



Research article

Development of a novel method to measure material surface staining by cigarette, e-cigarette or tobacco heating product aerosols

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ABSTRACT

Tobacco smoke (CS) may visually stain indoor surfaces including ceilings, walls and soft furnishings over time. Potentially reduced risk products (PRRPs) such as e-cigarettes (EC) and tobacco heating products (THP) produce chemically less complex aerosols with significantly reduced levels of toxicants, particles and odour. However, the potential effects of EC and THP aerosols on the staining of indoor surfaces are currently unknown. In this study, an exposure chamber was developed as a model system to enable the accelerated staining of wallpaper and cotton samples by a scientific reference cigarette (3R4F), three THP (gloTM, gloTM pro, gloTM sens) and an e-cigarette (iSwitch Maxx).

Exposure to 3R4F reference cigarettes caused the greatest level of staining, which was significantly higher than gloTM, gloTM pro, gloTM sens or iSwitch Maxx aerosols, all of which showed relatively little colour change. Exposure to 200–1000 puffs of 3R4F cigarette smoke resulted in a visible dose response effect to wallpaper and cotton samples which was not observed following exposure to gloTM, gloTM pro, gloTM sens or iSwitch Maxx aerosols. Aging of the samples for 4 weeks post-exposure resulted in changes to the staining levels, however PRRP staining levels were minimal and significantly lower than 3R4F exposed samples.

For the first time, diverse PRRPs across the tobacco and nicotine products risk continuum have been assessed *in vitro* for their impact on surface staining. CS exposure significantly increased the level of wallpaper and cotton staining, whereas exposure to gloTM, gloTM pro, gloTM sens or iSwitch Maxx aerosols resulted in significantly reduced levels of staining, staining levels were also comparable to untreated control samples.

1. Introduction

There has been significant global growth in the use of alternative consumer inhalation products for nicotine. Electronic cigarettes (EC) and more recently, tobacco heating products (THP) which heat rather than burn tobacco, are becoming more prevalent, but their geographical availability varies, depending on legislation. ECs are relatively simple devices, consisting of a battery, a microprocessor and an e-liquid tank which wicks e-liquid to a heating coil (Ayers et al., 2011; Etter et al., 2011; Pepper and Brewer, 2014; Sood et al., 2018; Papaefstathiou et al., 2019). Today ECs are available in a variety of geometries and battery power, as well as differing resistances for the heating coils. Liquids may be supplied in proprietary pod or cartridge systems or by refilling built-in tanks. Devices are also now available where the traditional heating coil is

replaced with technology that reduces potential over-heating (<http://www.govype.com/>). E-liquids in general consist of propylene glycol, vegetable glycerol, water and flavours, and can be purchased with or without nicotine.

Heat not-burn/THP are a newer addition to the market and are not, as yet, as broadly available as ECs. THP devices function by heating a tobacco consumable/stick to a temperature lower than 350 °C, significantly less than conventional cigarettes which can burn up to 950 °C and may smoulder between puffs at up to 650 °C (Baker and Proctor, 1990). Heating tobacco vaporizes the more volatile compounds, including nicotine, into an inhalable aerosol, but does not burn and pyrolyse the tobacco as in a conventional cigarette (Schaller et al., 2016; Forster et al., 2018).

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The burning of tobacco in a cigarette releases over 7,000 chemicals, including a number of known toxicants (Perfetti and Rodgman, 2013) whereas the heating of a THP tobacco consumable/stick at lower temperatures releases significantly less toxicants (Schaller et al., 2016; Forster et al., 2018; Mallock et al., 2018). E-liquids do not usually contain tobacco; studies have also confirmed that the heating of an e-liquid within an EC device also produces significantly fewer toxicants than CS (Tayyarah and Long, 2014; Margham et al., 2016). A lit cigarette produces two types of smoke: main-stream smoke, which is inhaled directly by consumers and portions exhaled to the environment, and side-stream smoke, which is produced as the cigarette smoulders between puffing. When used indoors, both main-stream and side-stream CS can contribute to surface staining, room environmental tobacco smoke (ETS) or odour (Noguchi et al., 2016).

The level of surface staining within a room could be influenced by room size, ventilation and the number of cigarettes smoked per day. In the laboratory, exposure conditions can easily be controlled, and surface staining assessed using exposure chambers. Exposure chambers can vary in size, the benefit of using a small chamber rather than a large chamber or room (Liu et al., 2017; Cancelada et al., 2019; Marcham et al., 2019) is the scope for accelerated staining to be assessed. In this study, a small-scale exposure chamber was developed to enable wallpaper and cotton samples to be exposed to main-stream aerosols by diffusion as would occur within a home or commercial premise.

CS deposited on indoor surfaces has been demonstrated to contain nicotine, polycyclic aromatic hydrocarbons (PAHs), and tobacco-specific nitrosamines (TSNAs) (Schick et al., 2014; Matt et al., 2016, 2019). ECs and THP do not produce side-stream aerosols and do not smoulder as a cigarette does. Furthermore, aerosols are only released after puffing on the THP consumable/stick or EC device mouthpiece by the consumer. Some EC also have buttons that are required to be pressed to release the aerosol. EC and THP use indoors could result in less surface staining and environmental exposure to non-consumers.

A number of published studies have highlighted potential health risks of ETS exposure (US EPA, 1992; IARC, 2004; California EPA, 2005). The health impact of exposure to environmental EC or THP aerosols are currently unknown; however, several studies have demonstrated improved air quality and a reduction of certain toxicants relative to conventional cigarettes (McAuley et al., 2012; Burstyn, 2014; O'Connell et al., 2015; Mitova et al., 2016; Liu et al., 2017; Ruprecht et al., 2017; Forster et al., 2018; Cancelada et al., 2019; Meišutović-Akhtarjeva et al., 2019).

The long-term health effects of EC and THP use are not currently known; however, there is a growing consensus that EC hold great potential for reducing the risk associated with cigarette smoking (McNeill et al., 2015, 2018) and should be promoted as smoking substitutes (Royal College of Physicians, 2016). There is less scientific data available for THP; however, since they produce aerosols with reduced levels of toxicants (Mitova et al., 2016; Ruprecht et al., 2017; Forster et al., 2018), the same consensus could be applied. Indeed, the Committees on Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and The Environment in 2017 (Committees on Toxicity, 2017) reviewed available THP data and concluded: "compared to conventional cigarette smoke, it is likely that there is a reduction in risk, though not to zero, to health for smokers who switch completely to heat-not-burn tobacco products".

