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Aerosol regional deposition of electronic cigarette emissions using an original *ex vivo* respiratory model

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Abstract

Aim: There is a need to bridge the data gap concerning the regional deposition of electronic nicotine delivery systems (ENDS) emissions in the airways. The present work aims to experimentally and precisely assess the aerosol regional deposition of ENDS emissions using an *ex vivo* model of lungs as a complementary approach to a deterministic computational model and as an alternative to animal or human *in vivo* experiments.

Methods: The refill liquid of a recent tank generation ENDS was radiolabelled with pertechnetate sodium. Particle size distributions of the emissions – in mass and in radioactivity – were determined using a cascade impaction technique. The aerosol regional deposition in the airways was both calculated with a deterministic computational model and experimentally assessed by gamma-camera imaging on a controlled breathing *ex vivo* model mimicking intrapleural depression thanks to generation of negative pressures in a sealed enclosure.

Results: The aerodynamic diameter of the ENDS emissions were $1.03 \pm 0.11 \mu\text{m}$ and $0.87 \pm 0.03 \mu\text{m}$ (mean \pm SD) in radioactivity and mass, respectively. The calculations led to a thoracic deposition of $92.89 \pm 0.03\%$ and $92.74 \pm 0.19\%$ in mass and radioactivity, respectively. The experimental data of thoracic deposition obtained by quantifying the deposited aerosol with planar scintigraphy of the *ex vivo* model were $91 \pm 4\%$.

Conclusions: The radiolabelling of the refill liquid did not affect the particle size distribution and allowed assessment of the aerosol regional deposition by two-dimensional gamma-camera imaging. When compared, no significant difference of the thoracic deposition between calculation and experimental data could be found. Moreover, data seemed to be in good accordance with previously published data. This work supports the conclusion that this preclinical respiratory model could be used to assess regional deposition of aerosol generated by an ENDS.

Introduction

Electronic nicotine delivery systems (ENDS) – also called electronic cigarettes or e-cigarettes – are a user-driven aerosol technology, which was first patented in early 2000's by Hon Lik (Grana Rachel et al., 2014). ENDS are a rapidly emerging and diversified product class (Williams & Talbot, 2019). Basically, ENDS are battery-powered personal vaporizers. The physical principle shared by all ENDS is an electrically powered heating element which vaporises a liquid solution. Thus, an aerosol is produced and available to be inhaled. The refill liquid of an ENDS contains humectants (*i.e.* glycerol

41 and propylene glycol) and optional ingredients in small quantities (nicotine, water, ethanol,
42 flavorings, etc.).

43 Claimed as a substitute for combustible cigarettes with a less harmful outcome, ENDS raised a
44 controversy in the scientific and medical community. Indeed, despite containing less carcinogenic
45 constituents than conventional cigarettes (Flora et al., 2016; Fuoco et al., 2014; Palazzolo, 2013; Sood
46 et al., 2018), health concerns are questioned due to the lack of robust clinical evidence about long-
47 term outcomes and especially about potential risks associated with ENDS use (Caldwell et al., 2012;
48 Flora et al., 2016; Fuoco et al., 2014; Kosmider et al., 2020; Olmedo et al., 2018; Palazzolo, 2013;
49 Smith et al., 2019; Visser et al., 2019). The vaping industry has seen a huge business increase during
50 the last decade, representing billions of dollars worldwide and millions users (Palazzolo, 2013). This
51 growing market is facing regulatory issues as regulators struggle to catch up with the fast
52 development of new ENDS (Fuoco et al., 2014; Palazzolo, 2013; Williams & Talbot, 2019). Currently,
53 most ENDS are regulated as general consumer products and not as medical devices. However, in
54 some countries such as the United Kingdom, some ENDS are licensed as a medical product for
55 smoking cessation – as a class IIa medical device – according to Directive 93/42/EC (MHRA, 2017; E-
56 Voke 10mg and 15mg Electronic Inhaler, 2015).

57 Therefore, this area of research concentrated interests from the aerosol sciences community to
58 bridge the data gap and reach a consensus concerning some scientific issues as the particle size
59 distribution of ENDS emissions and the aerosol regional deposition (Fuoco et al., 2014; Palazzolo,
60 2013). Indeed, one of the main pitfalls of ENDS assessment is intrinsically related to the aerosol
61 generated. This aerosol is composed of a dynamic mixture of gas and solvent droplets (Ingebrethsen
62 et al., 2012; Kleinstreuer & Feng, 2013), mainly composed of a mixture of propylene glycol (PG) and
63 vegetable glycerine (VG) acting as an airborne carrier for nicotine (Glasser et al., 2017; Thornburg,
64 2017). Thus, the experimental assessment of the particle size distribution of ENDS aerosol remains a
65 challenge because of the high hygroscopicity and volatile nature of the particulate matter
66 (Ingebrethsen et al., 2012; Manigrasso, Buonanno, Fuoco, et al., 2015; Manigrasso, Buonanno,
67 Stabile, et al., 2015; Manigrasso et al., 2017; Mikheev et al., 2016; Oldham et al., 2018; Son et al.,
68 2019; Sosnowski et al., 2018; Sosnowski & Kramek-Romanowska, 2016; Sundahl et al., 2017). These
69 obstacles seriously complicate the use of sizing techniques requiring a high degree of aerosol
70 dilution, which are expected to induce a significant particle evaporation. This corresponds to a
71 potential bias due to the alteration of the particle size distribution compared to the one provided to
72 the user. Additionally, the dynamic behaviour of the ENDS aerosol is expected after aerosol
73 generation and after inhalation. Particle size distribution and particle concentration are expected to
74 evolve due to condensational growth, particulate matter evaporation, coagulation and particle
75 deposition. Lastly, the assessment of the particle size distribution is also particularly tricky due to the
76 number of existing ENDS devices refill liquids, whose composition modifies of the aerodynamic
77 features (Ooi et al., 2019; Pourchez et al., 2018; Zervas et al., 2018; Zhao et al., 2018).

78 Determining the aerodynamic size of ENDS emissions has a strong importance because these data
79 are mandatory to run the existing semi-empirical and computational flow dynamic models estimating
80 the aerosol regional deposition into the lungs (Kleinstreuer & Feng, 2013; Thornburg, 2017). Another
81 entry needed for these models is the puffing regimen, which is also a controversial subject in
82 research papers (Kleinstreuer & Feng, 2013; Son et al., 2019; Vansickel et al., 2018). Therefore, it
83 remains difficult to use experimental aerodynamic data to calculate – using well-known
84 computational model – a precise exposure dose and lung regional deposition profile to estimate with
85 a high and robust confidence level the potential outcomes of ENDS use (Bertholon et al., 2013;

86 Manigrasso, Buonanno, Fuoco, et al., 2015; Palazzolo, 2013; Son et al., 2019; Sosnowski & Kramek-
87 Romanowska, 2016; Sundahl et al., 2017; Zhang et al., 2013).

88 As a complementary approach to semi-empirical and/or computational flow dynamics software, the
89 aim of this work was to experimentally and precisely assess the aerosol regional deposition of ENDS
90 emissions. Thus, an *ex vivo* respiratory model composed of an Ear-Nose-Throat (ENT) replica of
91 healthy adult upper airways and a porcine respiratory tract, placed in a sealed enclosure, was used
92 (Perinel et al., 2016, 2017). Passive ventilation was performed thanks to a depression generator,
93 producing negative pressures in the enclosure. First, we needed to radiolabel the refill liquids with a
94 radionuclide. This radiolabelling was validated by determining the aerodynamic size distribution of
95 radiolabelled aerosols generated by a recent tank generation ENDS. Then, we assessed the aerosol
96 regional deposition on the *ex vivo* model by gamma-camera imaging. The reliability of the obtained
97 data was evaluated by comparison with the literature and by running the multiple path particle
98 dosimetry (MPPD) model.

