Development of a method to measure cigarette, e-cigarette and tobacco heating product skin staining

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Introduction

- Potential reduced risk products (PRRPs), such as electronic cigarettes (EC) and tobacco heating products (THP), hold great potential for reducing the risks associated with cigarette smoking.[1-2]
- We have recently demonstrated that exposure to EC and THP aerosols *in vitro* results in minimal teeth staining.[3]
- PRRPs may have additional cosmetic and hygiene benefits for consumers, such as reduced skin staining.

Aim

- To develop a method to enable skin staining to be assessed following exposure to a scientific reference cigarette (3R4F), a novel vapour product (NVP)/EC or a commercial THP (glo™).
- To use developed method to assess skin staining levels of two novel products, iFUSE II THP and iSwitch EC.

Products

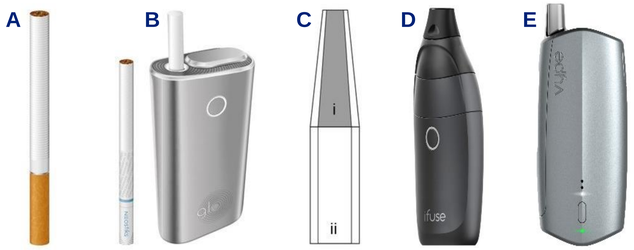

Figure 1: Products assessed. A) 3R4F Kentucky scientific reference cigarette (https://ctrp.uky.edu/), B) glo™ commercial THP with Neostick™, C) Schematic of BAT NVP, i: e-liquid consumable (5 mg/mL nicotine and flavours), , ii: battery, D) iFUSE II THP, E) iSwitch Max EC.

Methods

Particulate matter (PM) generation

- Products were attached to linear smoke machines and smoke/aerosol captured on Cambridge filter pads (CFP).
- The puffing regimes used were:
  - 3R4F cigarette and Glo™: Health Canada Intense[4]
  - NVP, iFUSEII and iSwitch Max: CORESTA recommended method No. 81[5].
- CFP captured smoke/aerosols were eluted using DMSO.

Figure 2: A) Representative CFP from 3R4F (10 puffs), Glo™ (48 puffs), e-cig (500 puffs) and blank. B) 3R4F PM eluted in DMSO.

Skin sample preparation and exposure

- Abattoir obtained porcine skin samples were prepare by clipping hair, removing excess subcutaneous fat and dermatoming to 500 to 750 µm.[6]
- Samples (0.8 cm^2) were prepared using a cylindrical biopsy punch and stored in Hanks' Balanced Salt solution (HBSS) for 10 minutes before baseline colour measurements.
- Three skin samples were incubated epidermis down, in 500 µL of test article or DMSO control for 0.25, 0.5, 1, 2, 4, 6 hours.

Colour measurement

- Samples were removed, washed in HBSS and colour readings (L*, a*, b*) were measured using a Konica Minolta CM-700d.
- Staining was determined as change in (ΔE) between baseline and exposure time ($\Delta E = \sqrt{((\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2)}$).
- One-way ANOVA was used to assess differences in ΔE means. The Tukey procedure was used for pairwise comparisons.

Results

- Staining was apparent after 1 hour exposure to 3R4F PM and continued to increase until 6 hours (Figures 4 and 5).
- After 1 hour, the mean ΔE values induced by 3R4F PM was statistically significantly ($p<0.0001$) higher than glo™, NVP, iFUSE II and iSwitch (Table 1).
- Staining levels for glo™, NVP, iFUSE II and iSwitch were minimal and comparable to DMSO control (Figures 4 and 5).

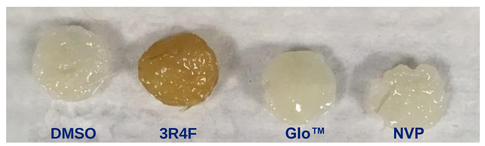

Figure 3: Skin samples following 6 hours exposure to DMSO, 3R4F, glo™, NVP.

Product	Hours	0.25	0.5	1	2	4	6
Study 1							
3R4F	Mean	9.44	12.51	15.57	17.45	19.87	21.77
	SD	2.19	2.24	1.99	2.33	2.60	2.84
glo™	Mean	8.04	9.3	10.64	9.78	8.98	8.39
	SD	2.54	2.20	2.48	2.17	2.49	2.97
NVP	Mean	8.86	10.34	11.37	10.64	10.33	10.01
	SD	2.75	1.74	1.83	2.00	2.02	2.56
DMSO	Mean	8.45	9.82	10.73	9.85	9.53	9.23
	SD	2.71	2.89	3.03	3.21	2.99	2.87
Study 2							
3R4F	Mean	10.78	12.73	15.25	17.51	18.93	18.66
	SD	1.18	2.18	1.89	1.74	1.16	1.60
iSwitch	Mean	8.67	8.95	9.99	9.61	8.36	8.13
	SD	2.60	2.28	3.25	3.28	3.05	2.59
iFUSE II	Mean	7.34	8.56	9.03	8.87	7.73	6.98
	SD	2.52	3.03	5.15	4.41	4.42	4.04
DMSO	Mean	6.96	8.68	10.04	9.41	8.23	8.46
	SD	2.81	2.99	3.88	2.17	2.38	3.00

Table 1: Delta E values following exposure to product PM or DMSO control.