In 2013, McNeill and Munafò (2013) placed tobacco and nicotine products on a risk continuum based on product toxicant emissions. Recent publications have added EC and THP to the risk continuum based on product toxicant emissions (Lowe et al., 2015), pre-clinical (Iskandar et al., 2017; Taylor et al., 2017, 2018; Jaunky et al., 2018; Thorne et al., 2018a, 2018b) and clinical data (Cravo et al., 2016; D'Ruiz et al., 2016; Martin et al., 2016; Haziza et al., 2016; Shahab et al., 2017; Gale et al., 2018). Long term clinical studies, where smokers switch solely to PRRPs for a number of months (Newland et al., 2019), will enable PRRPs to be placed more accurately on the risk continuum.

Cigarette smoke is known to stain consumers' teeth and surfaces in their homes (Bergström, 2004; Alandia-Roman et al., 2012). A method was recently published that quantified the level of enamel sample staining *in vitro* following exposure to CS, EC and THP aerosols (Dalrymple et al., 2018). In the current study, the method developed for enamel sample staining was modified to enable the accelerated staining of cotton and wallpaper samples to be assessed following exposure to emissions from 3R4F scientific reference cigarettes and PRRPs across the risk continuum; glo™, glo™ pro, glo™ sens and iSwitch Maxx.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were obtained from Sigma-Aldrich (Gillingham, UK) unless otherwise stated.

2.2. Test articles

All products used in this study are detailed in Figure 1 and Table 1. Prior to use, 3R4F cigarettes and THP Neostiks™ were conditioned for a minimum of 48 h and a maximum of 10 days for 3R4F or 5 days for THP Neostiks™. Conditioning conditions were 22 ± 1 °C and $60 \pm 3\%$ relative humidity, according to International Organization for Standardization 3402 (International Organization for Standardization, 1999). The e-liquid cartridges and tobacco pods were stored at room temperature prior to use. All devices were fully charged before use. iSwitch Maxx was tested at the highest power level and glo™ pro was tested using the device's boost function.

2.3. Wallpaper and cotton sample preparation

Wallpaper was purchased from www.ilovewallpaper.co.uk/ (Part number: ILWLINE). White Cotton drill (Part number: 438) was purchased from Cotton Mill, Lancashire, UK. Four wallpaper and cotton samples (2 × 2 cm) were prepared per dose. Prior to exposure, baseline colour values of wallpaper and cotton samples were calculated as detailed below. To represent the exposure of walls and soft furnishing by aerosol diffusion within a room, wallpaper and cotton samples were attached to the vertical sides of a novel exposure chamber (Figure 1) using Pritt Multi Tack (WW-29772841, Staples, UK). The exposure chamber (Curbridge Engineering Limited, Southampton, UK) is a modified version of the BAT designed exposure chamber (Patent publication number WO 03/100417 A1) where the central cylinder is extended in length from 9.6 to 21.5 cm, thus increasing the internal volume from 196 mL to 885 mL. This approach was adopted as there was already data available on the settling characteristics of aerosol in the smaller chamber (Adamson et al., 2012).

2.4. Smoke/aerosol exposure

The exposure method used is described in full in Dalrymple et al. (2018). Briefly, 3R4F reference cigarettes, THP or EC aerosols were generated using LM20X or LM20E linear engines (Borgwaldt-KC, Hamburg, Germany). Specific puffing regimes were used for each product as detailed in Table 2. The machines were adapted to enable 5 ports to deliver 5 puffs co-currently to the exposure chamber. This set up enabled 275 mL of smoke/aerosol to be delivered to each chamber every 30 s.

The wallpaper and cotton samples (20 of each) were exposed to 200, 400, 600, 800 or 1000 puffs of undiluted CS, THP or EC aerosols. A minimum of 3 independent experiments were performed per product or dose. After delivery of 50 puffs to the chamber, a settling time of 5 min was included to allow for aerosol deposition by diffusion and sedimentation within the chamber. Following exposure to 200-puffs, four wallpaper and cotton samples were removed from the exposure chamber and staining levels measured as detailed below. The chamber was resealed, and remaining samples were exposed to an additional 200 puffs. Four

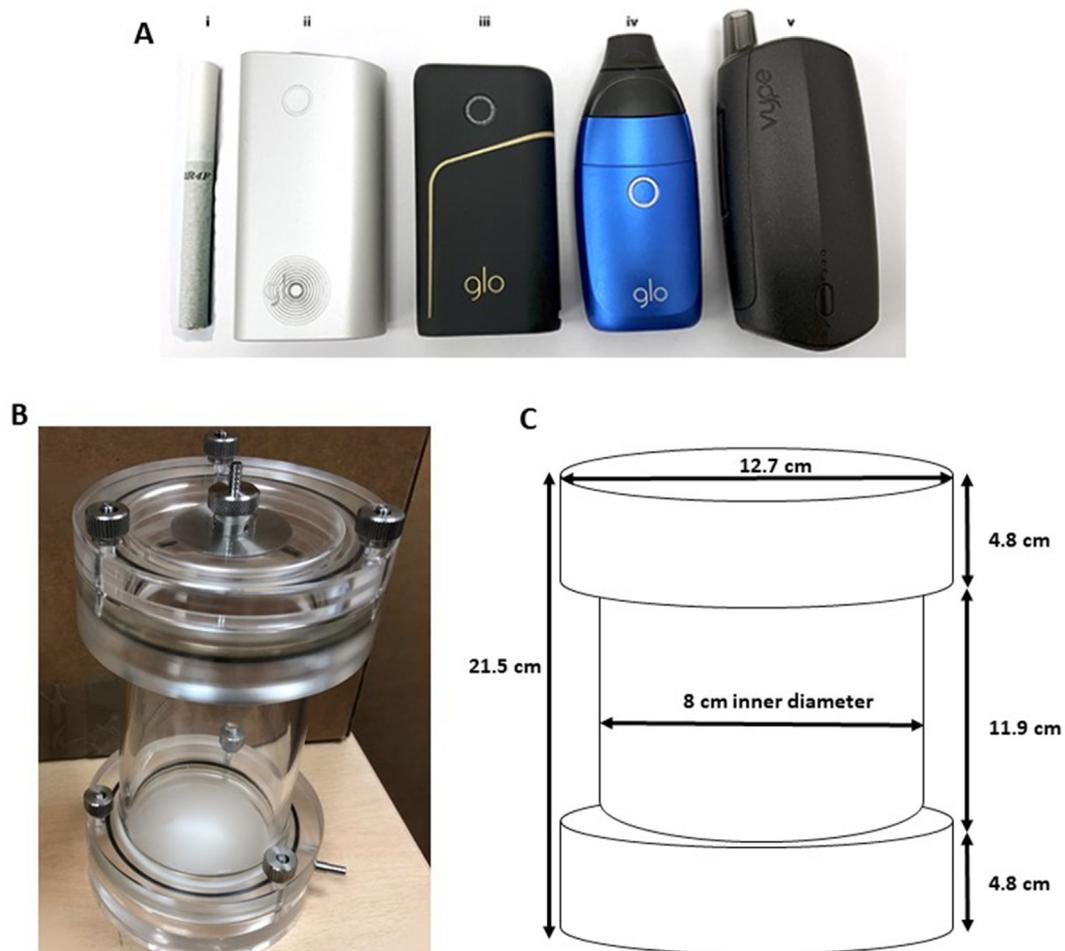


Figure 1. Products and aerosol exposure chamber. A) Products used for sample exposure, i: 3R4F reference cigarette (<https://ctrp.uky.edu/>), ii-iv: glo™, glo™ pro and glo™ sens, BAT's commercial tobacco heating products, v: iSwitch Maxx, BAT commercial electronic cigarette. B) The aerosol exposure chamber (Patent publication number WO 03/100417 A1) was modified to contain an additional 11.9 cm central cylinder section to enable wallpaper and cotton samples to be attached. C) Schematic of the modified chamber.

