

# A Laboratory Method to Measure Skin Surface Staining by Cigarette Smoke, Tobacco Heating Products and E-Cigarettes\*

by

Annette Dalrymple<sup>1</sup>, Emma-Jayne Bean<sup>1</sup>, Jesse Thissen<sup>1</sup>, Holger Behrsing<sup>2</sup>, Steven Coburn<sup>1</sup>, and James Murphy<sup>1</sup>

<sup>1</sup> British American Tobacco, R&D, Southampton, Hampshire, SO15 8TL, UK

<sup>2</sup> Institute for In Vitro Sciences, Gaithersburg, MD 20878, USA

## SUMMARY

Exhaled or side-stream cigarette smoke (CS) may visually stain a consumer's skin over time. Tobacco heating products (THPs) and e-cigarettes (ECs) have reduced staining potential because they do not produce side-stream aerosols and their exhaled aerosols have significantly reduced levels of toxicants, particles and odour. Here we assess discolouration of porcine skin *in vitro* after exposure to particulate matter (PM) or aerosols from CS (3R4F), two THPs (glo and glo sens) and an EC (iSwitch Maxx). PM was prepared by capturing aerosols on Cambridge filter pads and eluting with dimethyl sulfoxide (DMSO). Abattoir-obtained porcine skin samples were incubated with PM or DMSO control at 37 °C between 0 and 6.0 h. For aerosol assessment, porcine skin samples were exposed to between 50 and 400 puffs of the products, or air control, using a smoking machine. Colour profiles and staining levels of each skin sample were measured at different timepoints and puff thresholds using a spectrophotometer. Staining increased with time and dose, the greatest changes being observed following exposure to aerosols and PM from CS. THP, EC and control values were significantly different from CS after 0.5 h exposure to PM or 50 puffs of aerosols. The minimal staining induced by THPs and EC was comparable to controls. These data suggest that THPs and ECs could offer hygiene benefits to consumers who switch from

smoking cigarettes. Further studies are required to assess the longer-term effects of THPs and ECs on skin discolouration. [Contrib. Tob. Nicotine Res. 30 (2021) 158–166]

## KEYWORDS

Surface staining; cigarette; electronic cigarette/e-cigarette; tobacco heating product; hygiene; skin

## ZUSAMMENFASSUNG

Der exhalierte Rauch oder Nebenstromrauch von Zigaretten kann mit der Zeit die Haut der Konsumenten sichtbar verfärben. Tabakerhitzer und E-Zigaretten haben ein geringeres Verfärbungspotential, denn bei ihnen entstehen keine Nebenstrom-Aerosole und ihre exhalierten Aerosole haben einen signifikant niedrigeren Gehalt an Partikelmasse sowie an Gift- und Geruchsstoffen. In der vorliegenden Arbeit beurteilen wir *in vitro* die Verfärbung der Haut von Schweinen nach einer Exposition mit Partikelmasse aus dem Rauch einer herkömmlichen Zigarette (3R4F) bzw. mit Aerosolen aus zwei Tabakerhitzern (glo und glo sens) und einer E-Zigarette (iSwitch Maxx). Die Präparation der Partikelmasse erfolgte durch Auffangen der Aerosole mithilfe von Cambridge Filter Pads und Eluieren mit Dimethyl-

\*Received: 16<sup>th</sup> March 2021 – accepted: 20<sup>th</sup> August 2021

sulfoxid (DMSO). Schweinehautproben aus dem Schlachthof wurden jeweils mit Partikelmasse oder DMSO (Kontrolle) zwischen 0 und 6,0 h bei 37 °C inkubiert. Für die Analyse der Aerosole wurden die Schweinehautproben mithilfe einer Rauchmaschine 50 bis 400 Zügen der Produkte bzw. Luft (Kontrolle) ausgesetzt. Mit einem Spektralphotometer wurden für jede Hautprobe zu verschiedenen Zeitpunkten und Zugschwellenwerten Farbprofile und Verfärbungsgrade gemessen. Die Verfärbung nahm mit zunehmender Zeit und Dosis zu, wobei die größten Veränderungen nach Exposition mit Aerosolen und Partikelmasse aus Zigarettenrauch zu beobachten waren. Nach 0,5 h Exposition mit Partikelmasse bzw. 50 Zügen Aerosolen unterschieden sich die Werte für Tabakerhitzer und E-Zigaretten sowie die Kontrollwerte signifikant von denen für Zigarettenrauch. Die von Tabakerhitzen und E-Zigaretten verursachte minimale Verfärbung war mit den DMSO-Kontrollen vergleichbar. Diese Daten deuten darauf hin, dass Tabakerhitzer- und E-Zigaretten-Konsumenten, die von herkömmlichen Zigaretten auf diese Produkte umsteigen, Hygienevorteile erzielen könnten. Zur Beurteilung der langfristigen Wirkung von Tabakerhitzen und E-Zigaretten auf die Verfärbung der Haut sind weitere Untersuchungen erforderlich. [Contrib. Tob. Nicotine Res. 30 (2021) 158–166]

## RESUME

Au fil du temps, la fumée de cigarette exhalée ou latérale est susceptible de provoquer une coloration visible de la peau des consommateurs. Les produits de tabac chauffé et les cigarettes électroniques présentent un moindre potentiel de coloration puisqu'ils ne produisent pas d'aérosols latéraux et que leurs aérosols exhalés affichent des niveaux significativement réduits de substances toxiques, de matière particulaire et d'odeur. La présente étude analyse la décoloration de la peau de porc en milieu *in vitro* après exposition à de la matière particulaire ou à des aérosols émis par la fumée de cigarette traditionnelle (3R4F), par deux produits de tabac chauffé (glo et glo sens) et par une cigarette électronique (iSwitch Maxx). La matière particulaire fut préparée en capturant les aérosols sur des coussinets de filtre Cambridge et en l'éluant à l'aide de diméthylsulfoxyde (DMSO). Les échantillons de peau de porc obtenus auprès d'un abattoir furent incubés avec de la matière particulaire ou du DMSO (contrôle) à une température de 37 °C durant 0 à 6 heures. Afin d'évaluer les aérosols, les échantillons de peau de porc furent, à l'aide d'une machine à fumer, exposés à entre 50 et 400 bouffées des produits ou d'air (contrôle). Les profils colorimétriques et les niveaux de coloration de chaque échantillon de peau furent évalués à différents moments et à divers seuils de bouffée à l'aide d'un spectrophotomètre. La coloration s'intensifie avec le temps d'exposition et selon le dosage ; les modifications les plus marquantes furent observées dans le cas d'une exposition aux aérosols et à la matière particulaire contenus dans la fumée de cigarette. Les valeurs pour les produits de tabac chauffé, les cigarettes électroniques ainsi que les valeurs des contrôles furent significativement différentes de celles de la fumée de cigarette après une exposition de 0,5 heures à la matière