99 1. Material and methods

100 1. Materials

101 As previously described (Pourchez et al., 2018), the high-power ENDS used for this study was
102 composed of a lithium-ion battery iStick TC40W (2600 mAh, Eleaf, Shenzhen, China), a GS Air 2
103 atomiser (Eleaf, Shenzhen, China) and a GS Air pure cotton head wick (0.75 Ω , kanthal heating wire,
104 Eleaf, Shenzhen, China). All components were purchased in a local specialised store. For all
105 experiments, the atomiser was filled with at least 2 g of refill liquid, the power of the ENDS was set at
106 10 W and the airflow ring was removed to prevent any “dry hit” (*i.e.* user puffing without sufficient
107 amount of refill liquid in the wick, leading to overheat and potential damages).

108 The refill liquid was prepared from 100-PG base and 100-VG base (Arômes et Liquides, Andrézieux-
109 Bouthéon, France). Both bases were purchased from a local supplier as commercial grade to be as
110 close as possible to real use. The surfactant Tween[®] 80 was purchased from Sigma-Aldrich (CAS 9005-
111 65-6, Saint-Louis, Mo., USA). About 100 MBq of sodium pertechnetate (^{99m}Tc) were taken off from a
112 Tekcis[®] generator (Curium, Paris, France) located in the Nuclear Medicine department of the Saint-
113 Etienne hospital. Absolute ethanol (EtOH) was purchased from VWR (Fontenay-sous-Bois, France).

114 2. Radiolabelling of ENDS refill liquid

115 The refill liquid was prepared from bulk components in the following mass proportions: 74% PG, 14%
116 VG, 5% ^{99m}Tc, 4% Tween[®] 80 and 3% EtOH.

117 Bulk components were weighed with precision scales (Adventurer Pro, OHAUS, Parsippany, USA) and
118 homogenised thanks to a stirring plate and magnetic bar in a 20 mL scintillation vial. First, PG, VG,
119 Tween[®] 80 and EtOH were homogenised and kept under constant stirring. Then, ^{99m}Tc was added in
120 the radiopharmacy laboratory in the Nuclear Medicine department of the Saint-Etienne University
121 Hospital. The mixture was stirred for at least 10 minutes. Just before experiments, at least 2 g of
122 radiolabelled refill liquid were placed in the reservoir of the atomiser.

123 3. Puffing regimen

124 An in-house interface was previously developed to reproducibly introduce a puff that was well-
125 controlled for duration and volume (Bertrand et al., 2018; Pourchez et al., 2017; Prévôt et al., 2017).
126 The puffing regimen – called AFNOR regimen – was as follows: 55-mL puff volume, 4 s puff duration,
127 30-s inter-puff interval, 25 puffs per series, 300-s inter-series interval as described in the AFNOR
128 standard XP D90-300-1.

129 4. Particle size determination

130 The methodology was previously described and validated (Bertrand et al., 2018; Pourchez et al.,
131 2017, 2018; Prévôt et al., 2017). The assessment of the aerodynamic size distribution was performed
132 in triplicate using a DLPI cascade impactor (Dekati Low Pressure Impactor, Dekati Ltd., Kangasala,
133 Finland) operating at an airflow of 10 L/min. The DLPI allowed aerosolised particles to be sorted into
134 12 aerodynamic size groups (from 30 nm to 10 µm).

135 The aerosol generated by the e-cigarette was sampled and transferred to the DLPI through a metal
136 United States Pharmacopeia (USP) like artificial throat (height 112 mm; width 42 mm; internal
137 diameter 19 mm) with a previously described in-house interface (Pourchez et al., 2017, 2018). The
138 deposited fraction on each DLPI stage was collected on an impermeable plastic cover. This cover was
139 then removed and the amount of ^{99m}Tc quantified using Packard Cobra II auto-gamma counting
140 system (Perkin-Elmer, Waltham, MA, USA). Finally, the activity median aerodynamic diameter
141 (AMAD) and the geometric standard deviation (GSD) of the aerosolised particles were calculated,
142 according to European Pharmacopeia 2.9.18. GSD is determined by the ratio of the provisional size at
143 the 84th percentile of the distribution to the provisional size at the 16th percentile. Between each puff,
144 the DPLI was turned off to reduce potential evaporation of the collected particles. The mass median
145 aerodynamic diameter (MMAD) and the subsequent GSD were determined by gravimetric
146 measurement of each stage. For these experiments, 25 puffs with AFNOR regimen were performed.

147 5. Calculation of aerosol deposition using computational model

148 Estimation of the deposition of ENDS emissions in the human airways was carried out using the
149 Multiple Path Particle Dosimetry software (MPPD v3.04, ARA, Arlington, VA, USA). Introduced
150 parameters were obtained from the work of Son *et al.* (Son et al., 2019). Some parameters such as
151 puff volume, aerosol density, AMAD/MMAD and GSD were obtained from data generated in the
152 present study.

153 We used the Yeh & Schum symmetric airway morphometry with a functional residual capacity of
154 3300 mL and upper respiratory tract volume of 50 mL. The density of the aerosol was set at 1.07086
155 g/mL, while the MMAD and GSD was obtained from experimental data. Shape factor was set at 1. We
156 used constant exposure scenario, allowing the supine position inhalation. Aerosol concentration was
157 set at 36181 according to experimental data of cascade impaction. Breathing frequency was set at 15
158 cycle/min with 500 mL of tidal volume and an inspiratory fraction of 0.33 with oral breathing,
159 according to the experimental settings.

160 6. Experimental assessment of regional aerosol deposition using an *ex vivo* 161 respiratory model

162 To assess the regional deposition of the aerosol generated with an ENDS, the refill liquid was labelled
163 with 400 MBq of sodium pertechnetate. To assure enough deposition and, thus, a quantitative
164 assessment of the deposited dose, 100 puffs were sampled – according to the puffing regimen
165 described above – to be inhaled by the *ex vivo* respiratory model. To ensure inhalation of ENDS
166 emissions, each puff was manually synchronized with a deep inspiration over 4s. The human-like
167 model is composed of a 3D-printed Ear-Nose-Throat (ENT) replica (female subject, 27 years old)
168 connected to a sealed enclosure. The ENT presents a naso-buccal breathing but for the purposes of
169 this study, nasal orifices were occluded to ensure strict oral breathing. Within this enclosure, an *ex*
170 *vivo* porcine respiratory tract is passively ventilated thanks to a SuperDimension® depression
171 generator (Covidien, Dusseldorf, Germany), which applied negative pressure in the enclosure (mean
172 depression -9 kPa). Respiratory parameters were previously described (Crémillieux et al., 2020;
173 Montigaud, Perinel-Ragey, et al., 2019): 15 cycles per min, with 1.33 s inspiratory time and 2.66 s

174 expiratory time leading to inspiratory/expiratory ratio of 1:2 inducing tidal volume about 500 mL and
175 dynamic compliance about 90 cmH₂O/mL. All experiments with the *ex vivo* model were performed at
176 isothermal laboratory temperature.