Table 1. Product assessed for wallpaper and cotton staining.

Product	BAT device Code	Source	Consumable	E-liquid Nicotine mg/ml	Puffs per product/cartridge	Puffs per tobacco pod
3R4F	N/A	UoK*	N/A	N/A	10	N/A
glo™	THP1.0	BAT	Bright Tobacco Neostiks™	N/A	8	N/A
glo™ pro	THPEX0.0.BF3	BAT	Rich Tobacco Neostiks™	N/A	7	N/A
glo™ sens	IFU2.0	BAT	Mixed Fruit	0	150	50
iSwitch Maxx	ISMD1.020W	BAT	Virginia Tobacco	5	80	N/A

* Center for Tobacco Reference Products, University of Kentucky, Lexington, USA (<https://ctrp.uky.edu/>).

Table 2. Product puffing regimes.

Product	Regime	Puff Vol (mL)	Puff duration (sec)	Intensity (sec)	Vent blocking	Puff profile
3R4F	HCI	55	2	30	100%	Bell
glo™	HCI ^m	55	2	30	No	Bell
glo™ pro	HCI ^m	55	2	30	No	Bell
glo™ sens	CRM81	55	3	30	No	Square
iSwitch Maxx	CRM81	55	3	30	No	Square

HCI = Health Canada Intense (Health Canada Official Method T-115, 1999).

HCI^m = HCI modified (no vent blocking).

CRM81 = CORESTA recommended method No 81 (CORESTA, No. 81, 2015).

samples were then removed, staining levels were measured, and this process continued until the remaining four samples were exposed to 1000 puffs.

2.5. Colour measurements

Prior to exposure and after 200, 400, 600, 800 and 1000 puffs, colour readings (L*, a*, b*) were measured at 4 orientations on each wallpaper and cotton sample, using a Konica Minolta CM-700d Spectrophotometer (Konica Minolta Sensing Europe B.V., Nieuwegein, Netherlands). L* is a measure of the lightness, black has a L* value of 0 and white has a value of 100, a* is the green-red axis, with green in the negative direction and red in the positive direction and b* blue-yellow axis with blue in the negative direction and yellow in the positive direction. ΔL* Δa* Δb* values are calculated by subtracting the baseline L* a* b* values from the L* a* b* values after treatment/exposure. A negative ΔL* value indicates that a sample has darkened after exposure, whereas a negative Δa* value indicates a green colour change and negative Δb* value indicates a blue colour change. A positive ΔL* value indicates that a sample has lightened after exposure, whereas a positive Δa* value indicates a red colour change and positive Δb* value indicates a yellow colour change.

Data were captured using a ColourCalc Excel data capture spreadsheet (Chameleon colour services, UK). The CM-700d was calibrated daily using a white reference tile and a 3-mm aperture in SCI mode. To determine if staining changed over time, samples from the tobacco containing products (all except iSwitch Maxx) were also measured after 4 weeks' storage in the dark. The ΔE value, the change in colour space

before and after a treatment was determined in Excel by using the following equation:

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

2.6. Statistical methods

A one-way Analysis of Variance (ANOVA) was used to assess the differences in ΔL*, Δa*, Δb* and ΔE values between the products. Where residuals appear to be non-normally distributed, pairwise comparisons are carried out through Mann-Whitney U tests and T-tests. Values are compared at a significance level (α) of 0.05. Post-hoc Tukey adjustment for pairwise comparisons was also used.

3. Results

Exposure to 3R4F CS resulted in wallpaper sample staining; dose dependent changes were also observed. After exposure of samples to 200 puffs of 3R4F CS, ΔL*, Δa*, Δb* and ΔE values were significantly different (p < 0.05) to all THP and EC values (Table 3, Figure 2). The ΔL* Δa* Δb* values were calculated by subtracting exposure sample baseline L* a* b* values from samples L* a* b* values following exposure to 200, 400, 800 and 1000 puffs of product aerosol. A negative ΔL* value indicates that a sample darkened after exposure, whereas a negative Δa* value indicates the sample had a green colour and negative Δb* value a blue colour. A positive ΔL* value indicates that the sample lightened, a positive Δa*

Table 3. Mean ΔL*, Δa*, Δb* and ΔE values following exposure of wallpaper samples to product aerosols. Mean ΔL*, Δa*, Δb* and ΔE mean and standard deviation values following the exposure of wallpaper samples to 200–1000 puffs of 3R4F, glo™, glo™ pro, glo™ sens or iSwitch Maxx aerosol. Wallpaper samples exposed to 1000 puffs of all tobacco containing products were also analysed 28 days post exposure.