particulaire ou 50 bouffées d'aérosols. La coloration minimale induite par les produits de tabac chauffé et la cigarette électronique fut comparable aux valeurs de contrôle. Ces données laissent à penser que les produits de tabac chauffé et la cigarette électronique pourraient présenter des avantages en termes d'hygiène pour les consommateurs qui délaissent la cigarette traditionnelle. Des études complémentaires sont indispensables afin de déterminer les effets à long terme des produits de tabac chauffé et des cigarettes électroniques sur la décoloration cutanée. [Contrib. Tob. Nicotine Res. 30 (2021) 158–166]

## ABBREVIATIONS

3R4F	Kentucky Reference Cigarette
CS	cigarette smoke
CFP	Cambridge filter pad
DMSO	dimethyl sulfoxide
EC	electronic cigarette
PM	particulate matter
THP	tobacco heating product

## INTRODUCTION

Electronic cigarettes (ECs) have become widely available and have increased in popularity since their introduction to the market. More recently, tobacco heating products (THPs) have become commercially available, but so far are used less widely as availability varies by country and regulation. ECs are relatively simple devices that heat an e-liquid, creating an inhalable aerosol. Most e-liquids consist of 1,2-propylene glycol, vegetable glycerol, water and flavours, and can be purchased with or without nicotine. THPs heat tobacco rods (often known as consumables or sticks) to temperatures of 200–350 °C, which vaporise nicotine and other volatile compounds without the combustion/ pyrolysis of the tobacco (1, 2). Both classes of device s release the aerosol only when inhaled by the consumer. By contrast, conventional cigarettes burn at temperatures up to 950 °C, producing mainstream smoke, but also smoulder between puffs, producing side-stream smoke. Overall, cigarette smoke (CS) releases more than 7,000 chemicals, including many known toxicants (3, 4) and can contribute to room odour and staining (5, 6) as well as staining of consumers' teeth (7–9) and skin (10). THP and EC aerosols contain significantly fewer toxicants than CS (1, 2, 11–13), and there is growing consensus that THPs and ECs hold the potential to reduce the health risks associated with smoking (14–18). In addition to risk reduction, THPs and ECs might have hygiene or social benefits for smokers who switch. Laboratory methods have been developed to quantify tooth enamel sample and surface staining following exposure to CS or THP and EC aerosols (6, 9). Consistently, CS stains samples, whereas changes with THP and EC aerosols are minimal and close to controls. As far as we are aware, no comparison of skin staining by CS, THP and EC aerosols has been performed. In the current study, a laboratory method was developed to assess the exposure of porcine skin samples to emissions from CS, THP and EC aerosols.

## MATERIALS AND METHODS

### Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (Gillingham, UK, or St Louis, MO, USA) unless otherwise stated.

### Test articles

Four test products were used in this study: 3R4F Kentucky reference cigarettes, iSwitch Maxx e-cigarettes (British American Tobacco, Southampton, UK) and the glo and glo sens THP (British American Tobacco; Table 1). Prior to use, the 3R4F cigarettes were conditioned for a minimum of 48 h and a maximum of 10 days and the glo THP tobacco rods (Neostiks) were conditioned for 48 h to 5 days by storing at  $22 \pm 1$  °C and  $60 \pm 3\%$  relative humidity, according to the ISO 3402:1999 standard (19). The EC e-liquid cartridges and glo sens THP tobacco pods were stored at room temperature. All devices were fully charged before use.

### Porcine sample preparation

*Ex vivo* pig abdominal skin was retrieved in an abattoir and immediately placed on ice. In the laboratory, the skin was prepared by clipping the surface hair and removing excess subcutaneous fat. Slices of 500–750  $\mu\text{m}$  were cut with a dermatome, from which cylindrical biopsy punches of 5 mm diameter were obtained. Prepared skin punch samples were stored at  $-20 \pm 5$  °C until required. On the day of analysis, skin punches were removed from the freezer, brought to room temperature and examined for obvious defects, such as tears, fissures or scratches. Prior to use, skin punches were incubated for 10 min at standard

culture conditions ( $5 \pm 1\%$  CO<sub>2</sub> and  $37 \pm 1$  °C) in phenol-free Hanks' Balanced Salt Solution, containing calcium and magnesium (Thermo Fisher Scientific, Grand Island, NY, USA, 14025-092), and then washed in calcium- and magnesium-free Dulbecco's Phosphate Buffered Saline (PBS) (Thermo Fisher Scientific, 14190-144).

### Particulate matter preparation

3R4F CS and aerosols from glo, glo sens or iSwitch Maxx were generated using LM20X or LM20E linear smoking machines (Borgwaldt, Hamburg, Germany). The iSwitch Maxx was tested at the highest power level. Specific puffing regimes were used for each product (Table 2). The particulate fraction of CS or aerosol from each product was collected on 44-mm Cambridge filter pads (CFPs, Whatman, Maidstone, UK) and the particulate matter (PM) was eluted with dimethylsulfoxide (DMSO) as described previously (20, 21). Briefly, CFPs were weighed before and after aerosol collection to determine the weight of the captured aerosol. CFPs were placed in 100-mL glass bottles and DMSO added to achieve a concentration of 24 mg/mL. Bottles were covered with foil and placed, at room temperature, on an orbital shaker set at 150 rpm for 25 min. The PM was then extracted from each CFP under vacuum, 1-mL aliquots were prepared and stored in glass vials at  $-80$  °C until required.