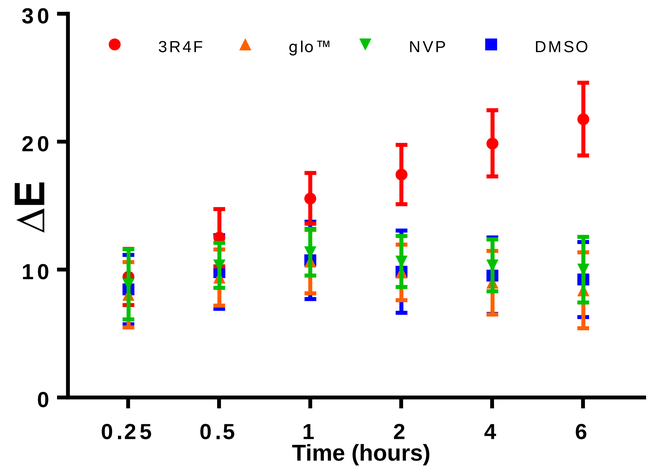

Figure 4: Mean ΔE values of skin samples following 0.25-6 hours exposure to 3R4F, NVP, Glo™ PM or DMSO.

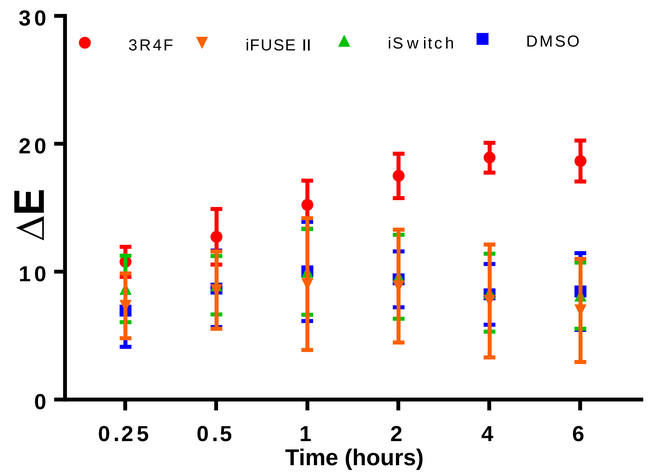

Figure 5: Mean ΔE values of skin samples following 0.25-6 hours exposure to 3R4F, iFUSE II, iSwitch PM or DMSO.

Conclusions

- Cigarette smoke particulate matter exposure significantly increased the level of staining of the skin samples.
- EC or the THPs induced little or no staining of skin samples; values were comparable to the DMSO control.
- For the first time, diverse PRRPs across the risk continuum have been assessed *in vitro* for their impact on skin staining.
- Further studies are required to assess the long-term impact of PRRP aerosols on skin staining.

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Abstract

Next generation tobacco and nicotine products (NGPs) such as electronic cigarettes (EC) and tobacco heating products (THP) have reduced toxicity and hold great potential for reducing the risks associated with cigarette smoking. NGPs may also have hygiene benefits for consumers that switch to these products. In this study, the level of skin staining was assessed following exposure to a scientific reference cigarette (3R4F) and NGPs across the risk continuum; a prototype EC or a commercial THP (glo™).

Test articles (TAs) were prepared by capturing cigarette smoke or EC/THP aerosol on Cambridge filter pads followed by elution with Dimethyl Sulfoxide (DMSO). Abattoir-obtained porcine skin (ø 0.5) samples were incubated at 37°C with each TA or DMSO control for 0, 0.25, 0.5, 1, 4 and 6h. Colour readings (L*, a*, b*) were measured for individual skin samples using a Konica Minolta CM-700d Spectrophotometer and mean colour change (ΔE) for each TA compared.

The reference cigarette 3R4F TA showed the greatest colour change, which was significantly higher than the EC and THP TAs, both of which showed relatively little colour change. The mean ΔE values at 6 hrs were: 21.78 ± 2.80, 8.38 ± 2.93, 10.01 ± 2.53 and 9.23 ± 2.87 for 3R4F, a prototype EC, glo™ or DMSO respectively.

The cigarette smoke extract significantly increased the level of staining of the skin samples whereas EC or the THP TAs induced little or no staining with values comparable to the DMSO control. For the first time, diverse NGPs across the risk continuum have been assessed in vitro for their impact on skin staining. Further studies are required to assess the long-term impact on skin staining of NGP aerosols.

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