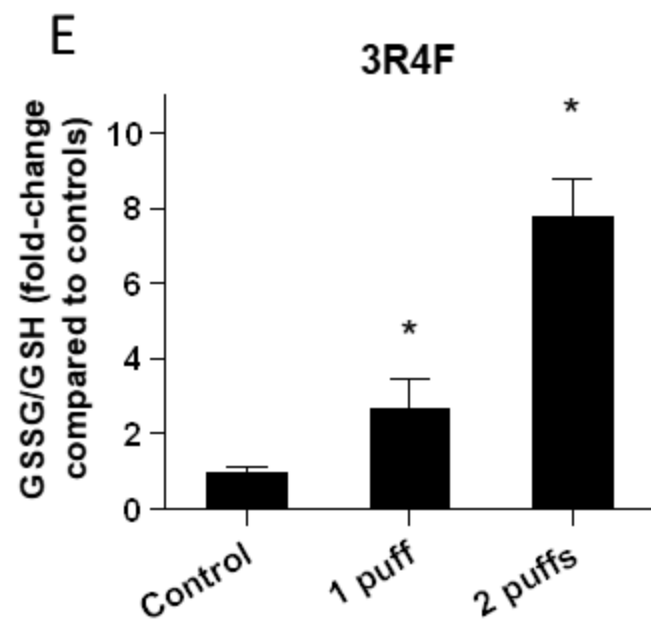
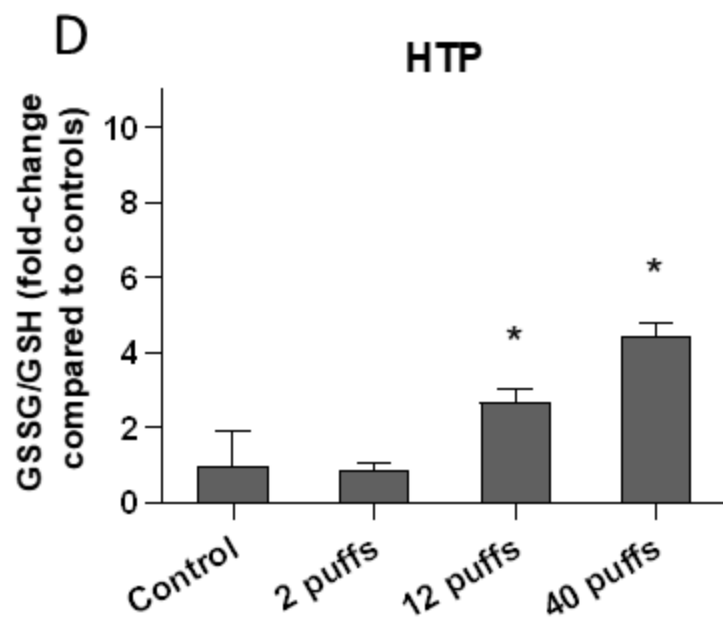
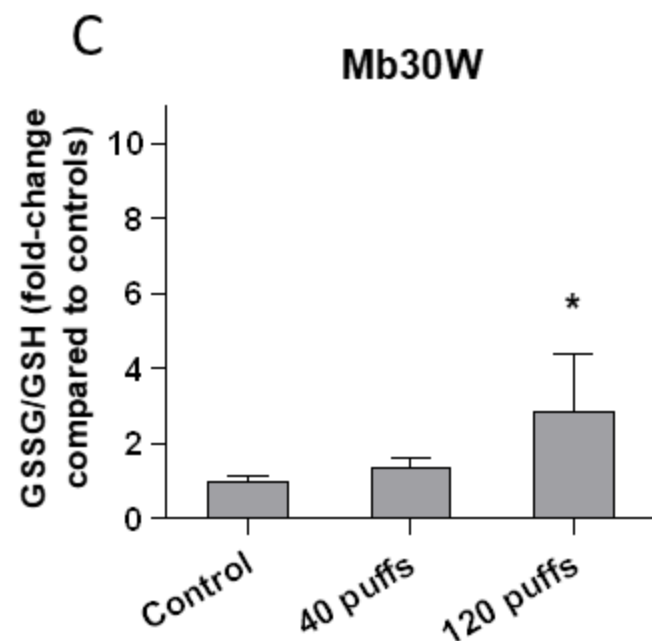
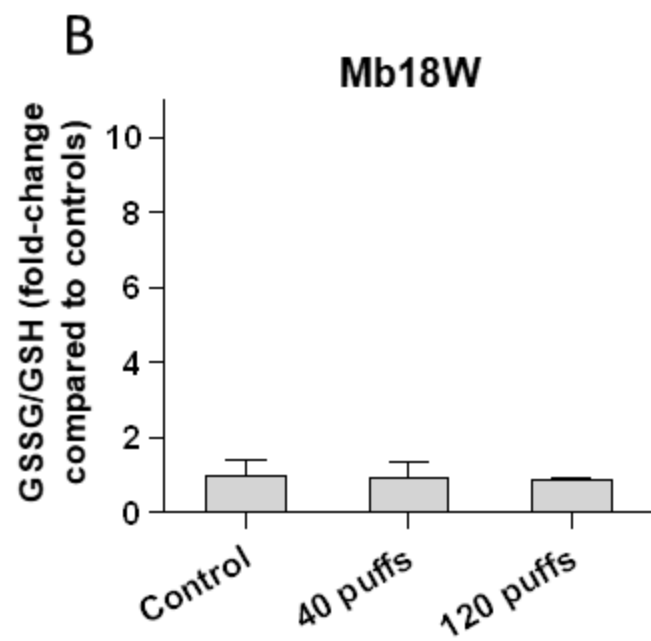
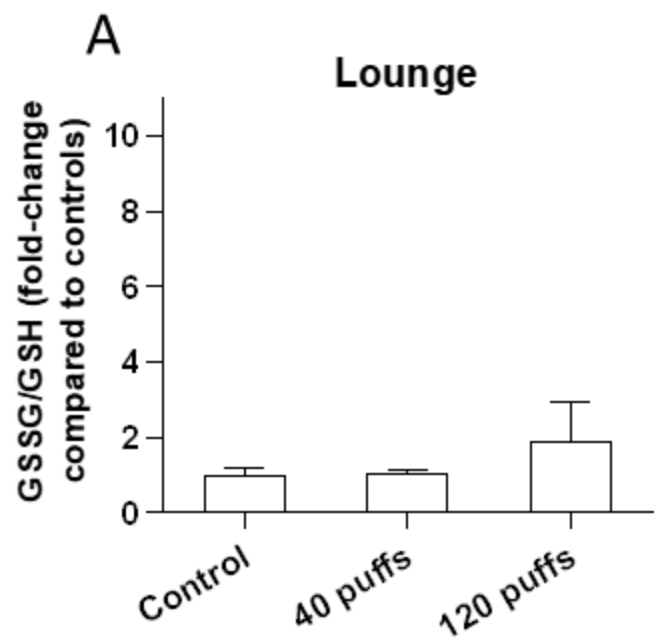
1020 **Figure 6.** Expression of mRNAs encoding genes related to metabolism [*CYP1A1* (A), *CYP1B1*
1021 (B)] and oxidative stress [*HMOX1* (C), *NQO1* (D)] in BEAS-2B cells. The gene expression was
1022 analysed 4 h or 24 h after exposure to 120 puffs of e-cig (Lounge, Mb18W or Mb30W), 12
1023 puffs of HTP or 1 puff of 3R4F cigarette. Data represent the mean \pm SD of three independent
1024 culture replicates. Results are expressed as fold-change relative to control cells, arbitrarily
1025 set at a value of 1. Data represent the mean \pm SD of three independent culture replicates. *
1026 $p < 0.05$ compared to control cells.