### PM exposure

Cylindrical punch biopsy skin samples were placed dermis down into 500  $\mu\text{L}$  of product PM or DMSO and incubated under standard culture conditions ( $5 \pm 1\%$  CO<sub>2</sub> and  $37 \pm 1$  °C). Samples were removed at 0.25, 0.5, 1.0, 3.0 and 6.0 h (three skin samples per timepoint) and colorimetric readings were taken. Each experiment was repeated at least three times.

**Table 1. Products assessed for skin staining.**

Product	Source	Consumable	E-liquid nicotine (mg/mL)	Puffs per product/cartridge	Puffs per tobacco pod
3R4F reference cigarette	University of Kentucky	N/A	N/A	10	N/A
iSwitch Maxx e-cigarette	British American Tobacco	Virginia tobacco	5	80	N/A
glo THP	British American Tobacco	Bright tobacco Neostiks	N/A	8	N/A
glo sens THP	British American Tobacco	Mixed fruit	0	150	50

**Table 2. Product puffing regimes.**

Product	Regime	Puff volume (mL)	Puff duration (s)	Puff interval/frequency (s)	Vent blocking	Puff profile
3R4F reference cigarette	HCI	55	2	30	100%	Bell
iSwitch Maxx e-cigarette	CRM81	55	3	30	None	Square
glo THP	HCI <sup>m</sup>	55	2	30	None	Bell
glo sens THP	CRM81	55	3	30	None	Square

#### Abbreviations:

CRM81: CORESTA recommended method no. 81 (30)

HCI: Health Canada intense smoking regime (29)

HCI<sup>m</sup>: Health Canada intense smoking regime modified with no vent blocking

N/A: Not Applicable

THP: Tobacco Heating Product

Cylindrical biopsy skin punches were placed into 12-well hanging inserts (Transwell®, Corning, Lowell, MA, USA) and then into a VITROCELL® 12/6 CF module (Waldkirch, Germany) with Hanks' Buffered Salt Solution at ambient room temperature. Using product-specific puffing regimes (Table 2) and a VITROCELL® VC1® engine, samples were exposed to 50, 100, 200 or 400 puffs of 3R4F CS, glo sens, iSwitch Maxx or air control (three skin samples per dose). Three or more independent experiments were performed for each product or control, glo was not assessed as an aerosol.

#### Colour measurements

Prior to exposure, the colour profile of each pig skin punch sample was determined using a CM-700d spectrophotometer (Konica Minolta Business Solutions, Greenville, SC, USA) with 5-mm aperture that was calibrated using the manufacturer-supplied white tile before use. Four measurements per skin samples were taken and the sample was rotated 90° between each measurement. Throughout the colorimetric analysis, the operator maintained a uniform specimen measuring port-to-tissue surface distance and ambient lighting to minimize variability and bias in measurements. Colour readings were captured, stored in the CM-700d using the SpectraMagic NX software (Konica Minolta Business Solutions) and the results were exported to a Microsoft Excel document. Samples exposed to PM or DMSO were rinsed in 500 µL phosphate-buffered saline before analysis. Those exposed to aerosol or air were removed from the exposure chamber and placed directly on to the CM-700d spectrophotometer aperture.

Colour profiles and staining levels were calculated at baseline and at every timepoint or puff number using the "Commission Internationale de L'éclairage L\*a\*b\* method". L\* is a measure of lightness and a\* and b\* are measures of green-red and blue-yellow colour components, respectively (6, 9, 22). Changes in values from baseline and between treatments were determined in Excel by calculating  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$  and  $\Delta E$  (total difference) with the following equation:

$$\Delta E = \sqrt{\left((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right)}$$

#### Statistical methods

The data analysis for this paper was generated using SAS software, Version 9.4 of the SAS System for Windows (Copyright © 2021 SAS Institute Inc., SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.) Generalised linear models were used to assess the differences in  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$  and  $\Delta E$  values between the products and reference cigarettes. The significance threshold for difference ( $\alpha$ ) was set at  $p = 0.05$ . Post-hoc Tukey adjustment for pairwise comparisons was also used.

## RESULTS

#### Particulate matter skin sample exposure

Exposure to 3R4F PM resulted in darkening and discoloration of the skin samples, with effects increasing over time. After 0.25 h, 3R4F  $\Delta L^*$  values were significantly lower than glo sens and DMSO values ( $p < 0.05$ , Table 3) indicating darkening of the skin samples. After 0.5 h, 3R4F  $\Delta L^*$  values were also significantly lower than glo and iSwitch Maxx values ( $p < 0.05$ ). After 0.25 h exposure, 3R4F  $\Delta a^*$  values (green to red), were significantly higher than glo and DMSO ( $p < 0.0001$ ) demonstrating reddening of the skin following the exposure. At 0.5 h, all products and DMSO  $\Delta a^*$  values were significantly lower ( $p < 0.0001$ ) than 3R4F. The  $\Delta b^*$  values (blue to yellow) following 3R4F exposure were significantly higher than glo, glo sens and DMSO control from 0.25 h demonstrating that the skin yellows with exposure. From 0.5 h, iSwitch Maxx and all other products  $\Delta b^*$  values were significantly lower than 3R4F ( $p < 0.0001$ ). From 0.25 h, total colour changes, shown by the  $\Delta E$  value (Figure 1, a), were significantly higher for 3R4F than DMSO and all products except iSwitch. At 0.5 h, glo, glo sens, iSwitch Maxx and DMSO control  $\Delta E$  values were significantly lower than the 3R4F value ( $p < 0.0001$ ). All THP and EC values were similar to those for the DMSO control throughout the timepoints assessed (Figure 1, a and b).