Product	Puffs	200	400	600	800	1000	28 days [§]
ΔL*							
3R4F	Mean (SD)	-2.67 (0.99)	-5.47 (1.74)	-7.16 (2.04)	-9.14 (2.71)	-10.56 (2.99)	-10.51 (2.37)
glo™ [‡]	Mean (SD)	-0.23 (1.07) ^a	-0.85 (1.01) ^a	-0.99 (1.01) ^a	-0.74 (1.18) ^a	-0.44 (0.62) ^a	-0.63 (0.67) ^a
glo™ pro	Mean (SD)	0.05 (0.10) ^a	0.07 (0.06) ^a	0.15 (0.07) ^a	0.21 (0.05) ^a	0.21 (0.09) ^a	0.16 (0.08) ^a
glo™ sens	Mean (SD)	1.50 (0.99) ^a	1.28 (1.21) ^a	1.04 (1.38) ^a	2.14 (0.12) ^a	1.58 (1.15) ^a	-0.18 (0.21) ^a
iSwitch Maxx	Mean (SD)	-1.59 (1.17) ^a	-1.60 (1.20) ^a	-1.44 (1.42) ^a	-1.72 (1.27) ^a	-1.54 (1.19) ^a	-
Δa*							
3R4F	Mean (SD)	0.55 (0.24)	1.65 (0.64)	2.45 (0.74)	3.27 (1.06)	4.03 (1.28)	4.30 (1.12)
glo™ [‡]	Mean (SD)	0.09 (0.11) ^a	0.09 (0.13) ^a	0.07 (0.11) ^a	0.04 (0.14) ^a	-0.03 (0.11) ^a	0.07 (0.12) ^a
glo™ pro	Mean (SD)	-0.02 (0.01) ^a	-0.06 (0.01) ^a	-0.11 (0.02) ^a	-0.13 (0.03) ^a	-0.17 (0.02) ^a	-0.15 (0.00) ^a
glo™ sens	Mean (SD)	-0.38 (0.32) ^a	-0.38 (0.31) ^a	-0.37 (0.31) ^a	-0.46 (0.07) ^a	-0.40 (0.21) ^a	-0.01 (0.00) ^{a,b}
iSwitch Maxx	Mean (SD)	0.32 (0.26) ^a	0.29 (0.18) ^a	0.28 (0.21) ^a	0.29 (0.18) ^a	0.29 (0.18) ^a	-
Δb*							
3R4F	Mean (SD)	6.32 (1.86)	11.51 (2.50)	14.63 (2.71)	17.55 (2.97)	20.07 (3.11)	22.17 (2.87) ^b
glo™ [‡]	Mean (SD)	-0.19 (0.12) ^a	-0.34 (0.09) ^a	-0.08 (0.35) ^a	0.06 (0.52) ^a	-0.09 (0.46) ^a	0.84 (0.71) ^{a,b}
glo™ pro	Mean (SD)	-0.12 (0.04) ^a	-0.13 (0.06) ^a	-0.14 (0.04) ^a	-0.22 (0.06) ^a	-0.10 (0.07) ^a	0.59 (0.05) ^{a,b}
glo™ sens	Mean (SD)	-0.07 (0.46) ^a	-0.10 (0.23) ^a	-0.02 (0.35) ^a	0.08 (0.14) ^a	0.21 (0.21) ^a	0.43 (0.25) ^a
iSwitch Maxx	Mean (SD)	0.29 (0.36) ^a	0.14 (0.27) ^a	0.31 (0.27) ^a	0.08 (0.25) ^a	0.16 (0.27) ^a	-
ΔE							
3R4F	Mean (SD)	6.90 (2.08)	12.87 (3.03)	16.49 (3.37)	20.09 (3.99)	23.07 (4.30)	24.93 (3.72)
glo™ [‡]	Mean (SD)	0.91 (0.62) ^a	1.05 (0.87) ^a	1.24 (0.74) ^a	1.23 (0.79) ^a	0.80 (0.38) ^a	1.28 (0.61) ^{a,b}
glo™ pro	Mean (SD)	0.16 (0.05) ^a	0.18 (0.04) ^a	0.23 (0.06) ^a	0.34 (0.05) ^a	0.30 (0.07) ^a	0.63 (0.05) ^{a,b}
glo™ sens	Mean (SD)	1.67 (0.94) ^a	1.59 (0.89) ^a	1.62 (0.75) ^a	2.20 (0.11) ^a	1.90 (0.61) ^a	0.51 (0.14) ^{a,c}
iSwitch Maxx	Mean (SD)	1.69 (1.18) ^a	1.73 (1.10) ^a	1.85 (0.93) ^a	1.83 (1.18) ^a	1.67 (1.09) ^a	-

^a Significantly different from 3R4F (p < 0.05).

^b Significantly higher after storage (p < 0.05).

^c Significantly lower after storage (p < 0.05).

[‡] glo™ was assessed at 160, 480, 640, 800 and 1040 puffs.

[§] Samples exposed to 1000 puffs were reassessed 28 days after initial exposure.

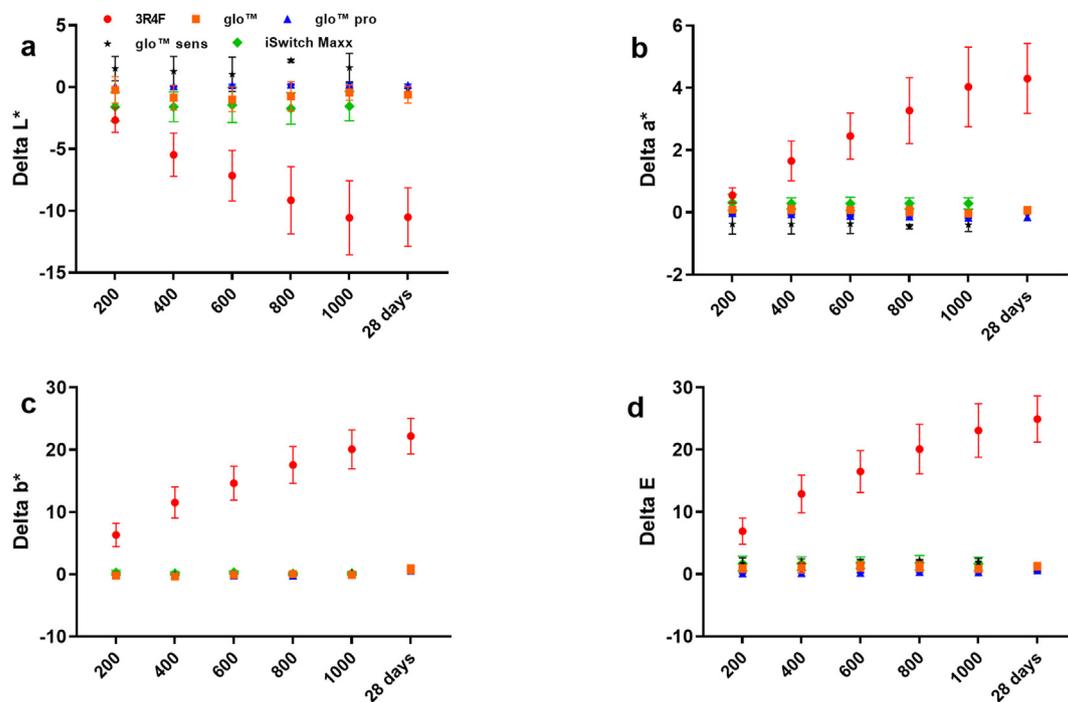


Figure 2. ΔL^* , Δa^* , Δb^* and ΔE values following exposure of wallpaper samples to product aerosols. ΔL^* , Δa^* , Δb^* and ΔE mean and standard deviation values following the exposure of wallpaper samples to 3R4F, glo[™], glo[™] pro, glo[™] sens or iSwitch Maxx. Graphs show the data obtained after 200–1000 puffs and following the storage of the 1000 puff exposed tobacco containing samples in the dark for 28 days. (a) ΔL^* (lightness), (b) Δa^* (green and red colour component), (c) Δb^* (blue and yellow colour components) and (d) ΔE (colour change) graphs.

value indicates the sample had a red colour and positive Δb^* value a yellowing of the sample.