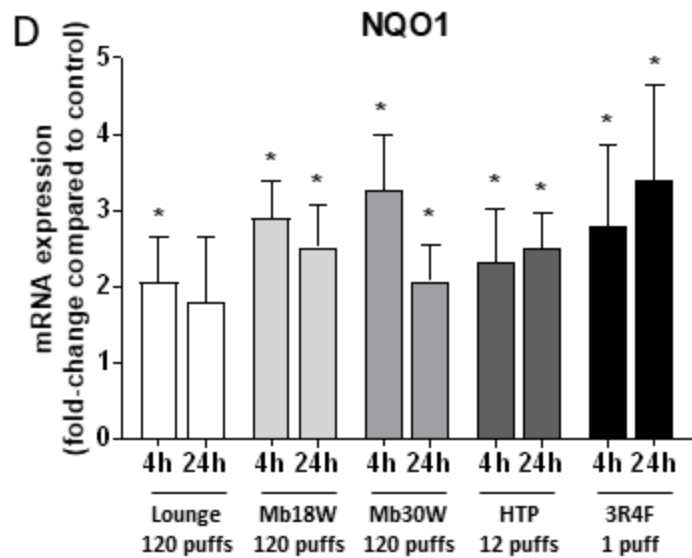
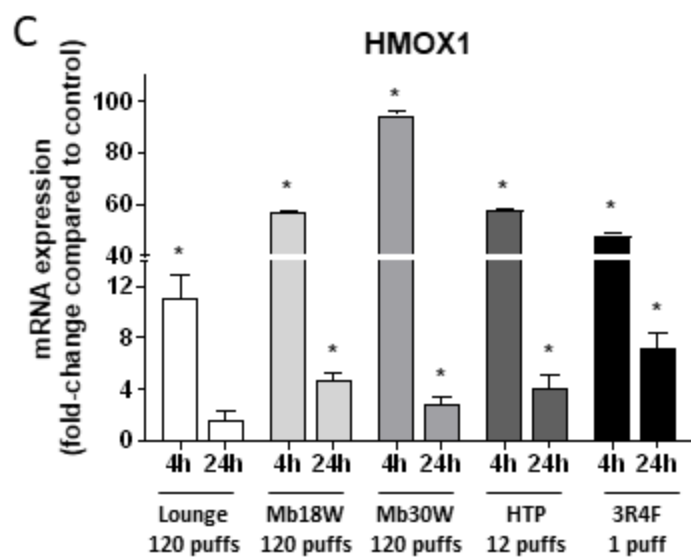
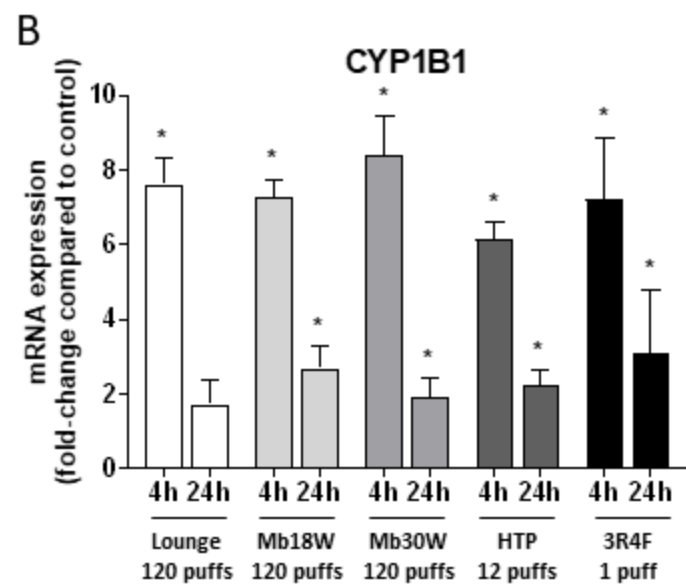
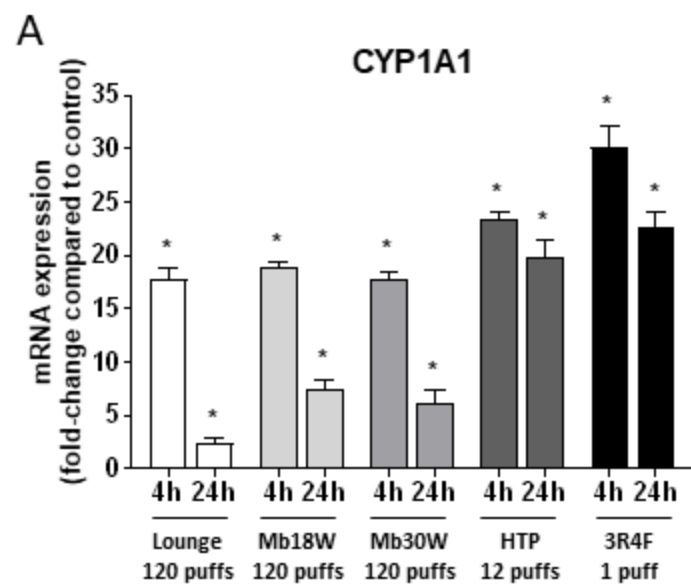


Table 1. Carbonyl concentrations (in ng/puff) in e-cig (Lounge, Mb18W or Mb30W), HTP and 3R4F cigarette aerosols. Data represent the mean \pm SD of four independent measurements. “~”: undetectable as $<$ to LOD.