#### Aerosol skin exposure

Exposure to 3R4F CS aerosol resulted in darkening and discoloration of punch skin samples, with dose-dependent changes observed for  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta E^*$  values (Table 4). After 50 puffs, 3R4F CS  $\Delta L^*$  values were significantly lower than those for glo sens, iSwitch Maxx and air control (all  $p < 0.0001$ ). Skin reddening was seen with 3R4F CS exposure compared with the other products and control, with the difference in  $\Delta a^*$  values becoming significant from 50 puffs (all doses  $p < 0.0001$ ). Skin yellowing, represented by  $\Delta b^*$  values, increased following 3R4F exposure, differing significantly from glo sens, iSwitch Maxx and air control values at all puff numbers (all  $p < 0.0001$ ). However, 3R4F  $\Delta b^*$  values reached a plateau at 200 puffs and decreased at 400 puffs. The  $\Delta E$  values (Figure 1 c), indicating overall colour change, were significantly higher for 3R4F CS than for glo sens, iSwitch Maxx and air control at 50–400 puffs (all  $p < 0.0001$ ). As for exposure to PM, all THP and EC values were comparable to air control at all doses (Figure 1, c and d).

## DISCUSSION

In this study, significant differences were noticed for skin darkening and discoloration after exposure to CS *versus* aerosol from THP and EC. By contrast, changes with THP and EC exposure remained similar to those seen with DMSO and air controls by time and dose. Value changes indicated darkening, reddening and yellowing of skin after CS exposure.

**Table 3. Mean  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$  and  $\Delta E$  and standard deviation values following the exposure of skin samples for 0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 h to particulate matter generated from 3R4F cigarettes, glo and glo sens THP, iSwitch Maxx EC or DMSO as a control.**

Hours	3R4F		glo		glo sens		iSwitch Maxx		DMSO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$\Delta L^*$ (lightness)										
0.25	-9.00	2.19	-7.76	2.45	-6.98 <sup>b</sup>	2.86	-8.41	2.60	-7.30 <sup>b</sup>	2.80
0.5	-10.76	2.60	-9.02 <sup>b</sup>	2.27	-8.35 <sup>b</sup>	3.16	-8.61 <sup>b</sup>	2.40	-8.64 <sup>a</sup>	3.27
1.0	-12.87	2.50	-10.26 <sup>b</sup>	2.63	-8.76 <sup>a</sup>	5.29	-9.70 <sup>b</sup>	3.65	-10.01 <sup>a</sup>	3.69
2.0	-14.19	2.48	-9.45 <sup>a</sup>	2.21	-8.77 <sup>a</sup>	4.50	-9.33 <sup>a</sup>	3.58	-9.20 <sup>a</sup>	2.79
4.0	-15.62	2.28	-8.74 <sup>a</sup>	2.45	-7.63 <sup>a</sup>	4.48	-8.22 <sup>a</sup>	3.05	-8.68 <sup>a</sup>	2.70
6.0	-16.76	2.54	-8.13 <sup>a</sup>	2.88	-6.89 <sup>a</sup>	4.13	-7.75 <sup>a</sup>	2.91	-8.29 <sup>a</sup>	3.11
$\Delta a^*$ (green-red)										
0.25	0.49	0.83	-0.29 <sup>a</sup>	0.34	0.25	0.55	0.52	0.48	-0.22 <sup>a</sup>	0.75
0.5	1.34	0.82	0.52 <sup>a</sup>	0.85	0.33 <sup>a</sup>	0.54	0.45 <sup>a</sup>	0.66	0.35 <sup>a</sup>	0.98
1.0	1.78	0.81	0.16 <sup>a</sup>	1.10	0.24 <sup>a</sup>	0.95	0.13 <sup>a</sup>	0.56	0.35 <sup>a</sup>	0.98
2.0	2.72	0.80	-0.28 <sup>a</sup>	1.30	-0.03 <sup>a</sup>	0.63	0.08 <sup>a</sup>	0.53	0.11 <sup>a</sup>	1.01
4.0	4.07	0.75	-0.07 <sup>a</sup>	1.35	0.03 <sup>a</sup>	0.60	-0.02 <sup>a</sup>	0.47	0.18 <sup>a</sup>	0.81
6.0	4.98	0.79	0.12 <sup>a</sup>	1.27	0.21 <sup>a</sup>	0.49	0.29 <sup>a</sup>	0.58	0.41 <sup>a</sup>	0.95
$\Delta b^*$ (blue-yellow)										
0.25	2.66	3.49	-0.70 <sup>a</sup>	2.02	1.21 <sup>b</sup>	1.26	1.41	1.35	0.13 <sup>a</sup>	2.39
0.5	5.86	2.17	0.70 <sup>a</sup>	2.27	1.05 <sup>a</sup>	1.11	1.17 <sup>a</sup>	1.91	0.84 <sup>a</sup>	2.71
1.0	7.90	1.84	0.70 <sup>a</sup>	2.37	0.05 <sup>a</sup>	1.51	1.61 <sup>a</sup>	0.86	0.49 <sup>a</sup>	2.24
2.0	9.35	2.59	0.64 <sup>a</sup>	2.06	0.38 <sup>a</sup>	0.74	1.49 <sup>a</sup>	1.02	0.34 <sup>a</sup>	2.61
4.0	10.20	3.21	-0.10 <sup>a</sup>	1.70	-0.31 <sup>a</sup>	0.78	0.72 <sup>a</sup>	1.28	-0.36 <sup>a</sup>	1.79
6.0	9.64	3.32	-0.69 <sup>a</sup>	1.63	-0.45 <sup>a</sup>	0.45	-0.38 <sup>a</sup>	2.00	-1.06 <sup>a</sup>	2.55
$\Delta E$ (total difference)										
0.25	10.11	1.87	8.03 <sup>b</sup>	2.53	7.34 <sup>a</sup>	2.52	8.66	2.59	7.70 <sup>a</sup>	2.85
0.5	12.62	2.19	9.39 <sup>a</sup>	2.19	8.56 <sup>a</sup>	3.04	8.96 <sup>a</sup>	2.28	9.25 <sup>a</sup>	2.97
1.0	15.41	1.94	10.64 <sup>a</sup>	2.48	9.02 <sup>a</sup>	5.15	9.99 <sup>a</sup>	3.35	10.39 <sup>a</sup>	3.47
2.0	17.47	2.03	9.78 <sup>a</sup>	2.17	8.87 <sup>a</sup>	4.42	9.62 <sup>a</sup>	3.28	9.63 <sup>a</sup>	2.74
4.0	19.40	2.05	8.99 <sup>a</sup>	2.48	7.73 <sup>a</sup>	4.42	8.36 <sup>a</sup>	3.06	8.88 <sup>a</sup>	2.76
6.0	20.22	2.77	8.38 <sup>a</sup>	2.97	6.99 <sup>a</sup>	4.04	8.14 <sup>a</sup>	2.59	8.84 <sup>a</sup>	2.96