3R4F exposure changed wallpaper sample ΔL^* , Δa^* , Δb^* and ΔE values in a dose dependent manner, demonstrating a direct correlation between puff number and sample discolouration. Following 3R4F exposure, ΔL^* values reduced signifying that samples darkened with CS exposure, values were also negative as baseline samples were lighter in colour. After 1000 puffs of EC and THP aerosols, the ΔL^* values of the wallpaper samples were minimal, values were also within the normal range of untreated samples. ΔL^* values also did not change in a dose dependent manner following exposure to 200–1000 puffs of EC and THP aerosols. The wallpaper sample Δa^* and Δb^* values were also significantly increased following 3R4F exposure, indicating a reddening and yellowing of the samples. The Δa^* and Δb^* values, following exposure to EC and THP aerosols, again were minimal and all values, apart from the Δa^* glo[™] pro values, did not change in a dose dependent manner. The Δa^* values for glo[™] pro decreased in a dose dependent manner indicating a green coloration, however values were -0.02 and -0.17 units at 200 puffs and 1000 puffs respectively. These values were significantly reduced compared to 3R4F, which were 0.55 and 4.03 units at 200 puffs and 1000 puffs respectively. The ΔE , total colour change, increased following 3R4F exposure in a dose dependent manner. The ΔE values following exposure to all EC and THP aerosols were significantly reduced compared to 3R4F values and did not increase in a dose dependent manner, the highest ΔE at 1000 puffs was 1.90 units for glo[™] sens, significantly less than 3R4F which had a ΔE value at 1000 puffs of 23.07 units. The colour of the wallpaper samples following 1000 puff exposure to all products and untreated control samples can be observed in Figure 3.

Exposure of cotton samples to 3R4F CS resulted in sample staining; exposure also changed most ΔL^* , Δa^* , Δb^* and ΔE values in a dose dependent manner demonstrating a direct correlation between puff number and sample discolouration. After exposure of cotton samples to 400–800 puffs of 3R4F CS, ΔL^* , Δa^* , Δb^* and ΔE values were

significantly different ($p < 0.05$) from all THP and EC values (Table 4, Figure 4). At 200 puffs, the ΔL^* values for all products, apart from iSwitch Maxx, were significantly different from the 3R4F value. After 400 puffs, ΔL^* values for all THP and EC were significantly different ($p < 0.05$) from 3R4F values (Table 4, Figure 4). The L^* values for 3R4F reduced with each dose signifying that samples darkened with CS exposure, values are also negative as baseline samples were lighter in colour. After 1000 puffs of the EC and THP aerosols, the ΔL^* values of the cotton samples were minimal, and values also did not change in a dose dependent manner, which would imply that the samples had not darkened following EC and THP aerosol exposure. The Δa^* values obtained for all products, including 3R4F, was minimal. In the case of 3R4F, exposure did decrease the Δa^* values and values were negative indicating a green colouration; however, changes were not dose related. The Δa^* values for 3R4F were significantly lower than the EC and THP values at 200–800 puffs and iSwitch Maxx value at 1000 puffs. The majority of Δa^* values for EC and THP aerosols were minimal and negative values recorded indicating a green colour. The Δa^* values for glo[™] and glo[™] pro did decrease in a dose dependent manner, however the values at 1000 puffs were -0.14 and -0.24 units respectively. The Δa^* values for iSwitch Maxx were positive, indicating a red colour, however values were similar at all doses and 0.24 units at 1000 puffs. The Δb^* values increased following 3R4F exposure, indicating a yellowing of the samples. The Δb^* values following exposure to EC and THP aerosols were again minimal and significantly lower than the 3R4F Δb^* values at all doses. Exposure to glo[™] and glo[™] pro aerosols did result in changes to Δb^* values in a dose dependent manner, however values were minimal, 0.81 and 0.92 units respectively at 1000 puffs and significantly less than the value for 3R4F which was 20.69 units at 1000 puffs. The ΔE , total colour change, increased following 3R4F exposure in a dose dependent manner and values were significantly different from EC and THP exposed samples at all doses. The level of staining following exposure to the EC and THP aerosols was minimal and all, apart from glo[™] or glo[™] pro did not increase in a dose dependent manner. However, the ΔE values for glo[™] and



Figure 3. Wallpaper staining following exposure to product aerosols. Wallpaper samples were exposed to 1000 puffs of aerosols generated, from top to bottom, 3R4F CS, glo™, glo™ pro, glo™ sens and iSwitch Maxx. Control samples are unexposed samples.

glo™ pro at 1000 puffs were 1.81 and 1.01 units respectively, significantly less than the 3R4F value which was 22.27 units.

This study also assessed the aging of samples exposed to 1000 puffs of tobacco containing products (Figures 2 and 4, Tables 3 and 4). Significant differences ($p < 0.05$) were seen with some PRRP following storage of samples, however the values obtained directly after exposure and following 28 days storage were all significantly less than recorded for 3R4F exposure and all ΔE values before and after storage were less than 2 units. The Δa^* value for glo™ sens increased at day 28, suggesting a reddening of the sample, however the change was by 0.4 units. At 28 days, Δb^* values for 3R4F, glo™ and glo™ pro exposed samples significantly increased indicating a yellowing of the wallpaper samples with aging. However, Δb^* values were 0.84 and 0.59 units for glo™ and glo™ pro respectively, significantly lower than the value of 22.17 units recorded for 3R4F. The ΔE values for glo™ and glo™ pro also increased after 28 days storage, with values of 1.28 and 0.63 units respectively, which were significantly lower than the 3R4F value of 24.93 units. The ΔE values for 3R4F also significantly increased at 28 days, whereas the value for glo™ sens significantly reduced. The wallpaper samples included in Figure 3 were photographed after 28 days storage in the dark and detail the comparability of PRRP exposed wallpaper to untreated control samples.

Significant differences ($p < 0.05$) were also recorded for PRRP exposed cloth samples following storage. However, the values obtained directly after exposure or following 28 days storage were significantly less than recorded for 3R4F exposure. In addition, all PRRP exposed cloth sample ΔE values before and after storage were less than 2 units. In the case of 3R4F exposed cloth samples, the ΔL^* value had significantly increased following storage, indicating lightening of the samples, however Δa^* , Δb^* and ΔE values were not significantly different from the values measured directly after exposure. In the case of PRRP exposure to cloth, storage significantly reduced the ΔL^* and ΔE values of glo™ sens exposed samples however the values were -0.58 and 0.71 units, significantly less than the values of -6.43 and 20.82 units for 3R4F. Following glo™ pro exposure and storage for 28 days significant changes were observed, Δa^* values decreased, whereas Δb^* and ΔE values increased indicating a reduction in red colour, yellowing of the samples and increased staining with storage, however values were significantly lower than values recorded with 3R4F exposure. The ΔE values for glo™ pro and 3R4F at 28 days were 1.80 and 20.82 units respectively. Following glo™ exposure and storage for 28 days the Δb^* value increased, indicating yellowing of samples, however the ΔE values before and after storage were comparable.