177 Detailed collection, preparation and storage protocols were extensively described in previously work
178 of our group (Montigaud, Georges, et al., 2019, 2019; Perinel et al., 2016, 2017). Briefly, all
179 respiratory tracts, collected from a local slaughterhouse, passed all quality controls according to
180 French regulation. At slaughtering time, swine were 6 months old, with 44% of female and 56% of
181 male from three different species: large white, pietrain and landrace (with mainly large white). After
182 removal of viscera, carcasses weighted 90 to 93kg. Visual controls of wounds and sutures were
183 achieved, and a bronchoscopy was performed to ensure the absence of significant obstruction of
184 proximal bronchi.

185 The gamma-camera imaging was performed as previously described (Montigaud, Perinel-Ragey, et
186 al., 2019; Perinel et al., 2016). Briefly, two-dimension scintigraphies were conducted on 3 respiratory
187 tracts with a variable angle dual detector Single Photon Emission Computed Tomography/Computed
188 Tomography (SPECT-CT, SYMBIA T2; Siemens, Knoxville, TN, USA). After inhalation of the aerosol,
189 each component of the system was imaged with 3-min anterior/posterior exposition: e-cigarette,
190 expiratory filter, ENT replica and respiratory tract. The count of each part was determined with
191 corrections for background radiation, radioactivity decay and tissue attenuation (correction factor
192 calculated for each component). Results were expressed in terms of the total deposited dose of
193 radioactivity in the respiratory tract and the ENT or as proportion of inhaled dose, which corresponds
194 to the sum of deposited aerosol in the ENT, the respiratory tract and the exhaled filter. The central-
195 to-peripheral ratio (C/P ratio) and its inverse, the penetration index (PI), were calculated as
196 previously described (Montigaud, Georges, et al., 2019; Newman et al., 2012).

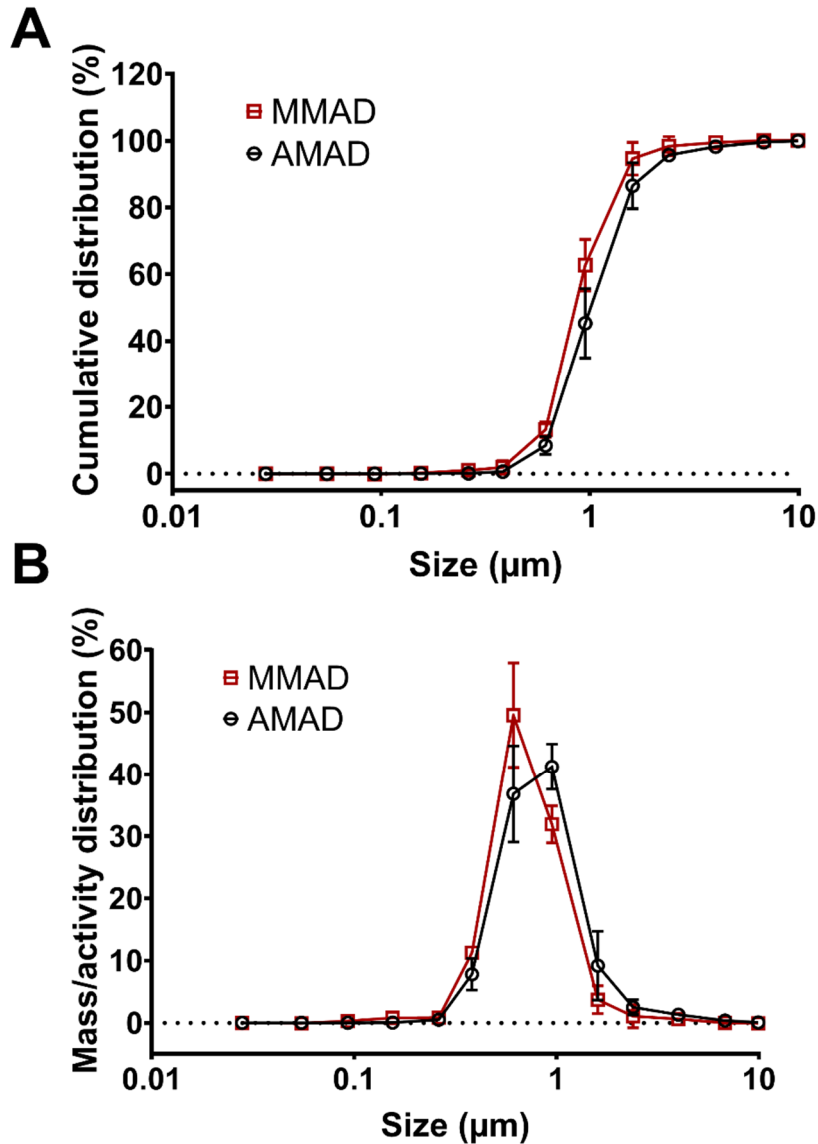
197 7. Statistical analysis

198 Statistical analyses were performed with GraphPad Prism 8 software. For the aerodynamic size
199 distribution, a non-parametric Kolmogorov-Smirnov test was used. For the comparison of the
200 regional deposition experimentally obtained and estimated with the MPPD software, a two-way
201 ANOVA was used with a Tukey's multiple comparison *post-hoc* test.

202 Results

203 1. Particle size distribution

204 The aerodynamic size distribution was used to determine the MMAD and the AMAD of the ENDS
205 emissions. Figure 1 presents the distribution of the radiolabelled droplets thanks to a DLPI cascade
206 impactor. As easily seen, there is a strong recovery of the 2 curves. Table 1 presents the obtained
207 AMAD and MMAD, with respective GSD. No significant difference between the two curves was
208 observed ($p=0.5696$).



209

210 *Figure 1 - A: cumulative frequency of mass or radioactivity distribution (n=3). B: frequency of mass or radioactivity*
 211 *distribution. Data are presented as mean ± standard deviation (SD) of the total deposited fraction in the apparatus (n=3).*
 212 *MMAD: Mass Median Aerodynamic Diameter. AMAD: Activity Median Aerodynamic Diameter.*

213 *Table 1 – Aerodynamic size assessment of aerosols emitted with an ENDS. Data are presented as mean ± standard deviation*
 214 *(SD) of a triplicate. MMAD: Mass Median Aerodynamic Diameter. AMAD: Activity Median Aerodynamic Diameter.*

	MMAD	AMAD
Size (µm)	0.87 ± 0.03	1.03 ± 0.11
GSD	1.47 ± 0.05	1.53 ± 0.05

215

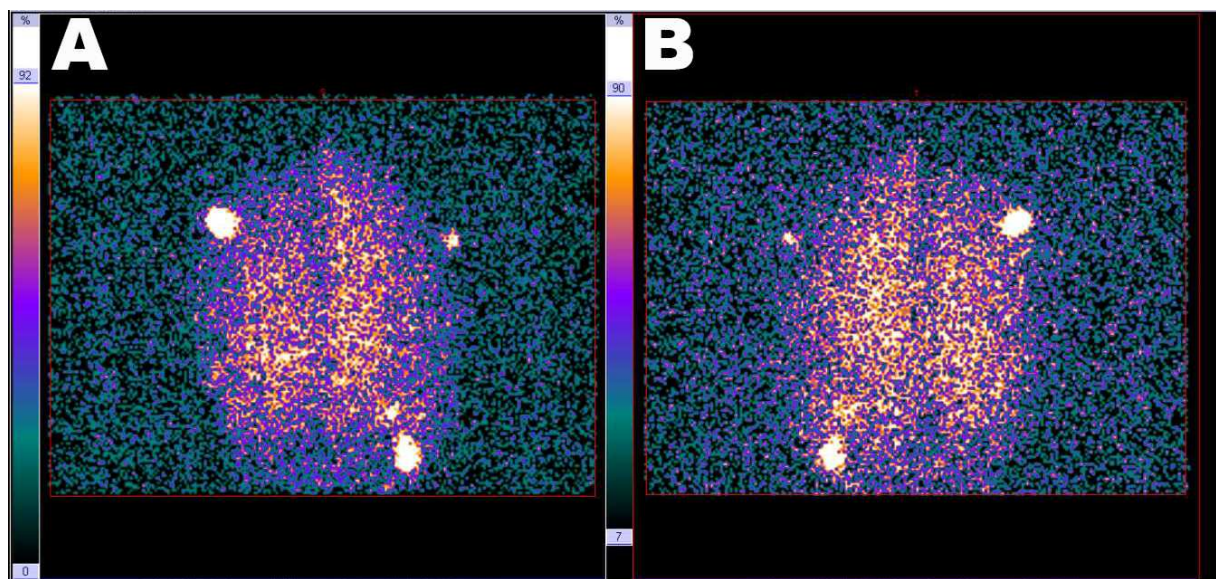
216 2. Aerosol regional deposition using an *ex vivo* respiratory model