**Table 4. Mean  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$  and  $\Delta E$  and standard deviation values following the exposure of skin samples to 50–400 puffs of 3R4F cigarettes, glo sens THP, iSwitch Maxx EC or air as a control.**

Puffs	3R4F		glo sens		iSwitch Maxx		Air	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$\Delta L^*$ (lightness)								
50	-9.11	1.88	1.22 <sup>a</sup>	1.69	-0.15 <sup>a</sup>	1.79	1.36 <sup>a</sup>	2.42
100	-8.90	2.86	0.72 <sup>a</sup>	1.53	-0.04 <sup>a</sup>	1.96	1.52 <sup>a</sup>	1.39
200	-15.55	2.84	0.28 <sup>a</sup>	1.24	-0.86 <sup>a</sup>	2.07	1.75 <sup>a</sup>	1.67
400	-22.73	3.05	0.69 <sup>a</sup>	1.35	-2.11 <sup>a</sup>	2.74	2.11 <sup>a</sup>	2.66
$\Delta a^*$ (green-red)								
50	5.39	1.24	-0.15 <sup>a</sup>	0.49	-0.16 <sup>a</sup>	0.18	-0.15 <sup>a</sup>	0.29
100	4.48	1.69	-0.03 <sup>a</sup>	0.25	-0.04 <sup>a</sup>	0.20	-0.07 <sup>a</sup>	0.35
200	8.93	1.72	-0.09 <sup>a</sup>	0.27	-0.20 <sup>a</sup>	0.29	-0.14 <sup>a</sup>	0.35
400	11.50	1.39	-0.21 <sup>a</sup>	0.32	-0.49 <sup>a</sup>	0.36	-0.21 <sup>a</sup>	0.39
$\Delta b^*$ (blue-yellow)								
50	18.35	2.68	-0.65 <sup>a</sup>	0.68	-1.10 <sup>a</sup>	0.77	-0.66 <sup>a</sup>	0.96
100	17.90	3.18	-0.24 <sup>a</sup>	0.45	-0.15 <sup>a</sup>	0.76	-0.44 <sup>a</sup>	0.84
200	19.68	2.73	-0.88 <sup>a</sup>	1.11	-0.89 <sup>a</sup>	1.24	-0.93 <sup>a</sup>	0.95
400	14.53	3.92	-1.35 <sup>a</sup>	0.69	-1.64 <sup>a</sup>	1.20	-1.33 <sup>a</sup>	1.20
$\Delta E$ (total difference)								
50	18.04	1.50	1.89 <sup>a</sup>	1.37	1.94 <sup>a</sup>	1.11	2.32 <sup>a</sup>	2.03
100	21.57	3.01	1.38 <sup>a</sup>	1.11	1.77 <sup>a</sup>	1.12	2.19 <sup>a</sup>	1.93
200	26.86	2.44	1.62 <sup>a</sup>	1.02	2.26 <sup>a</sup>	1.51	2.62 <sup>a</sup>	1.90
400	29.68	2.31	1.99 <sup>a</sup>	0.87	3.44 <sup>a</sup>	2.13	3.23 <sup>a</sup>	1.79