	Lounge		Mb18W		Mb30W		HTP		3R4F	
Formaldehyde	6.0	\pm 0.7	25.8	\pm 2.8	64.5	\pm 23.7	156.9	\pm 9.4	255.5	\pm 60.8
Acetaldehyde	32.9	\pm 5.4	63.0	\pm 10.3	160.9	\pm 46.4	26687.7	\pm 657.8	166345.0	\pm 59540.1
Propanone	3.9	\pm 2.7	13.8	\pm 3.0	28.5	\pm 8.1	3132.3	\pm 149.1	36075.8	\pm 7896.5
Propanal	2.1	\pm 0.7	8.4	\pm 2.4	23.2	\pm 5.6	1400.1	\pm 205.8	6924.8	\pm 1688.2
Methyl vinyl ketone	0.2	\pm 0.1	6.4	\pm 4.2	6.4	\pm 2.1	443.1	\pm 42.1	1341.1	\pm 219.3
Crotonaldehyde	2.4	\pm 0.1	16.1	\pm 3.3	38.8	\pm 8.1	139.9	\pm 10.2	1697.4	\pm 794.5
Methyl ethyl ketone	0.8	\pm 1.6	34.7	\pm 23.6	23.5	\pm 9.5	625.6	\pm 26.9	9005.1	\pm 1097.8
Methylpropenal	~	\pm ~	~	\pm ~	~	\pm ~	334.8	\pm 20.6	842.4	\pm 350.7
Butanal	0.1	\pm 0.1	2.0	\pm 0.1	2.4	\pm 0.1	985.9	\pm 94.7	3653.9	\pm 1055.0
Benzaldehyde	0.5	\pm 0.1	2.5	\pm 0.3	3.2	\pm 0.1	58.9	\pm 2.8	63.6	\pm 59.3
Isopentanal	0.7	\pm 0.1	7.9	\pm 1.1	11.5	\pm 0.6	391.3	\pm 37.6	2084.9	\pm 599.0
Pentanal	0.5	\pm 1.1	1.0	\pm 0.2	0.4	\pm 0.1	25.2	\pm 1.4	172.0	\pm 50.5
Glyoxal	0.6	\pm 0.4	0.6	\pm 0.0	0.7	\pm 0.0	40.7	\pm 9.2	308.2	\pm 92.0
o-Tolualdehyde	0.7	\pm 0.1	2.9	\pm 0.5	2.8	\pm 0.5	6.3	\pm 0.4	29.0	\pm 2.8
m-Tolualdehyde	~	\pm ~	1.0	\pm 0.6	1.1	\pm 0.8	~	\pm ~	~	\pm ~
p-Tolualdehyde	1.7	\pm 0.4	0.9	\pm 0.6	0.6	\pm 0.7	115.0	\pm 26.4	291.8	\pm 195.8
Methylglyoxal	25.2	\pm 3.1	12.2	\pm 1.1	44.1	\pm 10.9	490.1	\pm 69.8	982.0	\pm 249.0
Hexanal	0.5	\pm 0.1	1.5	\pm 0.1	1.8	\pm 0.1	22.1	\pm 11.8	10.4	\pm 12.1
2,5-Dimethylbenzaldehyde	~	\pm ~	0.6	\pm 0.1	0.7	\pm 0.1	~	\pm ~	~	\pm ~
Total carbonyl compounds	79	\pm 10	201	\pm 48	415	\pm 63	35056	\pm 825	230083	\pm 70153

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

	Lounge			Mb18W			Mb30W			HTP			3R4F		
Naphthalene	61.5	\pm	9.5	75.9	\pm	5.6	92.2	\pm	6.2	71.2	\pm	38.8	3598.6	\pm	735.4
Acenaphthene	0.2	\pm	0.1	2.6	\pm	1.1	5.0	\pm	1.4	12.5	\pm	13.6	1318.2	\pm	397.5
Fluorene	6.7	\pm	3.3	6.7	\pm	1.5	5.0	\pm	1.3	26.0	\pm	22.4	1976.7	\pm	387.6
Phenanthrene	7.2	\pm	0.7	25.2	\pm	8.2	22.8	\pm	3.5	55.9	\pm	34.7	2829.4	\pm	533.3
Anthracene	0.6	\pm	0.1	1.7	\pm	0.4	2.8	\pm	3.7	4.7	\pm	2.3	1356.2	\pm	266.7
Fluoranthene	9.2	\pm	1.4	20.1	\pm	11.8	11.5	\pm	11.8	131.0	\pm	79.0	1463.5	\pm	288.7
