Electronic Cigarette E-Liquids: An Assessment of Dermal Irritancy Potential

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1. Introduction and Objectives

1.0 Introduction

In 2016, the European Commission identified that e-liquids have the potential to cause skin reactions following accidental contact to nicotine and "other skin irritants"[1]. The risk of accidental exposure to e-liquids will be minimised as set out in the EUTPD2. E-Liquids are typically composed of Nicotine, Propylene glycol, Glycerol and Flavours. Nicotine is a known skin irritant and skin irritation is frequently reported by users of nicotine replacement therapies patches. Glycerol has been observed to cause slight dermal irritation in human volunteer studies, with Propylene Glycol exposure resulting in little to no irritant reaction in equivalent studies[2,3].

The OECD has written a Technical Guideline (TG 404) for the assessment of acute dermal irritation and corrosion[4]. It is recommended that *in vivo* studies are only commissioned once all of the existing data have been evaluated in a "weight of evidence" examination. Should this "weight of evidence" prove to be inconclusive, then additional testing should be initiated, starting with *in vitro* methods. In support of this *in vitro* models of reconstructed human epidermis have been developed, validated and gained regulatory acceptance in Europe (OECD test guideline 439)[5]. The OECD TG 439 can only discriminate between irritants and non-irritants, it cannot provide any indication of substance potency. Therefore, for the assessment of dermal irritation potential of e-liquids it was decided that the MTT Effective Time-50 protocol should be employed to categorise potency of different ingredient combinations. See Figure 1 for a comparison of OECD 439 and MTT ET-50 experimental protocols. The ability of this method to assess e-liquid mixtures had also yet to be determined.

Protocol		
Methods Available	Standard skin irritation test (distinguishes between (UN GHS Category 2) skin irritation and (UN GHS No Category) non skin irritation at 1hr exposure	ET_{50} skin irritation test (uses 3-5 exposure times over 1hr – 48 hr to provide potency score)
Skin Model Used	MatTek EpiDerm™ 3D human skin tissue model	MatTek EpiDerm™ 3D human skin tissue model
Number of Replicates	3	1 - 3
Exposure Times	1 hr	1 hr - 48 hr
Post Exposure Incubation	42 hr	42 hr
Positive Control	5% sodium dodecyl sulphate (SDS)	5% sodium dodecyl sulphate (SDS)
Negative Control	Dulbecco's Phosphate Buffered Saline (PBS)	Dulbecco's Phosphate Buffered Saline (PBS)
Endpoints	MTT IL-1α release (optional) H&E stained histology slide (optional)	MTT IL-1α release (optional) H&E stained histology slide (optional)
Scientific Endorsement	ECVAM	
International Regulatory Acceptance	OECD Test Guideline 439	

Figure 1: Comparison of testing protocols for OECD TG 439 and MTT Effective Time-50 methods

1.1 Objectives

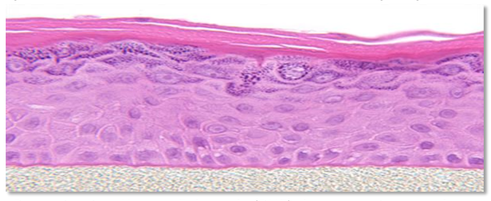
The aim of the study was to assess the irritant potential of e-liquids in the EpiDerm model (MTT Effective Time-50 protocol), and to determine the influence of varying concentrations of nicotine plus the influence of flavours. Therefore, three experimental (Base liquid (50:50 Propylene Glycol, Vegetable Glycerin), ± 2.4% or 4.5% Nicotine) and two commercial (containing 1.2% or 1.8% Nicotine) e-liquids were assessed.

The irritant potential was determined by assessing EpiDerm tissue viability after various exposure times to controls and test articles for protocol optimization.

2. Materials and Methods

2.0 Test System

The *in vitro* skin irritation test following the Effective Time-50 [ET50] protocol developed by MatTek was performed. Briefly, the test consists of a topical exposure of the test material to a reconstructed human epidermis (RhE) model (EpiDerm™) followed by a cell viability test. The EpiDerm™ model closely resembles the physiological and biochemical properties of the upper parts of the human skin. The enzymatic conversion of the vital dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, (MTT) by reductase in viable cell mitochondria into a blue formazan salt is quantitatively measured at 570 nm after the isopropanol extraction from tissues.



Reconstructed Human Epidermis (RHE), is a ready-to-use, highly differentiated 3D tissue model consisting of normal, human-derived epidermal keratinocytes cultured in serum free medium on specially prepared tissue culture inserts (MatTek)

2.1 Controls

The Dulbecco's phosphate buffered saline (DPBS) served as a negative control and 1% solution of sodium dodecyl sulfate (SDS), a known dermal irritant, in ultrapure water (18.2 MΩ-cm), served as a positive control.