<sup>a</sup> = Significantly different from 3R4F p < 0.0001

<sup>b</sup> = Significantly different from 3R4F p < 0.05

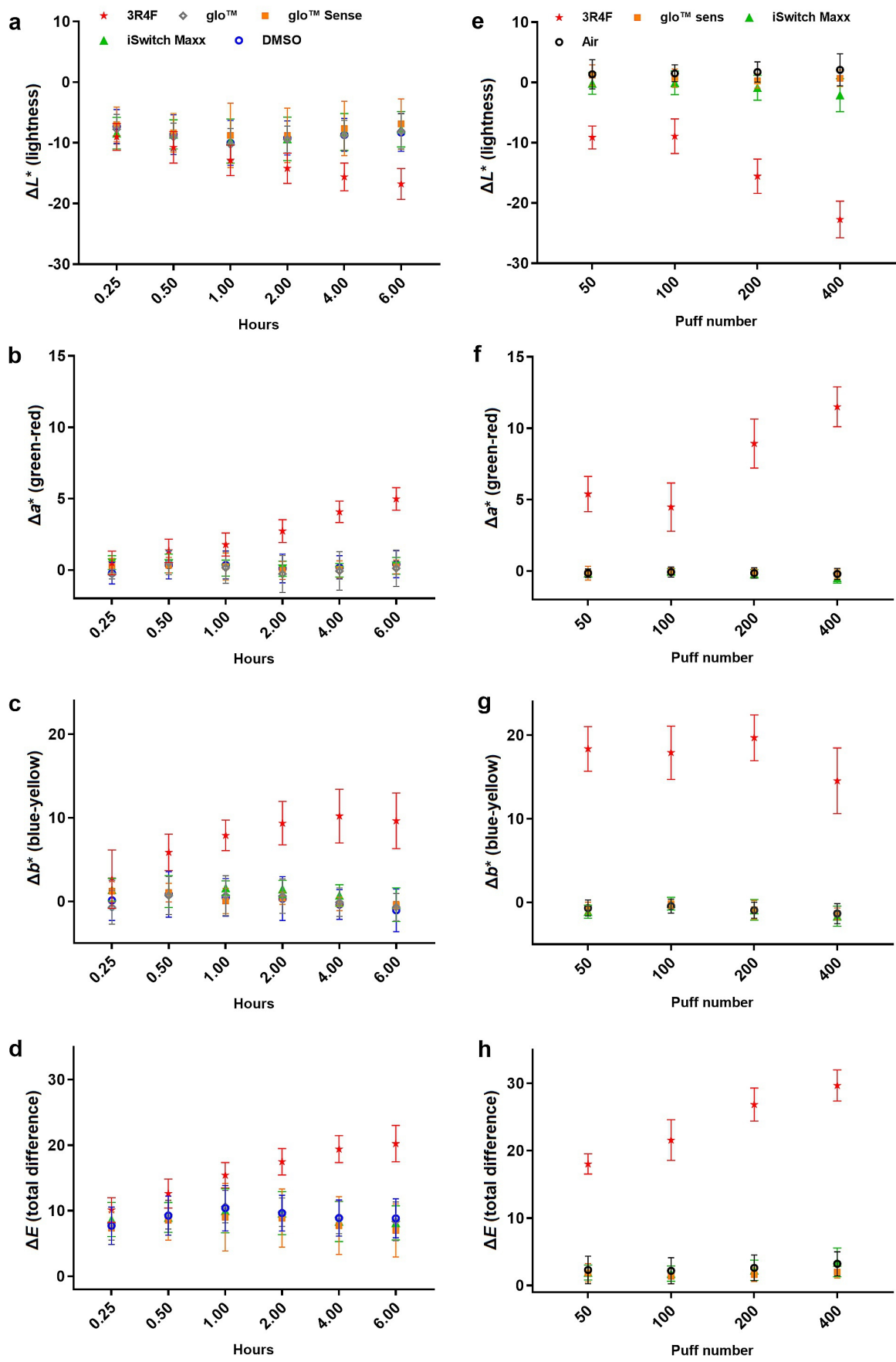


Figure 1. Changes in porcine skin sample colour following exposure to particulate matter or aerosol from cigarettes, tobacco heating products or e-cigarettes. Values are means and standard deviations.

Consensus is growing that THPs and ECs hold great potential for reducing the health risk associated with cigarette smoking (14–18). The aerosols produced by THPs and ECs differ greatly from CS, and studies have confirmed they contain significantly less toxicants (1, 2, 4, 11–13). In addition to risk reductions, there could be hygiene and/or social consideration benefits for smokers who switch to THPs and ECs, which seem to be of importance to consumers. A recent survey of Japanese THP consumers highlighted social consideration and hygiene as motivations for switching from smoking to using THPs. Consumers also believed that THPs are less harmful to people around them and have reduced odour (23).

Numerous countries now restrict smoking indoors. Before these bans, the impact of CS could be easily visualised as yellow or brown staining on surfaces and a characteristic odour left on hair, clothing and furnishing fabrics. Staining and odour are due to exhaled and side-stream CS produced as a cigarette burns between puffs. CS is composed of two phases, the particulate, also known as “tar”, and the vapor phase (3, 4). The particulate colour is thought to come from the burning of the tobacco in the cigarette, which then deposit on surfaces resulting in yellowing or brown staining (5–10). Unlike a burning cigarette, THPs and ECs release an aerosol only when consumers inhale on the product, this lack of side-stream aerosol might reduce staining of surfaces such as furnishing fabric and wallpaper (6) and also the staining of consumers’ hands. THP and EC reduced staining levels are also possibly due to the fact that THP devices heat rather than burn the tobacco contained in the consumable and that the majority of EC e-liquids do not contain tobacco.

In this study, the accelerated staining methods developed for enamel, wallpaper and cotton samples (6, 9) were adapted to enable the exposure of porcine skin samples. Porcine skin was selected, as samples are routinely used for *in vitro* testing due to structural and functional similarities to human skin (24, 25). Two exposure methods were used – submerging in PM extracts and exposure to aerosol. The capture of the particulate fraction of CS is widely used to assess tobacco products *in vitro* and also THP and EC products (20, 21, 26). In this study, the contributions of CS to skin darkening and discoloration was confirmed by PM exposure and indicated a time-related effect. Likewise, aerosol studies, which are more aligned to consumer exposure, showed dose-related increases in skin sample darkening and discoloration with CS. Limited staining or discoloration was observed following exposure to the THP and EC PM or aerosol. In the current aerosol study, a dose response was not observed for 3R4F  $\Delta b^*$  values, but was observed for 3R4F  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta E$  values. Increasing  $\Delta b^*$  values with 3R4F dose was observed for the PM study and also in previous aerosol studies (6, 9), differences could be due to the surface of the skin which is not as uniform as enamel, wallpaper or cotton.

A limitation of this study is that the experimental method delivers mainstream, but not side-stream CS and the ECs were operated at the highest power during aerosol collection, which might have over-represented THP and EC

exposure and under-represented CS exposure. Nevertheless, clear and significant differences seen with mainstream CS suggest that staining levels would also differ with side-stream CS.

The data produced in this study support published findings that detail yellowing of the skin, fingernails and facial hair by CS (10). Although we assessed short-term exposures, studies looking at long-term CS exposure have proposed that prominent wrinkles, gauntness and a grey colour to the facial skin are due to CS. Twin studies in which one twin is a smoker and the other a non-smoker also highlight CS-induced changes to the skin (10, 27, 28). Long-term switching studies would enable a further understanding of long-term effects of THP and EC on skin structure and allow investigation of whether the effects of CS exposure are reversible.

## CONCLUSIONS

We describe a novel method developed to assess skin sample staining by CS, THP or EC aerosols. CS exposure significantly increased the level of skin sample staining in a dose-dependent manner, whereas the THP and EC aerosol exposure resulted in minimal staining. These data suggest that THPs and ECs may have hygiene benefits for consumers who switch to exclusive use of these products. Further studies are required to assess the long-term impact on skin of consumers who switch from smoking to using ECs or THPs.