217 The regional aerosol deposition was assessed with our innovative *ex vivo* respiratory model. One
 218 hundred puffs were used with the AFNOR regimen. Figure 2 presents the images obtained with
 219 planar scintigraphies. A homogenous deposition could be observed in the respiratory tract, as also
 220 shown by the Table S1. It is important to note that the hot-spots in Figure 2 correspond to cuts in the
 221 respiratory tract due to the sacrifice of the animal at the slaughterhouse. Therefore, it collected more
 222 aerosol due to the decreased resistance in these areas. This point corresponds to an intrinsic artefact

223 of the respiratory model that was extensively acknowledged in previously published works but does
224 not significantly modify the overall aerosol regional deposition within the model (Montigaud,
225 Georges, et al., 2019; Montigaud, Périnel-Ragey, et al., 2019; Perinel et al., 2016, 2017).

226 When expressed as a percentage of the total aerosol deposited dose in the respiratory tract, the
227 fraction in the lungs represents about 90% of the deposited dose, while the ENT collected 10% of the
228 deposited dose (Table 2). The C/P ratio of the *ex vivo* model was 0.75 of the deposited dose in the
229 respiratory tract.

230 When expressed as a proportion of total inhaled dose (Table 3), the deposition pattern remained
231 similar: most of the aerosol found in the lungs ($84\% \pm 8$), while the rest of the aerosol is partitioned
232 between exhaled fraction ($7\% \pm 6$) and deposited fraction in the interface and upper airways ($8\% \pm$
233 3).



234
235 *Figure 2 – Planar scintigraphies of a respiratory tract after inhalation of 100 radiolabelled puffs ENDS emissions. A: anterior*
236 *acquisition. B: posterior acquisition. Hot spots correspond to cuts in the respiratory tract and, therefore, collected more*
237 *aerosol due to the decreased resistance in these areas.*

238 3. Estimation of aerosol deposition using computational model

239 The estimation of the deposited doses in the respiratory tract was performed with the MPPD
240 implemented with the AFNOR puffing regimen and the AMAD or the MMAD of the emitted aerosol.
241 The other parameters were taken from a published paper (Son et al., 2019). Figure 3 presents the
242 different deposited fractions of the aerosol along the airways up to the pulmonary region. As
243 presented, over 70% of the aerosol is exhaled, while the remaining aerosols is partitioned between
244 the head, the tracheobronchial tree and the pulmonary region. Figure 3 presents the comparison of
245 experimental and estimated data. We only considered the deposited doses in the *ex vivo* model in
246 this comparison. No significant difference could be found between the estimated deposition
247 obtained with AMAD and MMAD as entries, as well as for our experimental data ($p > 0.9999$; Table 2
248 and Figure 3). Penetration of the aerosol was assessed by the determination of the C/P ratio, which
249 were 0.7495 ± 0.0203 and 0.6942 ± 0.0365 for the estimation based on the MMAD and the AMAD,
250 respectively, as expressed as a proportion of the total deposited dose in the respiratory tract (Table
251 3).

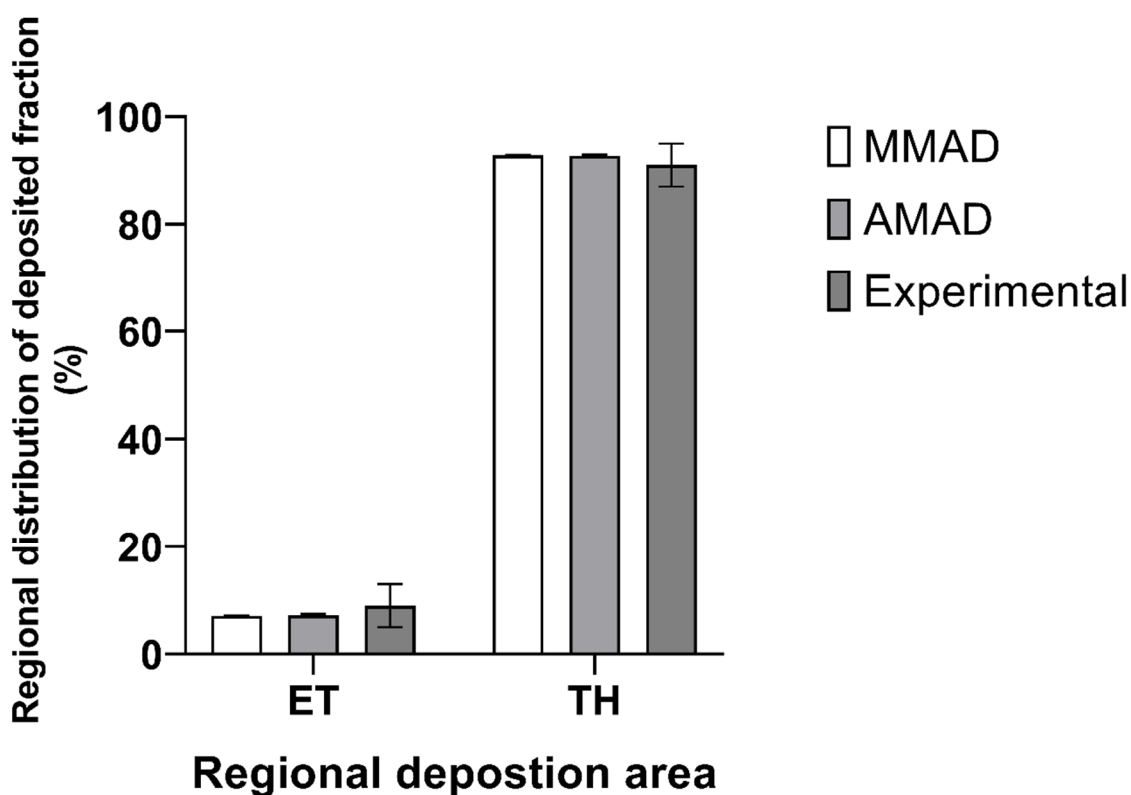
252 When the deposition pattern is expressed as proportion of the inhaled dose, as presented in Table 3,
253 we could see that the exhaled fraction is over 80% ($85.24\% \pm 0.72$ and $82.86\% \pm 1.79$ for the

254 estimation based on the MMAD and the AMAD, respectively). Lung deposition is about 15% (13.71%
 255 ± 0.67 and 15.90% ± 1.64 for the estimation based on the MMAD and the AMAD, respectively). The
 256 upper airway deposition is about 1% (1.05% ± 0.06 and 1.25% ± 0.15 for the estimation based on the
 257 MMAD and the AMAD, respectively).