Pyrene	17.9	\pm	4.3	30.9	\pm	9.2	30.9	\pm	10.9	153.0	\pm	98.6	1752.4	\pm	304.4
Benzo(c)phenanthrene	1.9	\pm	0.6	4.5	\pm	2.1	3.1	\pm	4.4	10.2	\pm	6.9	1.5	\pm	0.6
Benzo(a)anthracene	0.2	\pm	0.0	2.6	\pm	0.8	3.2	\pm	4.0	43.8	\pm	23.3	542.5	\pm	150.1
Chrysene	0.4	\pm	0.3	1.6	\pm	0.2	2.5	\pm	4.0	26.3	\pm	13.8	471.7	\pm	72.5
5-Methylchrysene	1.5	\pm	0.4	1.0	\pm	0.7	0.6	\pm	0.4	1.7	\pm	1.0	1130.5	\pm	293.9
Benzo(e)pyrene	1.9	\pm	0.2	6.1	\pm	2.5	5.3	\pm	3.6	22.9	\pm	17.1	1343.9	\pm	303.1
Benzo(b)fluoranthene	0.3	\pm	0.1	1.2	\pm	0.2	4.2	\pm	7.1	18.9	\pm	8.9	358.9	\pm	125.6
Benzo(k)fluoranthene	0.2	\pm	0.1	0.5	\pm	0.1	1.4	\pm	2.2	18.4	\pm	11.1	99.6	\pm	28.1
Benzo(a)pyrene	0.6	\pm	0.2	0.6	\pm	0.2	1.1	\pm	0.3	25.6	\pm	13.8	457.6	\pm	114.5
Dibenzo(a,l)pyrene	0.1	\pm	0.0	0.0	\pm	0.0	0.1	\pm	0.0	0.3	\pm	0.1	0.6	\pm	0.2
Dibenzo(a,h)anthracene	0.0	\pm	0.0	0.0	\pm	0.0	0.2	\pm	0.3	0.8	\pm	0.4	38.4	\pm	11.8
Benzo(g,h,i)perylene	1.5	\pm	0.7	0.9	\pm	0.2	4.8	\pm	3.1	16.6	\pm	8.9	276.2	\pm	55.9
Indeno(1,2,3-c,d)pyrene	0.3	\pm	0.1	0.2	\pm	0.0	1.5	\pm	2.0	6.6	\pm	5.6	214.0	\pm	81.4
Dibenzo(a,e)pyrene	0.1	\pm	0.0	0.0	\pm	0.0	0.2	\pm	0.3	0.5	\pm	0.3	92.4	\pm	43.5
Anthanthrene	0.3	\pm	0.1	0.2	\pm	0.1	0.4	\pm	0.1	11.7	\pm	6.7	233.9	\pm	52.6
Coronene	0.3	\pm	0.1	0.5	\pm	0.1	2.8	\pm	1.0	5.5	\pm	1.8	25.6	\pm	5.4
Cyclopenta(c,d)pyrene	~	\pm	~	~	\pm	~	~	\pm	~	~	\pm	~	~	\pm	~
Total PAHs	113	\pm	16	183	\pm	29	202	\pm	57	664	\pm	389	19582	\pm	400

Table 3. Profile of inflammatory mediators (IL-6, IL-8, MCP-1 and GRO α) 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 \pm 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 \pm 0.6	0.8 \pm 0.3	0.7 \pm 0.2	0.7 \pm 0.1
120 puffs	2.2 \pm 1.0	0.6 \pm 0.1	0.6 \pm 0.2	0.6 \pm 0.1
Mb18W				
40 puffs	2.5 \pm 1.1	1.1 \pm 0.6	1.0 \pm 0.1	1.0 \pm 0.2
120 puffs	2.3 \pm 0.6	1.0 \pm 0.3	0.8 \pm 0.1	0.8 \pm 0.1
Mb30W				
40 puffs	3.3 \pm 0.4	0.9 \pm 0.1	0.8 \pm 0.1	0.7 \pm 0.2
120 puffs	2.4 \pm 0.1	0.6 \pm 0.1	0.6 \pm 0.1	0.4 \pm 0.1
HTP				
2 puffs	0.4 \pm 0.0	1.0 \pm 0.0	0.6 \pm 0.0	0.9 \pm 0.1
12 puffs	3.0 \pm 3.0	3.3 \pm 0.4	0.6 \pm 0.1	0.7 \pm 0.1
40 puffs	1.3 \pm 1.0	1.3 \pm 1.5	0.2 \pm 0.1	0.2 \pm 0.1
3R4F				
1 puff	1.4 \pm 0.5	1.4 \pm 0.3	1.0 \pm 0.1	0.7 \pm 0.1
2 puffs	1.5 \pm 0.5	1.8 \pm 0.8	0.2 \pm 0.1	0.2 \pm 0.1