Table 4. Mean ΔL^* , Δa^* , Δb^* and ΔE values following cotton sample exposure to product aerosols. Mean ΔL^* , Δa^* , Δb^* and ΔE mean and standard deviation values following the exposure of cotton samples to 200–1000 puffs of 3R4F, gloTM, gloTM pro, gloTM sens or iSwitch Maxx aerosol. Cotton samples exposed to 1000 puffs of all tobacco containing products were also analysed 28 days post exposure.

Product	Puffs	200	400	600	800	1000	28 days [§]
ΔL^*							
3R4F	Mean (SD)	-1.53 (0.51)	-3.22 (1.06)	-4.73 (1.44)	-5.89 (1.60)	-8.09 (2.83)	-6.43 (1.65) ^b
glo TM [‡]	Mean (SD)	-0.42 (0.46) ^a	-1.23 (0.44) ^a	-1.04 (0.56) ^a	-1.03 (0.60) ^a	-1.46 (1.23) ^a	-0.70 (0.69) ^a
glo TM pro	Mean (SD)	-0.10 (0.13) ^a	-0.10 (0.12) ^a	-0.12 (0.11) ^a	-0.28 (0.15) ^a	-0.21 (0.27) ^a	-0.32 (0.20) ^a
glo TM sens	Mean (SD)	1.59 (1.00) ^a	1.48 (1.72) ^a	1.38 (1.73) ^a	2.37 (0.35) ^a	1.52 (1.55) ^a	-0.58 (0.23) ^{a,c}
iSwitch Maxx	Mean (SD)	-1.94 (1.21)	-1.84 (1.36) ^a	-1.57 (1.41) ^a	-1.72 (1.53) ^a	-1.59 (1.52) ^a	-
Δa^*							
3R4F	Mean (SD)	-0.74 (0.18)	-1.04 (0.15)	-1.05 (0.21)	-0.94 (0.31)	-0.38 (0.78)	-0.57 (0.51)
glo TM [‡]	Mean (SD)	-0.04 (0.02) ^a	-0.03 (0.10) ^a	-0.05 (0.12) ^a	-0.11 (0.13) ^a	-0.14 (0.16)	-0.19 (0.18) ^a
glo TM pro	Mean (SD)	-0.03 (0.01) ^a	-0.08 (0.03) ^a	-0.15 (0.04) ^a	-0.18 (0.03) ^a	-0.24 (0.03)	-0.39 (0.01) ^c
glo TM sens	Mean (SD)	-0.24 (0.21) ^a	-0.16 (0.16) ^a	-0.13 (0.15) ^a	-0.23 (0.07) ^a	-0.10 (0.16)	0.00 (0.04) ^a
iSwitch Maxx	Mean (SD)	0.28 (0.13) ^a	0.28 (0.12) ^a	0.27 (0.14) ^a	0.25 (0.16) ^a	0.24 (0.15) ^a	-
Δb^*							
3R4F	Mean (SD)	5.47 (1.46)	10.49 (2.62)	14.16 (3.43)	17.22 (3.06)	20.69 (4.08)	19.78 (3.48)
glo TM [‡]	Mean (SD)	0.21 (0.07) ^a	0.43 (0.11) ^a	0.52 (0.21) ^a	0.70 (0.25) ^a	0.81 (0.25) ^a	1.00 (0.67) ^{a,b}
glo TM pro	Mean (SD)	0.17 (0.05) ^a	0.37 (0.13) ^a	0.59 (0.15) ^a	0.72 (0.14) ^a	0.92 (0.11) ^a	1.72 (0.02) ^{a,b}
glo TM sens	Mean (SD)	-0.34 (0.30) ^a	-0.38 (0.31) ^a	-0.33 (0.29) ^a	-0.55 (0.13) ^a	-0.42 (0.26) ^a	0.41 (0.05) ^a
iSwitch Maxx	Mean (SD)	0.29 (0.26) ^a	0.27 (0.30) ^a	0.21 (0.29) ^a	0.24 (0.25) ^a	0.27 (0.27) ^a	-
ΔE							
3R4F	Mean (SD)	5.74 (1.49)	11.04 (2.75)	14.99 (3.63)	18.24 (3.36)	22.27 (4.78)	20.82 (3.81)
glo TM [‡]	Mean (SD)	0.51 (0.42) ^a	1.32 (0.42) ^a	1.20 (0.53) ^a	1.33 (0.47) ^a	1.81 (1.05) ^a	1.44 (0.59) ^a
glo TM pro	Mean (SD)	0.22 (0.09) ^a	0.41 (0.14) ^a	0.63 (0.16) ^a	0.81 (0.17) ^a	1.01 (0.12) ^a	1.80 (0.05) ^{a,b}
glo TM sens	Mean (SD)	1.67 (1.02) ^a	2.08 (0.96) ^a	2.04 (0.89) ^a	2.45 (0.33) ^a	1.93 (1.06) ^a	0.71 (0.21) ^{a,c}
iSwitch Maxx	Mean (SD)	2.00 (1.22) ^a	1.95 (1.28) ^a	1.85 (1.09) ^a	1.99 (1.21) ^a	1.93 (1.11) ^a	-

^a Significantly different from 3R4F (p < 0.05).

^b Significantly higher after storage (p < 0.05).

^c Significantly lower after storage (p < 0.05).

[‡] gloTM was assessed at 160, 480, 640, 800 and 1040 puffs.

[§] Samples exposed to 1000 puffs were reassessed 28 days after initial exposure.