2.2 Test Materials

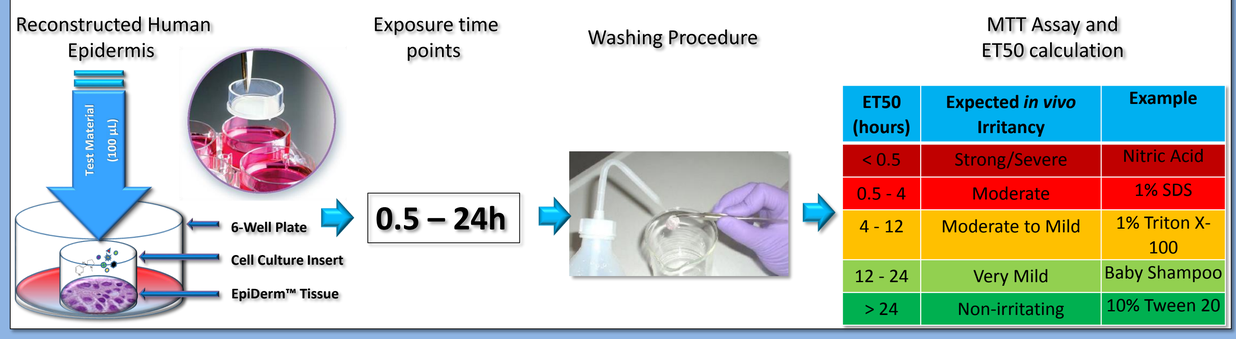
Base Liquid (PG/VG: 50/50% W/W), Base Liquid + 2.4% Nicotine (PG/VG/Nic: 48/48/2.4% W/W), Base Liquid + 4.5% Nicotine (PG/VG/Nic: 47.75 /47.75/4.5% W/W) and two commercially available e-liquids (Commercial Sample 1: 1.8% Nicotine and Commercial Sample 2: 1.2% Nicotine).

2.3 Exposure

For the exposure, 100 µL of each test material was applied into the cell culture insert atop the EpiDerm™ model. All tissues were then placed into the incubator at 37°C and 5% CO_2 for 30 minutes, 2 hours, 6 hours, 12 hours and 24 hours.

2.4 Post Exposure

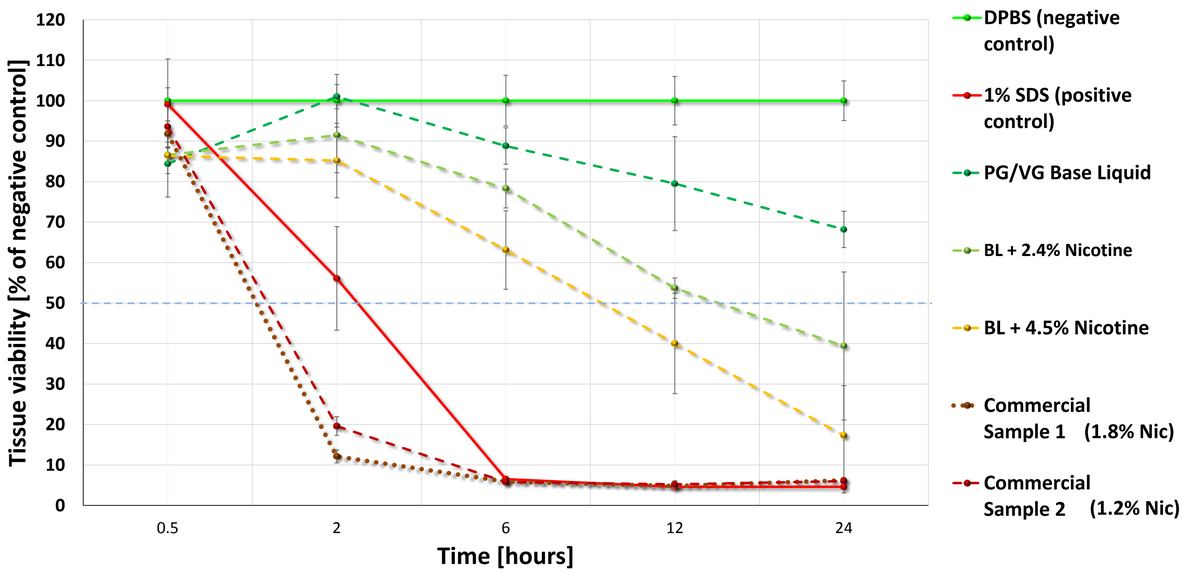
The tissues were rinsed up to 25 times with approximately 1 mL of DPBS. The apical surface was gently blotted with a cotton swab and cultures were immediately transferred to a 24-well plate containing MTT media. Standard MTT Assay protocol was used. All test article were evaluated to their potential to auto-reduce MTT prior the test. All MTT data were normalized to the negative control, DPBS. The assay fit the acceptance criteria as the OD of the negative control tissues was >1.0 (between 1.007 and 1.619). The positive control, 1% SDS, was effective, reducing tissue viability to 56.1% of control after 2 hours, and <10% of control after 6, 12 and 24 hours exposure. Relative cell viability was calculated for each tissue and presented as percent (%) of the mean of the negative control tissues. The ET_{50} values were calculated using GraphPad Prism (Version 5.01, San Diego, CA).