258 *Table 2 - Regional aerosol deposition expressed as mean \pm standard deviation (SD) of the percentage of total deposited*
 259 *aerosol for computational model with AMAD and MMAD as inputs or for experimental results presented in this study. Extra-*
 260 *thoracic: ENT replica + mouth interface equivalent to head deposition in the determinist computational model. Thoracic:*
 261 *respiratory tract equivalent to the sum of tracheobronchial and peripheral deposition in the determinist computational*
 262 *model.*

	MPPD estimation from MMAD	MPPD estimation from AMAD	Experimental results (n=3)
Extra-thoracic deposition	7.11% \pm 0.03	7.26% \pm 0.19	9% \pm 4
Thoracic deposition	92.89% \pm 0.03	92.74% \pm 0.19	91% \pm 4

263



264

265 *Figure 3 - MPPD estimation with MMAD or AMAD as input and experimental results of the deposition of emission of the*
 266 *chosen ENDS. Data are presented as mean \pm standard deviation (SD) of the percentage of total deposited aerosol. ET: extra-*
 267 *thoracic region (head and mouthpiece). TH: thoracic region (tracheobronchial tree + peripheral lungs). MMAD: Mass Median*
 268 *Aerodynamic Diameter. AMAD: Activity Median Aerodynamic Diameter.*

269

270 *Table 3 – Homogeneity and penetration of the aerosol deposition expressed as mean ± standard deviation (SD) of the*
 271 *percentage of total inhaled aerosol for computational model with AMAD and MMAD as inputs or for experimental results*
 272 *presented in this study. Interface: in-house mouth interface. ENT: human head replica. Lungs: tracheobronchial + pulmonary*
 273 *deposition. Exhaled: fraction collected on the respiratory filter or as the complementary to one of the deposited fractions.*
 274 *C/P: central-to-peripheral ratio. PI: penetration index.*

	Deposited fraction of the aerosol expressed as proportion of the inhaled dose (%)			Regionalisation of the deposition/penetration of the aerosol in the respiratory tract		
	Interface + ENT	Exhaled	Lungs	C/P ratio	PI	Left/right ratio
Experimental data	8 ± 3	7 ± 6	84 ± 8	0.75 (n=1)	1.33 (n=1)	1.00 (n=1)
Estimation from MMAD	1.05 ± 0.06	85.24 ± 0.72	13.71 ± 0.67	0.7495 ± 0.0203 (n=3)	1.3348 ± 0.0366 (n=3)	N/A
Estimation from AMAD	1.25 ± 0.15	82.86 ± 1.79	15.90 ± 1.64	0.6942 ± 0.0365 (n=3)	1.4432 ± 0.0759 (n=3)	N/A

275 Discussion

276 1. Particle size distribution

277 To our knowledge, only one study was conducted on radiolabelled aerosol emitted with an ENDS
 278 (Holbrook et al., 2018). In this work, the aerosol is radiolabelled by a complex process with
 279 technetium solid particles incorporated in the ENDS emissions. Despite being an ingenious way to
 280 radiolabel the aerosol, this method seems not to be suitable for extensive studies. The method
 281 presented in this work seems to be easier as it only needs to introduce a radionuclide –
 282 pertechnetate ions ($^{99m}\text{TcO}_4^-$) – in the refill liquid. With a MMAD of $0.87 \pm 0.03 \mu\text{m}$ and an AMAD of
 283 $1.03 \pm 0.11 \mu\text{m}$, we considered that the two aerodynamic sizes are equivalent. This assumption is
 284 supported by the absence of significant difference of the distributions obtained with a cascade
 285 impactor ($p=0.5696$). Therefore, one could assume that the radiolabelling was efficient and does not
 286 modify the airborne properties of the ENDS emissions. These aerodynamic sizes are coherent with
 287 data found in literature, when cascade impactors are used (Holbrook et al., 2018; Mulder et al., 2019;
 288 Oldham et al., 2018; Pourchez et al., 2018; Sundahl et al., 2017; Zhao et al., 2018).

289 While the presented data are coherent with literature for cascade impactor measurement, they fall
 290 in the wide controversy concerning the aerodynamic size determination and concerning the lack
 291 standardized methods for this assessment (Ingebrethsen et al., 2012). Indeed, when particle count
 292 and light scattering methods are used, the aerodynamic size distribution appeared to be bimodal
 293 with a mode composed of small nanoparticles between 10 to 100 nm and another of submicronic
 294 particles with a size 2-fold lower than the MMAD and AMAD found in this study (Nordlund et al.,
 295 2017; Pratte et al., 2016; Son et al., 2019). However, due to the high concentration of droplets in the
 296 aerosol, these methods required to dilute the aerosol and, thus, it is acknowledged that this
 297 particular point is a bias leading to an underestimation of the size of the droplets (Bertholon et al.,
 298 2013; Ingebrethsen et al., 2012). This is due to a dynamic evaporation process induced by the volatile

299 and semi-volatile components of the refill liquids (Caldwell et al., 2012; Glasser et al., 2017;
300 Sosnowski & Odziomek, 2018).

301 Lastly, it is important to be noted that there is an important variability of ENDS technical features and
302 refill liquids. The main point of variation of the ENDS is due to the power generated by the battery
303 and the coil, while the composition of the refill liquids would influence the dynamic behaviour of the
304 e-cigarette aerosols (Floyd et al., 2018; Mulder et al., 2019; Olmedo et al., 2018; Ooi et al., 2019).

305 2. Aerosol regional deposition of ENDS emissions: modelling vs. experimental

306 In this study, about 90% of the deposited dose was found to the thoracic regions (Table 2).
307 Moreover, our estimation based on the experimentally determined AMAD and MMAD led to similar
308 modelled results, which were not significantly different from the experimental data generated with
309 the *ex vivo* model. This findings seems to be coherent with some modelled data of the literature,
310 where e-cigarette and conventional cigarette aerosol deposition are estimated to deposit up to 90%
311 in the thoracic region (Son et al., 2019; Sundahl et al., 2017). However, these findings seem to be
312 similar when only expressed as proportion of the total deposited dose. Moreover, comparison should
313 be done cautiously, as the breathing pattern and the airway morphometry used to estimate the
314 values could be different, which would generate comparison biases.

315 Indeed, when the results are expressed as a proportion of the total inhaled dose (*i.e.* the sum of the
316 radioactivity found in the respiratory tract, the upper airways and in the exhaled filters). In this case
317 the experimental data did not match with estimated values obtained with the MPPD or the
318 literature, where 70 to 90 % of the aerosol is found to be exhaled (Son et al., 2019; Sundahl et al.,
319 2017). In the present study, only 7% is found in the exhaled filter for experimental data, while over
320 80% of the aerosol is exhaled according to the MPPD. (Table 3). These discrepancies between the
321 experimental and estimated values seem to be problematic because it is hard to determine, which
322 model is similar to reality. However, while most of the estimation are agreeing on a high exhaled
323 fraction, the study from St. Helen *et al.* showed interesting “deposition data” on healthy volunteers.
324 In their study, the retained dose of PG, VG and nicotine as well as the exhaled dose of the same
325 components were assessed. They found that the retained dose, equivalent to the deposited dose, of
326 the aerosol was about $93.8 \pm 14.4\%$ for the nicotine, $84.4 \pm 26.4\%$ for the VG and $91.7 \pm 15.9\%$ for
327 the PG (St. Helen et al., 2016). Interestingly, these real-life data are in opposition with estimated data
328 while they appeared to be similar to the present experimental values. One could think that
329 determinist computational model are not accurate enough, due to some biases in the model itself.