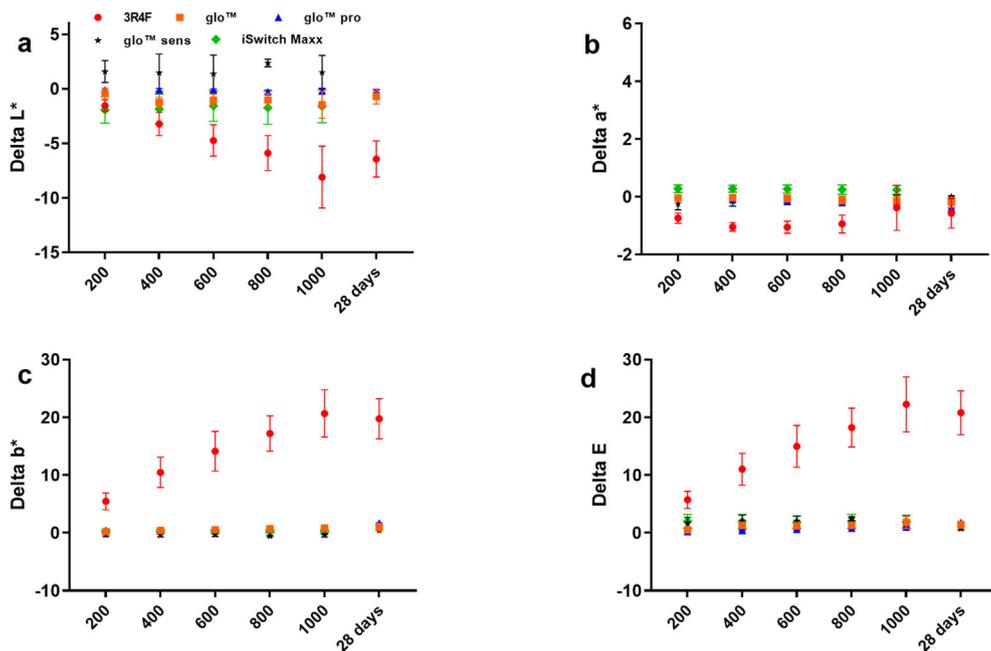


Figure 4. ΔL^* , Δa^* , Δb^* and ΔE values following exposure of cotton samples to product aerosols. ΔL^* , Δa^* , Δb^* and ΔE mean and standard deviation values following the exposure of cotton samples to 3R4F, gloTM, gloTM pro, gloTM sens or iSwitch Maxx. Graphs show the data obtained after 200–1000 puffs and following the storage of the 1000 puff exposed samples in the dark for 28 days. (a) ΔL^* (lightness), (b) Δa^* (green and red colour component), (c) Δb^* (blue and yellow colour components) and (d) ΔE (colour change) graphs.

4. Discussion

CS can deposit and then stain a number of surfaces including the walls and soft furnishings of the home and also commercial premises. EC and THP are relatively new products and propensity to cause staining when used indoors by consumers is unknown. In this study, the repeated *in vitro* exposure of wallpaper and cotton samples, to a scientific reference cigarette (3R4F), three THP (glo™, glo™ pro, glo™ sens) and an EC (iSwitch Maxx) were assessed in a novel small-scale exposure chamber. The data presented confirms that CS exposure stained wallpaper and cotton samples in a dose dependent manner. However, exposure of wallpaper and cotton samples to glo™, glo™ pro, glo™ sens or iSwitch Maxx aerosols induced significantly lower levels of staining compared to the 3R4F reference cigarette.

The developed exposure chamber enabled wallpaper and cotton samples to be exposed to the aerosol by diffusion as would occur within a home or commercial premise. Samples were attached to the inner walls of the exposure chamber to mimic wallpaper attached to walls or curtains hanging within a room. The developed chamber is based on an exposure chamber used for *in vitro* cell (Adamson et al., 2018; Jaunky et al., 2018) and 3D tissue (Haswell et al., 2017; Bishop et al., 2019) exposure to CS and PRRP aerosols. The original chamber was also selected as data is available on the settling characteristics of aerosols in the chamber (Adamson et al., 2012) and was used for enamel staining analysis (Dalrymple et al., 2018). The benefit of using a small chamber as described, rather than a full-size room or a larger chamber (Liu et al., 2017; Cancelada et al., 2019; Marcham et al., 2019) is the scope for accelerated exposure under more controlled conditions. The diffusional and settling behaviour of the test aerosols could also be calculated, enabling a degree of read across of different sets of data; and thus, the relative potential for staining from different products. Data generated from this exposure chamber could potentially be extrapolated to a larger space/room to understand the long-term impact of PRRP aerosols on indoor spaces and surfaces. However, chamber assessment, as used in this study, maybe an over-representation of potential room exposure and staining as the developed method does not take into consideration room ventilation or other lifestyle factors that may affect sample diffusion and deposition within a room.

The accelerated staining method developed for wallpaper and cotton samples is based on a recently published method that assessed bovine enamel sample staining levels *in vitro* following exposure to CS and a number of PRRPs (Dalrymple et al., 2018). In Dalrymple et al., 2018 and other studies assessing toothpaste or whitening/bleaching agents (Bazzi et al., 2012; Da Silva et al., 2018), CS staining levels were assessed using the Commission Internationale de L'éclairage (CIE) L*a*b* method (Wasilewski et al., 2010). L* is a measure of the lightness, whereas a* and b* are measures of the green-red and blue-yellow colour components respectively. The L*a*b* values then are used to calculate the ΔE value, the change in colour space before and after a treatment. In the case of enamel sample assessment, a ΔE of less than 3.3 is clinically acceptable staining (Villalta et al., 2006).

The ΔL^* , Δa^* , Δb^* and ΔE values obtained for wallpaper and cotton samples exposed to CS, THP or EC aerosols is consistent with published data for enamel samples (Dalrymple et al., 2018; Zanetti et al., 2019), dental resin composites (Zhao et al., 2017) and dentin (Zanetti et al., 2019) that were exposed to CS, THP or EC aerosols. These studies also demonstrated high levels of CS staining and significantly lower levels of dental samples staining when exposed to THP or EC aerosols. In the current study, the mean ΔE values following exposure to 1000 puffs of glo™, glo™ pro, glo™ sens or iSwitch Maxx aerosols were less than 2 demonstrating minimal staining of the wallpaper and cotton samples by the PRRPs. Whereas exposure to 3R4F CS resulted in dose related increases in wallpaper staining, a ΔE value of 23.07 units was recorded at 1000 puffs, which was significantly higher than all the PRRPs. CS

staining could also be easily visualised without the use of analytic equipment (Figure 3).

For the majority of THP and EC products, the ΔE values did not change with dose, the exception were glo™ and glo™ pro exposure of cotton samples, however, the ΔE value at 1000 puffs were 1.81 and 1.01 units respectively, significantly less than was recorded for 3R4F which had a ΔE value at 1000 puffs of 22.27 units. In addition, this dose response was also not observed for wallpaper samples exposed to glo™ or glo™ pro, staining levels at all doses were minimal and the ΔE value at 1000 puffs were 0.80 and 0.30 units respectively.

Sample storage resulted in some staining level differences and plateau effects. After 28 days storage, 3R4F wallpaper samples ΔL^* and Δa^* values were observed to plateau, values were not significantly different to values measured at 1000 puffs. Whereas, Δb^* and ΔE values for 3R4F, glo™ and glo™ pro exposed samples significantly increased at 28 days indicating a yellowing and increased staining of the wallpaper samples. However, glo™ and glo™ pro Δb^* and ΔE values were significantly lower than the values recorded for 3R4F. The Δa^* and ΔE values for glo™ sens exposed wallpaper samples significantly changed with storage, however values were minimal. Cloth ΔL^* values significantly changed for 3R4F and glo™ sens, increased and decreased values respectively. The Δa^* value decreased and Δb^* plus ΔE values increased for cloth samples exposed to glo™ pro, however values were significantly lower than 3R4F. In the case glo™, an increase with storage was only observed for the Δb^* value. Cloth Δa^* , Δb^* and ΔE values following 3R4F exposure also plateaued at 28 days. Plateau effects are probably not due to measurement saturation but indicate no change in colour as the equipment can read -100 to 100 in each axis. The spectrophotometry method used may not take into account additional sample deposition with time if the colour remains constant. The developed method also does not control for environmental factors such as room ventilation or discontinuation of product use in the room, both could result in room staining levels to appearing to plateau. Storage of samples for 28 days could result in aerosol evaporation, absorption/drying on the wallpaper or cloth sample or particle dislodgement during sample transfer resulting in changes to values, plateau effects or value reductions at day 28. The 28 day time-point was included in this study as nicotine changes from being colourless to yellow when exposed to air or light, therefore surface staining within a home or a commercial premise may not be observed instantly. The Δb^* values for 3R4F, glo™ and glo™ pro exposed wallpaper samples significantly increased at 28 days indicating a yellowing of the samples.