ET50 (hours)	Expected *in vivo* Irritancy	Example
< 0.5	Strong/Severe	Nitric Acid
0.5 - 4	Moderate	1% SDS
4 - 12	Moderate to Mild	1% Triton X-100
12 - 24	Very Mild	Baby Shampoo
> 24	Non-irritating	10% Tween 20

3. Results

Mean Tissue viability over time following exposure to controls or e-liquids

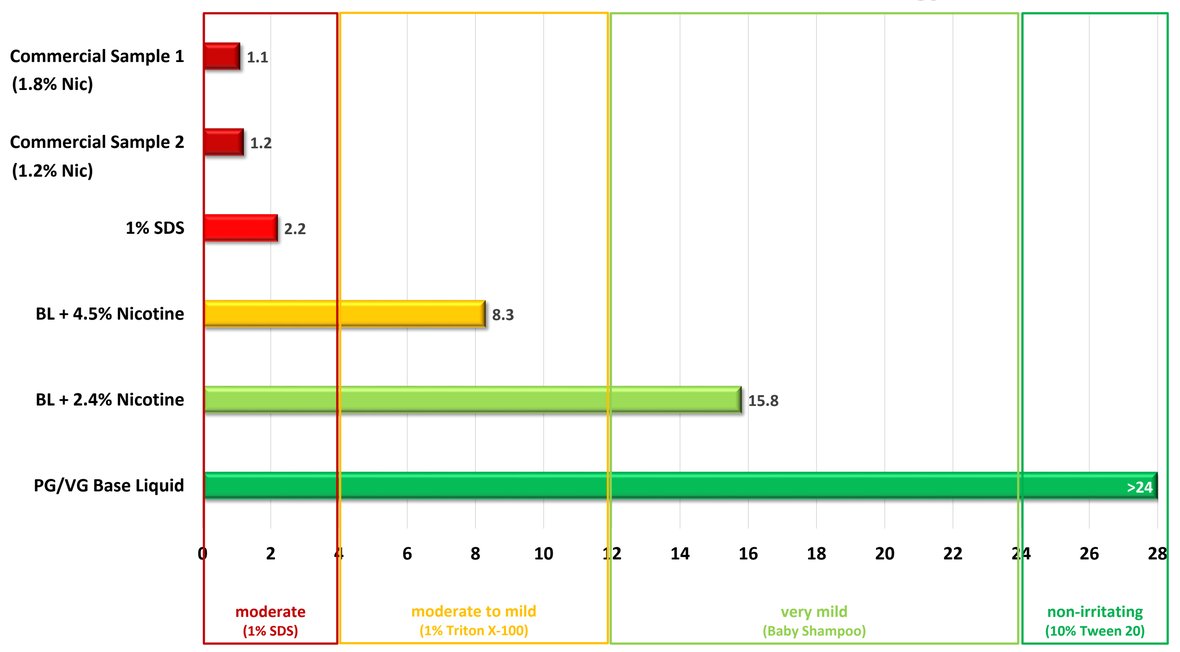


Data is expressed as mean cell viability (%) compared to negative control (n=3).

Statistically significant ($p<0.05$) reductions in tissue viability relative to negative control were observed after:

- 24 hours post exposure to Base Liquid (BL)
- 6 hours and each subsequent time point post exposure to BL+ 2.4% and BL+ 4.5% Nicotine
- 2 hours and each subsequent time point post exposure to the two commercial samples

Potency Categorisation of test articles using ET_{50} (hours)



The Base Liquid sample is categorised as non-irritating due to it's ET_{50} greater than 24 hours post exposure. Base liquids containing nicotine had lower ET_{50} values relative to higher nicotine concentrations. The two commercial products were categorized as moderate irritants, even though they had lower nicotine concentrations at 1.2 and 1.8%.

4. Conclusions

- Results indicate that the Base liquid (BL) is a 'non-irritant' (ET_{50} >24 hours).
- Unsurprisingly, with the addition of nicotine, a concentration dependent increase in irritation is observed. The Base Liquids with 2.4 and 4.5% Nicotine reduced the ET_{50} to 15.8 and 8.3 hours respectively.
- It is of note that the commercial products were identified as 'moderate irritants' (ET_{50} 1.1-1.2 hours), despite having lower nicotine concentrations than the experimental liquids.
- This suggests that certain flavours can contribute to the irritant potential, consistent with the findings from the Royal College of Physicians 2016, and these results demonstrate the utility of this assay as a screening tool for e-liquids.
- Future work should focus on the elucidation of the flavour components in the commercial samples which appear to be contributing to the irritant potential of the e-liquids.

References

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5. OECD (2015) Test Guideline 439, In Vitro Skin Irritation: Reconstituted Human Epidermis Test Method