## AUTHOR CONTRIBUTIONS

Annette Dalrymple, Emma-Jayne Bean, Jesse Thissen, Steven Coburn and James Murphy designed the studies, approved all the data and prepared the manuscript. Annette Dalrymple and Emma-Jayne Bean produced the particulate matter fractions and Jesse Thissen performed the statistical analysis. Holger Behrsing managed the studies at IIVS. All authors approved the final manuscript.

## ACKNOWLEDGMENTS

The authors acknowledge the following BAT employees: Damien Breheny, Simone Santopietro, Keiann Williams, Ana Boriesa and Joseph Brehm for particulate matter generation. We also thank the following IIVS employees: Pooja Desai, Nicholas Castro and Adam Wahab for experimental work.

## CONFLICTS OF INTEREST

This study was funded by British American Tobacco (BAT) R&D, Southampton, UK. Experimental work was performed at BAT R&D and Institute for In Vitro Sciences (IIVS), Gaithersburg, MD 20878, USA. All authors are employees of BAT or IIVS.

## REFERENCES

- Schaller, J.-P., D. Keller, L. Poget, P. Pratte, E. Kaelin, D. McHugh, G. Cudazzo, D. Smart, A.R. Tricker, L. Gautier, M. Yerly, R. Reis Pires, S. Le Bouhellec, D. Ghosh, I. Hofer, E. Garcia, P. Vanscheeuwijck, and S. Maeder: Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical Composition, Genotoxicity, Cytotoxicity, and Physical Properties of the Aerosol; *Regul. Toxicol. Pharmacol.* 81, Suppl. 2 (2016) S27–S47. DOI: 10.1016/j.yrtph.2016.10.001
- Forster, M., S. Fiebelkorn, C. Yurteri, D. Mariner, C. Liu, C. Wright, K. McAdam, J. Murphy, and C. Proctor: Assessment of Novel Tobacco Heating Product THP1.0. Part 3: Comprehensive Chemical Characterisation of Harmful and Potentially Harmful Aerosol Emissions; *Regul. Toxicol. Pharmacol.* 93 (2018) 14–33. DOI: 10.1016/j.yrtph.2017.10.006
- Baker, R.R. and C.J. Proctor: The Origins and Properties of Environmental Tobacco Smoke; *Environ. Int.* 16 (1990) 231–245. DOI: 10.1016/0160-4120(90)90117-O
- Rodgman, A. and T.A. Perfetti: *The Chemical Components of Tobacco and Tobacco Smoke*, 2<sup>nd</sup> Edition; CRC Press, Boca Raton, FL, USA, 2013.
- Noguchi, M., S. Tanaka, K. Watanabe, and A. Yamasaki: Correlation Between Odor Concentration and Volatile Organic Compounds (VOC) Composition of Environmental Tobacco Smoke (ETS); *Int. J. Environ. Res. Public Health* 13 (2016) 994. DOI: 10.3390/ijerph13100994
- Dalrymple, A., T.C. Badrock, A. Terry, E.-J. Bean, M. Barber, P.J. Hall, S. Coburn, J. McAughey, and J. Murphy: Development of a Novel Method to Measure Material Surface Staining by Cigarette, E-Cigarette or Tobacco Heating Product Aerosols; *Heliyon* 6 (2020) e05012. DOI: 10.1016/j.heliyon.2020.e05012
- Alandia-Roman, C.C., D.R. Cruvinel, A.B.S. Sousa, F.C.P. Pires-de-Souza, and H. Panzeri: Effect of Cigarette Smoke on Color Stability and Surface Roughness of Dental Composites; *J. Dent.* 41 (2013) e73–e79. DOI: 10.1016/j.jdent.2012.12.004
- Bergström, J.: Tobacco Smoking and Chronic Destructive Periodontal Disease; *Odontology* 92 (2004) 1–8. DOI: 10.1007/s10266-004-0043-4
- Dalrymple, A., T.C. Badrock, A. Terry, M. Barber, P.J. Hall, D. Thorne, M.D. Gaca, S. Coburn, and C. Proctor: Assessment of Enamel Discoloration *In Vitro* Following Exposure to Cigarette Smoke and Emissions from Novel Vapor and Tobacco Heating Products; *Am. J. Dent.* 31 (2018) 227–233.
- Ortiz, A. and S.A. Grando: Smoking and the Skin; *Int. J. Dermatol.* 51 (2012) 250–262. DOI: 10.1111/j.1365-4632.2011.05205.x
- Mallock, N., L. Böss, R. Burk, M. Danziger, T. Welsch, H. Hahn, H.L. Trieu, J. Hahn, E. Pieper, F. Henkler-Stephani, C. Hutzler, and A. Luch: Levels of Selected Analytes in the Emissions of “Heat Not Burn” Tobacco Products that are Relevant to Assess Human Health Risks; *Arch. Toxicol.* 92 (2018) 2145–2149. DOI: 10.1007/s00204-018-2215-y
- Margham, J., K. McAdam, M. Forster, C. Liu, C. Wright, D. Mariner, and C. Proctor: Chemical Composition of an E-Cigarette Aerosol - A Quantitative Comparison with Cigarette Smoke; *Chem. Res. Toxicol.* 29 (2016) 1662–1678. DOI: 10.1021/acs.chemrestox.6b00188
- Tayyarah, R. and G.A. Long: Comparison of Select Analytes in Aerosol from E-Cigarettes with Smoke from Conventional Cigarettes and with Ambient Air; *Regul. Toxicol. Pharmacol.* 70 (2014) 704–710. DOI: 10.1016/j.yrtph.2014.10.010
- Committees on Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COT, COC and COM): Statement on the Toxicological Evaluation of Novel Heat-Not-Burn Tobacco Products; 2017. Available at [https://cot.food.gov.uk/sites/default/files/heat\\_not\\_burn\\_tobacco\\_statement.pdf](https://cot.food.gov.uk/sites/default/files/heat_not_burn_tobacco_statement.pdf) (accessed October 19, 2020)
- Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT): Statement on the Potential Toxicological Risks from Electronic Nicotine (and Non-Nicotine) Delivery Systems (E(N)NDS - E-Cigarettes); 2020. Available at <https://cot.food.gov.uk/sites/default/files/2020-09/COT%20E%28N%29NDS%20statement%202020-04.pdf> (accessed September 17, 2020)
- Food and Drug Administration (FDA): FDA Permits Sale of IQOS Tobacco Heating System Through Premarket Tobacco Product Application Pathway; 2019. Available at <https://www.fda.gov/news-events/press-announcements/fda-permits-sale-iqos-tobacco-heating-system-through-premarket-tobacco-product-application-pathway> (accessed October 19, 2020)
- McNeill, A., L.S. Brose, R. Calder, S.C. Hitchman, P. Hajek, and H. McRobbie: E-Cigarettes: An Evidence Update. A Report Commissioned by Public Health England; 2015. Available at [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/457102/Ecigarettes\\_an\\_evidence\\_update\\_A\\_report\\_commissioned\\_by\\_Public\\_Health\\_England\\_FINAL.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/457102/Ecigarettes_an_evidence_update_A_report_commissioned_by_Public_Health_England_FINAL.pdf) (accessed October 19, 2020)
- McNeill, A., L.S. Brose, R. Calder, L. Bauld, and D. Robson: Evidence Review of E-Cigarettes and Heated Tobacco Products. A Report Commissioned by Public Health England; 2018. Available at [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/684963/Evidence\\_review\\_of\\_e-cigarettes\\_and\\_heated\\_tobacco\\_products\\_2018.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/684963/Evidence_review_of_e-cigarettes_and_heated_tobacco_products_2018.pdf) (accessed October 19, 2020)
- International Organization for Standardization (ISO): ISO 3402:1999 – Tobacco and Tobacco Products – Atmosphere for Conditioning and Testing; 1999. Available at <https://www.iso.org/standard/28324.html> (accessed October 19, 2020)
- Combes, R., K. Scott, I. Crooks, D. Dillon, C. Meredith, K. McAdam, and C. Proctor: The *In Vitro* Cytotoxicity and Genotoxicity of Cigarette Smoke Particulate Matter with Reduced Toxicant Yields; *Toxicol. In Vitro* 27 (2013) 1533–1541. DOI: 10.1016/j.tiv.2013.03.005
- Thorne, D., I. Crooks, M. Hollings, A. Seymour, C. Meredith, and M. Gaca: The Mutagenic Assessment of



