Acute toxicity of a flavoured e-liquid according to TPD 2 is related to e-cigarette vaporiser resistance and electrical power

Thomas Alexander Mrva¹, Ophélie Poirier², Peter C. Dartsch³

¹Happy People GmbH, Germany, ²Takasago Europe GmbH, Zülpich, Germany; ³Dartsch Scientific GmbH, Germany

Keywords: TPD 2, e-cigarette, flavour, human lung cells, cytotoxicity

Introduction
In contrast to cigarette smoking, the vapour of e-cigarettes is not the result of a combustion process and has been shown to have much lower health effects in vitro and in vivo (Misra et al. 2014). Prompted by that background we used a specially created e-liquid in accordance to TPD 2 to investigate whether the vaporiser resistance in combination with electrical power of the e-cigarette might be responsible for the degree of acute toxicity on cultured human lung cells after vaping.

Materials and methods
The flavoured e-liquid according to TPD 2 consisted of a base liquid of 70 % vegetable glycerol, 30 % propylene glycol, 1.2 % nicotine and a flavour named “Blueberry & Cheesecake”. The liquid was transferred to a specially designed vaping apparatus and 10 puffs with a duration of 4-5 seconds and a pause of 10 seconds between two puffs were applied to the liquid. A common e-cigarette was used (eGrip OLED CL 30 from Joyetech with two different vaporisers of 1.0 and 0.4 Ohm and an electrical power ranging from 6.5 to 30 Watts). The vapour was passed into 10 ml of HEPES-buffered cell culture medium. After sterile filtration the primary extract was added at different test concentrations to cultures of human lung cells (A-549) with a seeding density of 10,000 cells/well in 96 well-plates. After 24 hours cell vitality was measured enzymatically by cleavage of XTT (Xenometrix, Allschwil, Switzerland) by the activity of mitochondrial dehydrogenases.

Results
The results show that even an e-liquid according to TPD 2 can produce a marked acute toxicity on cultivated human lung cells when vaping beyond the usual limits. By using a vaporiser with a resistance of 1.0 Ohm, above 15 Watts cytotoxicity was observed causing nearly complete cell death within 24 hours. By using the subohm vaporiser with a resistance of 0.4 Ohm, even the highest power of 30 Watts did not cause a cytotoxic effect. The cytotoxic effect was mainly related to the production of free radicals as can be shown by cleavage of a tetrazolium dye (not depicted).

Conclusions
The results indicate that vaping of an e-liquid which is in accordance to TPD 2 also causes marked acute toxic effects when vaping at forced conditions. This effect seems to be mainly related to an excess of free radicals. However, at common and moderate user conditions there are no toxic effects which might cause acute cell death within the lung or the respiratory tract. Thus, we recommend a more detailed elucidation of e-cigarette users on these health effects when vaping at forced conditions.

Summary of the toxicological data on the acute toxicity of a flavoured e-liquid manufactured according to TPD 2 after vaping with a 1.0 Ohm vaporizer (left column) and a 0.4 Ohm vaporizer (right column) at different power settings of the e-cigarette. Data were obtained with cultured human lung cells. Data represent mean value ± standard deviation of three independent experiments (n=3).

References