330 However, further studies are needed to validate both the estimation by a determinist computational
331 model, such as the MPPD, and expedata obtained, such as St. Helen *et al.* or the present study, and
332 to reconcile computational and experimental data. Particularly, hygroscopic growth and
333 condensation phenomena, as well as vapour-gas equilibrium and deposition mechanisms involved
334 (James F. Pankow, 2001; James F. Pankow et al., 2003), should be better understood to predict the
335 outcomes of the exposure to an aerosol emitted by ENDS.

336 It is interesting to note that, even if the regionalisation of the deposition is not accurate between
337 experimental data, when expressed as proportion of the inhaled dose, the penetration of the aerosol
338 tend to be similar. Indeed, for the *ex vivo* model the C/P ratio was 0.75, while it was between 0.70-
339 0.75 for the estimation with the MPPD.

340 Therefore, despite being questionable to assess the exhaled dose, the *ex vivo* respiratory model
341 appeared to be a potential tool of interest to assess regional deposition and the penetration of
342 aerosol emitted with an ENDS. Moreover, this model already proved to be an interesting surrogate

343 preclinical model to animal experiments by respecting the 3Rs concept and therefore being ethically
344 less restricted (National Center for Replacement, Reduction and Refinement of Animal Research,
345 2018; Perinel et al., 2016, 2017). Despite the improvements that could be added to the model, such
346 as an automated aerosol sample or the vertical position of the respiratory tract. Indeed, the supine
347 position of the *ex vivo* model is a known limit of the model that could lead to overestimate the
348 deposited fraction in the respiratory tract. However, we hope that this new tool would be a new step
349 to assess the deposition of e-cigarette aerosols and to help to produce strong and reliable scientific
350 evidence concerning the outcomes of ENDS.

351 Conclusion

352 Through this study, we aimed to assess the regional deposition of an aerosol generated with a high-
353 power ENDS. First, we had to radiolabel the refill liquid with pertechnetate sodium solution, which
354 was achieved thanks to the use of a commonly used surfactant. To validate this point, we studied the
355 recovery of the MMAD and the AMAD using a DLPI cascade impactor. No significant difference could
356 be found between these two aerodynamic distributions. Then, we assessed the regional aerosol
357 deposition with a preclinical *ex vivo* respiratory model. Pulmonary deposition ($91 \pm 4\%$) was coherent
358 with data described in literature using semi-empirical/computational fluid dynamics software
359 (Sundahl et al., 2017; Zhang et al., 2013). Lastly, we compared the estimated deposition available in
360 published data with our experimental data and with estimated values obtained by running the MPPD
361 software, which appeared to be in the same range of commonly acknowledged values (Manigrasso,
362 Buonanno, Fuoco, et al., 2015; Manigrasso, Buonanno, Stabile, et al., 2015; Son et al., 2019;
363 Sosnowski & Kramek-Romanowska, 2016; Sundahl et al., 2017; Zhang et al., 2013). Therefore, we
364 supported that this preclinical model could be used to assess regional deposition of aerosol
365 generated with an ENDS. We hope that this work will allow the generation of strong evidences to fill
366 the data gap concerning the outcomes of ENDS.

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373 Declaration of interest

374 JP works as expert on electronic cigarette risk assessment for the French Agency for Food,
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376 Other authors report no conflict of interest concerning the present study.

377 References

378 Bertholon, J.-F., Becquemin, M. H., Roy, M., Roy, F., Ledur, D., Annesi Maesano, I., & Dautzenberg, B.
379 (2013). Comparaison de l'aérosol de la cigarette électronique à celui des cigarettes ordinaires
380 et de la chicha. *Revue Des Maladies Respiratoires*, 30(9), 752–757.
381 <https://doi.org/10.1016/j.rmr.2013.03.003>

382 Bertrand, P., Bonnarne, V., Piccirilli, A., Ayrault, P., Lemée, L., Frapper, G., & Pourchez, J. (2018).
383 Physical and chemical assessment of 1,3 Propanediol as a potential substitute of propylene
384 glycol in refill liquid for electronic cigarettes. *Scientific Reports*, 8(1), 1–10.
385 <https://doi.org/10.1038/s41598-018-29066-6>

386 Caldwell, B., Sumner, W., & Crane, J. (2012). A Systematic Review of Nicotine by Inhalation: Is There a
387 Role for the Inhaled Route? *Nicotine & Tobacco Research*, 14(10), 1127–1139.
388 <https://doi.org/10.1093/ntr/nts009>

389 Crémillieux, Y., Montigaud, Y., Bal, C., Pinaud, N., Pham, V., Perinel, S., Natuzzi, M., Lux, F., Tillement,
390 O., Ichinose, N., Zhang, B., & Pourchez, J. (2020). Three-dimensional quantitative MRI of
391 aerosolized gadolinium-based nanoparticles and contrast agents in isolated ventilated
392 porcine lungs. *Magnetic Resonance in Medicine*, 83(5), 1774–1782.
393 <https://doi.org/10.1002/mrm.28041>

394 Flora, J. W., Meruva, N., Huang, C. B., Wilkinson, C. T., Ballentine, R., Smith, D. C., Werley, M. S., &
395 McKinney, W. J. (2016). Characterization of potential impurities and degradation products in
396 electronic cigarette formulations and aerosols. *Regulatory Toxicology and Pharmacology*, 74,
397 1–11. <https://doi.org/10.1016/j.yrtph.2015.11.009>

398 Floyd, E. L., Queimado, L., Wang, J., Regens, J. L., & Johnson, D. L. (2018). Electronic cigarette power
399 affects count concentration and particle size distribution of vaping aerosol. *PLOS ONE*,
400 13(12), e0210147. <https://doi.org/10.1371/journal.pone.0210147>

401 Fuoco, F. C., Buonanno, G., Stabile, L., & Vigo, P. (2014). Influential parameters on particle
402 concentration and size distribution in the mainstream of e-cigarettes. *Environmental*
403 *Pollution*, 184, 523–529. <https://doi.org/10.1016/j.envpol.2013.10.010>

404 Glasser, A. M., Collins, L., Pearson, J. L., Abudayyeh, H., Niaura, R. S., Abrams, D. B., & Villanti, A. C.
405 (2017). Overview of Electronic Nicotine Delivery Systems: A Systematic Review. *American*
406 *Journal of Preventive Medicine*, 52(2), e33–e66.
407 <https://doi.org/10.1016/j.amepre.2016.10.036>

408 Grana Rachel, Benowitz Neal, & Glantz Stanton A. (2014). E-Cigarettes. *Circulation*, 129(19), 1972–
409 1986. <https://doi.org/10.1161/CIRCULATIONAHA.114.007667>

410 Holbrook, L. T., Zeman, K. L., Burke, A., Jaspers, I., & Bennett, W. D. (2018). Radiolabeling an
411 Electronic Cigarette Aerosol Using Technetium Carbon Ultrafine Particles. *Journal of Aerosol*
412 *Medicine and Pulmonary Drug Delivery*, 32(1), 47–53.
413 <https://doi.org/10.1089/jamp.2017.1442>

414 Ingebretsen, B. J., Cole, S. K., & Alderman, S. L. (2012). Electronic cigarette aerosol particle size
415 distribution measurements. *Inhalation Toxicology*, 24(14), 976–984.
416 <https://doi.org/10.3109/08958378.2012.744781>