- an Electronic-Cigarette and Reference Cigarette Smoke Using the Ames Assay in Strains TA98 and TA100; *Mutat. Res.* 812 (2016) 29–38.  
DOI: 10.1016/j.mrgentox.2016.10.005
22. de Souza Araujo Wasilewski, M., M.K. Takahashi, G.A. Kirsten, and E.M. de Souza: Effect of Cigarette Smoke and Whiskey on the Color Stability of Dental Composites; *Am. J. Dent.* 23 (2010) 4–8.
23. Adamson, J., C. Kanitscheider, K. Prasad, O.M. Camacho, E. Beyerlein, Y.K. Bhagavan, C. Proctor, and J. Murphy: Results from a 2018 Cross-Sectional Survey in Tokyo, Osaka and Sendai to Assess Tobacco and Nicotine Product Usage after the Introduction of Heated Tobacco Products (HTPs) in Japan; *Harm. Reduct. J.* 17 (2020) 32.  
DOI: 10.1186/s12954-020-00374-3
24. Summerfield, A., F. Meurens, and M.E. Ricklin: The Immunology of the Porcine Skin and its Value as a Model for Human Skin; *Mol. Immunol.* 66 (2015) 14–21. DOI: 10.1016/j.molimm.2014.10.023
25. Zatz, J.L.: *Scratching the Surface: Rationale and Approaches to Skin Permeation*; in: *Skin Permeation Fundamentals and Application*, edited by J. Zatz, Allured Publishing Corporation, Wheaton, IL, USA, 1993, pp. 11–31.
26. Thorne, D., D. Breheny, C. Proctor, and M. Gaca: Assessment of Novel Tobacco Heating Product THP1.0. Part 7: Comparative *In Vitro* Toxicological Evaluation; *Regul. Toxicol. Pharmacol.* 93 (2018) 71–83. DOI: 10.1016/j.yrtph.2017.08.017
27. Prieux, R., M. Eeman, B. Rothen-Rutishauser, and G. Valacchi: Mimicking Cigarette Smoke Exposure to Assess Cutaneous Toxicity; *Toxicol. In Vitro* 62 (2020) 104664. DOI: 10.1016/j.tiv.2019.104664
28. Soeur, J., J.-P. Belaïdi, C. Chollet, L. Denat, A. Dimitrov, C. Jones, P. Perez, M. Zanini, O. Zobiri, S. Mezzache, D. Erdmann, G. Lereaux, J. Eilstein, and L. Marrot: Photo-Pollution Stress in Skin: Traces of Pollutants (PAH and Particulate Matter) Impair Redox Homeostasis in Keratinocytes Exposed to UVA1; *J. Dermatol. Sci.* 86 (2017) 162–169.  
DOI: 10.1016/j.jdermsci.2017.01.007
29. Health Canada: Determination of “Tar”, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke; 1999. Available at <https://healthycanadians.gc.ca/en/open-information/tobacco/t100/nicotine> (accessed October 19, 2020)
30. Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA): CORESTA Recommended Method No. 81. Routine Analytical Machine for E-Cigarette Aerosol Generation and Collection – Definitions and Standard Conditions; 2015. Available at [https://www.coresta.org/sites/default/files/technical\\_documents/main/CRM\\_81.pdf](https://www.coresta.org/sites/default/files/technical_documents/main/CRM_81.pdf) (accessed October 19, 2020)

*Corresponding author:*

*Annette Dalrymple*  
*British American Tobacco, R&D*  
*Southampton, SO15 8TL, UK*  
*Tel: +44 (0) 2380 58 88766*  
*E-mail: annette\_dalrymple@bat.com*