Differences in staining levels between CS and PRRP aerosols are possibly due to the fact that EC do not contain tobacco and that the tobacco in THP stick/consumable is heated to temperatures less than 350 °C, which is significantly less than cigarettes which can burn up to 950 °C. The lower heating temperature of the THP product, could result in a lower level of pigments being released from the tobacco into the aerosol. Studies have confirmed that THP products produce aerosols with less particles (Forster et al., 2018), together, less pigments and reduced particles, could result in less staining. EC are not used with a tobacco stick/consumable but with an e-liquid composed of propylene glycol, vegetable glycerol, water and flavours, when heated the aerosol produced has very little colour. Indeed, when CS, THP or EC aerosols are captured onto Cambridge filter pads, the differences in aerosol colour can be easily observed (Dalrymple et al., 2018).

PRRPs when used indoors could result in reduced surface staining as they do not produce any side-stream aerosol. PRRP use indoors has been documented to result in improved air quality, when compared to cigarette use (McAuley et al., 2012; Burstyn, 2014; O'Connell et al., 2015; Mitova et al., 2016; Ruprecht et al., 2017; Forster et al., 2018; Cancelada et al., 2019; Meišutović-Akhtarjeva et al., 2019). When used indoors, both main-stream and side-stream CS can reduce air quality and contribute to room odour, surface deposition and staining (McAuley et al., 2012; Burstyn, 2014; O'Connell et al., 2015; Mitova et al., 2016;

Ruprecht et al., 2017; Forster et al., 2018; Cancelada et al., 2019; Matt et al., 2019; Meišutovič-Akhtarjeva et al., 2019).

In the current study, the experimental method developed delivered the whole CS, EC and THP puff to the exposure chamber, which is probably an overrepresentation of EC and THP room exposure, and underrepresents exposure for CS, as the experimental design/chamber did not account for the effect of CS side-stream exposure. However, the experimental design gives an indication of staining potential of different tobacco and nicotine products. Studies have demonstrated that EC exhalate does not contain the levels of nicotine that CS exhalate does (Czogala et al., 2014) as it is postulated that the majority of nicotine contained in an EC is inhaled and absorbed by the consumers' lungs (Bush and Goniewicz, 2015; Liu et al., 2017). A recent study quantified the deposition of nicotine on floors, walls and windows of cigarette smokers', EC consumers' and non-smokers' homes (Bush and Goniewicz, 2015). The authors concluded that the homes of EC consumers had over 200 times lower deposited nicotine levels in their homes than in cigarette smokers' (Bush and Goniewicz, 2015). This data is also aligned with the consensus that EC and THP use indoors has less impact on indoor air quality (McAuley et al., 2012; Burstyn, 2014; O'Connell et al., 2015; Mitova et al., 2016; Liu et al., 2017; Ruprecht et al., 2017; Forster et al., 2018; Meišutovič-Akhtarjeva et al., 2019) and therefore indoor use could result in reduced surface staining.

The puff number selected per dose, 200, was selected as this approximates to a consumer's use of one pack of cigarettes per day. The total number of puffs per experiment, 1000, could correspond to a smoker's consumption of 5 packs of cigarettes over 5 days. The method developed assessed the staining of 1000 puffs in a 275 mL chamber and gives an indication of staining potential. Staining levels in a home or commercial premises could be influenced by room size, ventilation, humidity and cigarettes, EC or THP number/puffs per day. The selected puff number per dose is also aligned to published EC consumer consumption studies. Robinson et al., in 2015 calculated the average puff number from 21 EC consumers to be 225 puffs per day (Robinson et al., 2015) and a more recent study with 34 consumers and three different e-liquids, the puff number per day was less than a 100 puffs for each e-liquid (Robinson et al., 2018). Differences in consumption could be due to differences in EC delivery; in the 2015 study consumers used a cig-a-like EC; whereas consumers in the 2019 study used a tank format EC.

In the current study, a novel staining method was developed to assess environmental exposure and surface staining. Nicotine can also be used as a marker of CS, EC and THP environmental exposure due to its stability (Bush and Goniewicz, 2015; Liu et al., 2017; Forster et al., 2018). As with staining levels, deposited nicotine levels can also be used to demonstrate the differences in CS and EC environmental exposure. Marcham et al. (2019) developed a chamber to model environmental exposure and EC deposition on glass and terry cloth (Marcham et al., 2019). The authors observed that terry cloth absorbed nicotine at higher levels; this is possible due to increased surface area compared to glass or the increased absorbance of the terry cloth (Marcham et al., 2019). In the current study, differences in Δa^* values were observed between the wallpaper and cotton samples; again, this could be due to differences in surfaces or absorption properties of the cotton.

5. Conclusions

This study has developed a novel method to assess wallpaper and cotton sample staining by CS or PRRPs aerosols. The method developed demonstrated that CS exposure significantly increased the level of wallpaper and cotton sample staining in a dose dependent manner, whereas glo™, glo™ pro, glo™ sens or iSwitch Maxx exposure resulted in significantly reduced levels of staining. This data suggests that PRRPs may have additional social benefits for consumers and others. Further studies are required to assess the long-term impact on the indoor spaces and surfaces when consumers switch from cigarettes to PRRPs.

Declarations

Author contribution statement

Annette Dalrymple, Emma-Jayne Bean: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Thomas C. Badrock: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Anya Terry, Mark Barber: Conceived and designed the experiments; Performed the experiments.

Peter J. Hall, John McAughey: Conceived and designed the experiments; Analyzed and interpreted the data.

Steven Coburn, James Murphy: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare the following conflict of interests: This study was funded by British American Tobacco (BAT) R&D, Southampton. Experimental work was performed at BAT R&D and Intertek CRS, UK. All authors are employees of BAT, Intertek CRS or Borgwaldt KC GmbH. Intertek CRS and Borgwaldt KC GmbH received funds from BAT to generate data that is included in this manuscript.

Additional information

No additional information is available for this paper.

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