417 Kleinstreuer, C., & Feng, Y. (2013). Lung Deposition Analyses of Inhaled Toxic Aerosols in
418 Conventional and Less Harmful Cigarette Smoke: A Review. *International Journal of*
419 *Environmental Research and Public Health*, 10(9), 4454–4485.
420 <https://doi.org/10.3390/ijerph10094454>

421 Kosmider, L., Cox, S., Zaciera, M., Kurek, J., Goniewicz, M. L., McRobbie, H., Kimber, C., & Dawkins, L.
422 (2020). Daily exposure to formaldehyde and acetaldehyde and potential health risk
423 associated with use of high and low nicotine e-liquid concentrations. *Scientific Reports*, 10(1),
424 1–10. <https://doi.org/10.1038/s41598-020-63292-1>

425 Manigrasso, M., Buonanno, G., Fuoco, F. C., Stabile, L., & Avino, P. (2015). Aerosol deposition doses
426 in the human respiratory tree of electronic cigarette smokers. *Environmental Pollution*, 196,
427 257–267. <https://doi.org/10.1016/j.envpol.2014.10.013>

428 Manigrasso, M., Buonanno, G., Fuoco, F. C., Stabile, L., & Avino, P. (2017). Electronic cigarettes: age-
429 specific generation-resolved pulmonary doses. *Environmental Science and Pollution*
430 *Research*, 24(14), 13068–13079. <https://doi.org/10.1007/s11356-017-8914-8>

431 Manigrasso, M., Buonanno, G., Stabile, L., Morawska, L., & Avino, P. (2015). Particle doses in the
432 pulmonary lobes of electronic and conventional cigarette users. *Environmental Pollution*,
433 202, 24–31. <https://doi.org/10.1016/j.envpol.2015.03.008>

434 e-Voke 10mg and 15mg Electronic Inhaler, Pub. L. No. PL 42601/0003-4, PL 42601/0003-4 PL
435 42601/0003-4 (2015). [https://mhraproductsprod.blob.core.windows.net/docs-](https://mhraproductsprod.blob.core.windows.net/docs-20200420/56f25daab2a2968139bc37075e194d1a5f12b33f)
436 [20200420/56f25daab2a2968139bc37075e194d1a5f12b33f](https://mhraproductsprod.blob.core.windows.net/docs-20200420/56f25daab2a2968139bc37075e194d1a5f12b33f)
437 MHRA. (2017, December 14). *E-cigarettes: regulations for consumer products*. GOV.UK.
438 <https://www.gov.uk/guidance/e-cigarettes-regulations-for-consumer-products>
439 Mikheev, V. B., Brinkman, M. C., Granville, C. A., Gordon, S. M., & Clark, P. I. (2016). Real-Time
440 Measurement of Electronic Cigarette Aerosol Size Distribution and Metals Content Analysis.
441 *Nicotine & Tobacco Research, 18*(9), 1895–1902. <https://doi.org/10.1093/ntr/ntw128>
442 Montigaud, Y., Georges, Q., Pourchez, J., Leclerc, L., Goy, C., Clotagatide, A., Prevot, N., & Perinel-
443 Ragey, S. (2019). Aerosol delivery during invasive mechanical ventilation: development of a
444 preclinical ex vivo respiratory model for aerosol regional deposition. *Scientific Reports, 9*(1),
445 1–9. <https://doi.org/10.1038/s41598-019-54480-9>
446 Montigaud, Y., Perinel-Ragey, S., Plantier, L., Leclerc, L., Goy, C., Clotagatide, A., Prévôt, N., &
447 Pourchez, J. (2019). Development of an ex vivo preclinical respiratory model of idiopathic
448 pulmonary fibrosis for aerosol regional studies. *Scientific Reports, 9*(1), 1–11.
449 <https://doi.org/10.1038/s41598-019-54479-2>
450 Mulder, H. A., Patterson, J. L., Halquist, M. S., Kosmider, L., Turner, J. B. M., Poklis, J. L., Poklis, A., &
451 Peace, M. R. (2019). The Effect of Electronic Cigarette User Modifications and E-liquid
452 Adulteration on the Particle Size Profile of an Aerosolized Product. *Scientific Reports, 9*.
453 <https://doi.org/10.1038/s41598-019-46387-2>
454 National Center for Replacement, Reduction and Refinement of Animal Research. (2018, August 13).
455 *The 3Rs | NC3Rs*. <https://www.nc3rs.org.uk/the-3rs>
456 Newman, S., Bennett, W. D., Biddiscombe, M., Devadason, S. G., Dolovich, M. B., Fleming, J.,
457 Haeussermann, S., Kietzig, C., Kuehl, P. J., Laube, B. L., Sommerer, K., Taylor, G., Usmani, O.
458 S., & Zeman, K. L. (2012). Standardization of Techniques for Using Planar (2D) Imaging for

459 Aerosol Deposition Assessment of Orally Inhaled Products. *Journal of Aerosol Medicine and*
460 *Pulmonary Drug Delivery*, 25(S1), S-10. <https://doi.org/10.1089/jamp.2012.1Su4>

461 Nordlund, M., Belka, M., Kuczaj, A. K., Lizal, F., Jedelsky, J., Elcner, J., Jicha, M., Sauser, Y., Bouhellec,
462 S. L., Cosandey, S., Majeed, S., Vuillaume, G., Peitsch, M. C., & Hoeng, J. (2017).
463 Multicomponent aerosol particle deposition in a realistic cast of the human upper respiratory
464 tract. *Inhalation Toxicology*, 29(3), 113–125.
465 <https://doi.org/10.1080/08958378.2017.1315196>

466 Oldham, M. J., Zhang, J., Rusyniak, M. J., Kane, D. B., & Gardner, W. P. (2018). Particle size
467 distribution of selected electronic nicotine delivery system products. *Food and Chemical*
468 *Toxicology*, 113, 236–240. <https://doi.org/10.1016/j.fct.2018.01.045>

469 Olmedo, P., Goessler, W., Tanda, S., Grau-Perez, M., Jarmul, S., Aherrera, A., Chen, R., Hilpert, M.,
470 Cohen, J. E., Navas-Acien, A., & Rule, A. M. (2018). Metal Concentrations in e-Cigarette Liquid
471 and Aerosol Samples: The Contribution of Metallic Coils. *Environmental Health Perspectives*,
472 126(2). <https://doi.org/10.1289/EHP2175>

473 Ooi, B. G., Dutta, D., Kazipeta, K., & Chong, N. S. (2019). Influence of the E-Cigarette Emission Profile
474 by the Ratio of Glycerol to Propylene Glycol in E-Liquid Composition. *ACS Omega*, 4(8),
475 13338–13348. <https://doi.org/10.1021/acsomega.9b01504>

476 Palazzolo, D. L. (2013). Electronic Cigarettes and Vaping: A New Challenge in Clinical Medicine and
477 Public Health. A Literature Review. *Frontiers in Public Health*, 1.
478 <https://doi.org/10.3389/fpubh.2013.00056>

479 Pankow, James F. (2001). A consideration of the role of gas/particle partitioning in the deposition of
480 nicotine and other tobacco smoke compounds in the respiratory tract. *Chemical Research in*
481 *Toxicology*, 14(11), 1465–1481. <https://doi.org/10.1021/tx0100901>

482 Pankow, James F., Tavakoli, A. D., Luo, W., & Isabelle, L. M. (2003). Percent free base nicotine in the
483 tobacco smoke particulate matter of selected commercial and reference cigarettes. *Chemical*
484 *Research in Toxicology*, 16(8), 1014–1018. <https://doi.org/10.1021/tx0340596>

