1. Introduction and Objectives

1.1 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”2. The risk of accidental exposure to e-liquids will be minimised as set out in the EUToxII. 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 Glycerol exposure resulting in little to no irritant reaction in equivalent studies3,4.

The OECD has written a Technical Guideline (TG 404) for the assessment of acute dermal irritation and corrosion5. 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 model of reconstructed human epidermis have been developed, validated and gained regulatory acceptance in Europe (OECD test guideline 428). The OECD TG 419 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 428 and MTT ET-50 experimental protocols. The ability of this method to assess e-liquid mixtures has also yet to be determined.

Figure 1: Comparison of testing protocols for OECD TG 428 and MTT Effective Time-50 methods

3. Results

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

| Time (hours) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 | 11.5 | 12.0 |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tissue viability [% of negative control] | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |

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:
1. 24 hours post exposure to Base Liquid (BL)
2. 6 hours and each subsequent time point post exposure to BL + 2.4% and BL + 4.5% Nicotine
3. 2 hours and each subsequent time point post exposure to the two commercial samples

4. Conclusions

Results indicate that the Base Liquid (BL) is a “non-irritant” (ET50 >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 ET50 to 15.8 and 8.3 hours respectively.

It is of note that the commercial products were categorised as ‘moderate irritants’ (ET50 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 the elucidation of the factors contributing to the irritant potential of the e-liquids.

References


Electronic Cigarette E-Liquids: An Assessment of Dermal Irritant Potential
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2. Materials and Methods

2.1 Controls

The Dulbecco’s phosphate buffered saline (DPBS) was served as a negative control and 1% solution of sodium dodecyl sulphate (SDS), a known dermal irritant, in ultrapure water (18.2 MO, 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/NC: 48/48/2.4% W/M), Base Liquid + 4.5% Nicotine (PG/VG/NC: 47.5/47.5/4.5% W/M) 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% CO2 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 pad and cultures were immediately transferred to a 24-well plate containing MTT media. Standard MTT Assay protocol was used. All test articles were evaluated to their potential to auto-mitrate prior to the test. All MTT data were normalised to the negative control, DPBS. The assay fit the acceptance criteria as the O/Negative control tissues as >3.0 (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 ET50 values were calculated using GraphPad Prism (Version 5.01, San Diego, CA).