485 Perinel, S., Leclerc, L., Prévôt, N., Deville, A., Cottier, M., Durand, M., Vergnon, J.-M., & Pourchez, J.
486 (2016). Micron-sized and submicron-sized aerosol deposition in a new ex vivo preclinical
487 model. *Respiratory Research*, 17. <https://doi.org/10.1186/s12931-016-0395-7>

488 Perinel, S., Pourchez, J., Leclerc, L., Avet, J., Durand, M., Prévôt, N., Cottier, M., & Vergnon, J. M.
489 (2017). Development of an ex vivo human-porcine respiratory model for preclinical studies.
490 *Scientific Reports*, 7. <https://doi.org/10.1038/srep43121>

491 Pourchez, J., de Oliveira, F., Perinel-Ragey, S., Basset, T., Vergnon, J.-M., & Prévôt, N. (2017).
492 Assessment of new-generation high-power electronic nicotine delivery system as thermal
493 aerosol generation device for inhaled bronchodilators. *International Journal of*
494 *Pharmaceutics*, 518(1), 264–269. <https://doi.org/10.1016/j.ijpharm.2017.01.009>

495 Pourchez, J., Parisse, S., Sarry, G., Perinel-Ragey, S., Vergnon, J.-M., Clotagatide, A., & Prévôt, N.
496 (2018). Impact of power level and refill liquid composition on the aerosol output and particle
497 size distribution generated by a new-generation e-cigarette device. *Aerosol Science and*
498 *Technology*, 52(4), 359–369. <https://doi.org/10.1080/02786826.2017.1422857>

499 Pratte, P., Cosandey, S., & Goujon-Ginglinger, C. (2016). A scattering methodology for droplet sizing
500 of e-cigarette aerosols. *Inhalation Toxicology*, 28(12), 537–545.
501 <https://doi.org/10.1080/08958378.2016.1224956>

502 Prévôt, N., Oliveira, F. de, Perinel-Ragey, S., Basset, T., Vergnon, J.-M., & Pourchez, J. (2017). Nicotine
503 delivery from the refill liquid to the aerosol via high-power e-cigarette device. *Scientific*
504 *Reports*, 7(1), 2592. <https://doi.org/10.1038/s41598-017-03008-0>

505 Smith, D. M., Schneller, L. M., O'Connor, R. J., & Goniewicz, M. L. (2019). Are E-Cigarette Flavors
506 Associated with Exposure to Nicotine and Toxicants? Findings from Wave 2 of the Population
507 Assessment of Tobacco and Health (PATH) Study. *International Journal of Environmental*
508 *Research and Public Health*, 16(24), 5055. <https://doi.org/10.3390/ijerph16245055>

509 Son, Y., Mainelis, G., Delnevo, C., Wackowski, O. A., Schwander, S., & Meng, Q. (2019). Investigating
510 E-Cigarette Particle Emissions and Human Airway Depositions under Various E-Cigarette-Use

511 Conditions. *Chemical Research in Toxicology.*
512 <https://doi.org/10.1021/acs.chemrestox.9b00243>

513 Sood, A. K., Kesic, M. J., & Hernandez, M. L. (2018). Electronic cigarettes: One size does not fit all.
514 *Journal of Allergy and Clinical Immunology, 141*(6), 1973–1982.
515 <https://doi.org/10.1016/j.jaci.2018.02.029>

516 Sosnowski, T. R., Jabłczyńska, K., Odziomek, M., Schlage, W. K., & Kuczaj, A. K. (2018).
517 Physicochemical studies of direct interactions between lung surfactant and components of
518 electronic cigarettes liquid mixtures. *Inhalation Toxicology, 30*(4–5), 159–168.
519 <https://doi.org/10.1080/08958378.2018.1478916>

520 Sosnowski, T. R., & Kramek-Romanowska, K. (2016). Predicted Deposition of E-Cigarette Aerosol in
521 the Human Lungs. *Journal of Aerosol Medicine and Pulmonary Drug Delivery, 29*(3), 299–309.
522 <https://doi.org/10.1089/jamp.2015.1268>

523 Sosnowski, T. R., & Odziomek, M. (2018). Particle Size Dynamics: Toward a Better Understanding of
524 Electronic Cigarette Aerosol Interactions With the Respiratory System. *Frontiers in*
525 *Physiology, 9*. <https://doi.org/10.3389/fphys.2018.00853>

526 St. Helen, G., Havel, C., Dempsey, D., Jacob, P., & Benowitz, N. L. (2016). Nicotine delivery, retention,
527 and pharmacokinetics from various electronic cigarettes. *Addiction (Abingdon, England),*
528 *111*(3), 535–544. <https://doi.org/10.1111/add.13183>

529 Sundahl, M., Berg, E., & Svensson, M. (2017). Aerodynamic particle size distribution and dynamic
530 properties in aerosols from electronic cigarettes. *Journal of Aerosol Science, 103*, 141–150.
531 <https://doi.org/10.1016/j.jaerosci.2016.10.009>

532 Thornburg, J. (2017). Chapter 3 - Exposures to e-Cigarette Vapor. In K. E. Farsalinos, I. G. Gillman, S. S.
533 Hecht, R. Polosa, & J. Thornburg (Eds.), *Analytical Assessment of E-Cigarettes* (pp. 37–58).
534 Elsevier. <https://doi.org/10.1016/B978-0-12-811241-0.00003-6>

535 Vansickel, A. R., Edmiston, J. S., Liang, Q., Duhon, C., Connell, C., Bennett, D., & Sarkar, M. (2018).
536 Characterization of puff topography of a prototype electronic cigarette in adult exclusive

537 cigarette smokers and adult exclusive electronic cigarette users. *Regulatory Toxicology and*
538 *Pharmacology*, 98, 250–256. <https://doi.org/10.1016/j.yrtph.2018.07.019>

539 Visser, W. F., Klerx, W. N., Cremers, H. W. J. M., Ramlal, R., Schwillens, P. L., & Talhout, R. (2019). The
540 Health Risks of Electronic Cigarette Use to Bystanders. *International Journal of Environmental*
541 *Research and Public Health*, 16(9), 1525. <https://doi.org/10.3390/ijerph16091525>

542 Williams, M., & Talbot, P. (2019). Design Features in Multiple Generations of Electronic Cigarette
543 Atomizers. *International Journal of Environmental Research and Public Health*, 16(16), 2904.
544 <https://doi.org/10.3390/ijerph16162904>

545 Zervas, E., Litsiou, E., Konstantopoulos, K., Pouloupoulos, S., & Katsaounou, P. (2018). Physical
546 characterization of the aerosol of an electronic cigarette: impact of refill liquids. *Inhalation*
547 *Toxicology*, 30(6), 218–223. <https://doi.org/10.1080/08958378.2018.1500662>

548 Zhang, Y., Sumner, W., & Chen, D.-R. (2013). In Vitro Particle Size Distributions in Electronic and
549 Conventional Cigarette Aerosols Suggest Comparable Deposition Patterns. *Nicotine &*
550 *Tobacco Research*, 15(2), 501–508. <https://doi.org/10.1093/ntr/nts165>

551 Zhao, J., Nelson, J., Dada, O., Pyrgiotakis, G., Kavouras, I. G., & Demokritou, P. (2018). Assessing
552 electronic cigarette emissions: linking physico-chemical properties to product brand, e-liquid
553 flavoring additives, operational voltage and user puffing patterns. *Inhalation Toxicology*,
554 30(2), 78–88. <https://doi.org/10.1080/08958378.2018.1450462>

555