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My-eLiquid
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TEST REPORT

Tobacco smoke vs. flavoured e-liquid vapour of the brand „My-eLiquid“ – Acute toxic effects on cultured human lung cells –

Background

In contrast to tobacco smoke, the vapour of an e-cigarette is not the result of a combustion process and is believed to have much lower health effects. Prompted by this background, the present study was performed with human lung cells to compare the acute toxic effects of tobacco smoke with the vapour of three completely different and flavoured e-liquids manufactured by My eLiquid, D-81379 München, Germany.

Tobacco cigarette and e-liquids

The investigations were done by using a common cigarette brand of medium strength with 10 mg tar, 0.8 mg nicotine und 10 mg carbon monoxide. For comparison, the following liquids from My eLiquid, D-81379 München, Germany, were examined:

- My-eLiquid „Erdbeer“ containing 18 mg/ml nicotine. Ingredients: glycerol, bidistilled water, flavours, nicotine, E1520.
- My eLiquid „Summersplash“ containing 18 mg/ml nicotine. Ingredients: glycerol, bidistilled water, flavours, nicotine, E1520, E1519, E1518.
- My-eLiquid „PINK Poisen“ containing 18 mg/ml nicotine. Ingredients: glycerol, bidistilled water, flavours, nicotine, ethanol, E260, E270, E300, E1520, E1519, E1518.

The liquids were mixed on purpose to contain various allowed food additives. In detail, the used food additives were: ethanol, E260 (acetic acid as preservative and acidifier), E270 (lactic acid as preservative and acidifier), E300 (ascorbic acid as antioxidant), E1518 (triacetin as carrier for flavours), E1519 (benzylalcohol as carrier for flavours) and E1520 (propylene glycol as carrier for flavours and vapour production).

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Simulation of smoking and vaping to obtain the primary extract

In order to simulate the real smoking or vaping conditions, a special apparatus was used which allows to vary the frequency, length and the depths of the puffs (Fig. 1). For smoking of two cigarettes, 20 puffs with a duration of 3 seconds and a pause of 30 seconds between two puffs was presumed. See Vansickel AR et al. (2010): A clinical laboratory model for evaluating the acute effects of electronic “cigarettes”: Nicotine delivery profile and cardiovascular and subjective effects. Cancer Epidemiology, Biomarkers, and Prevention 19:1945–1953. Comparable conditions were applied for the e-cigarette (EVOD, EU version, vaporiser 2.2 Ω and rechargeable battery 3.7 V; KangerTech), i.e. 20 puffs of 5 seconds and a pause of 30 seconds between two puffs. The smoke of the cigarettes and the vapour of the liquids were aspirated by a pump and piped into 20 ml of HEPES-buffered cell culture medium. The resulting primary extracts had a neutral pH value of 7.4 ± 0.3. The extract was brownish-yellow for cigarette smoke and colourless for all e-liquid vapours. Both primary extracts were filtrated sterile by pressing them through a sterile porous membrane (porous size 0.45 µm) and added to the lung cell cultures as described in the next chapter.

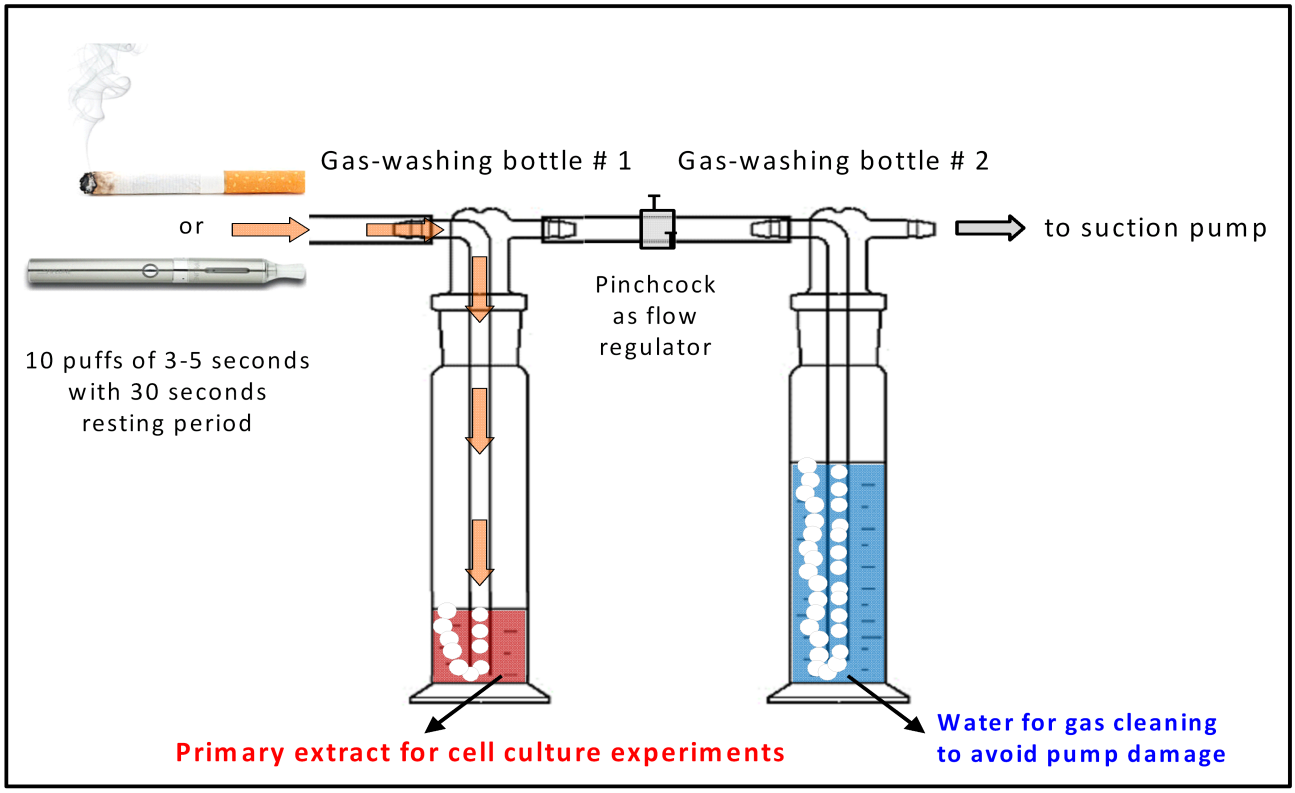

Figure 1: Experimental setup for simulation of smoking or vaping. The suction pump on the right generates an adjustable underpressure which aspirates the smoke or vapour and bubbles it into the culture medium in the left gas-washing bottle # 1. This yields the primary extract which is used either undiluted or diluted stepwise for further cell culture experiments. The right gas-washing bottle # 2 is only for gas cleaning to avoid pump damage.

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Experimental setup

The investigations were done with human lung carcinoma cells (cell line A549; ECACC, Salisbury, UK) which are widely used in current scientific research all over the world. See Cervellati F et al.(2014): Comparative effects between electronic and cigarette smoke in human keratinocytes and epithelial lung cells. Toxicology in Vitro 28: 999-1005.

Cells were routinely cultured as mass cultures in a Binder CO_2 incubator at 37 °C with a moist atmosphere of 5 % CO_2 and 95 % air. Culture medium was DMEM/Ham's F12 (1:1) supplemented with 10 % fetal bovine serum and 100 Units/ml of penicillin & 100 µg/ml of streptomycin. All cell culture reagents were from GE Healthcare Life Sciences, D-35091 Cölbe, Germany.

For the experiments, cells were taken from 80 to 90 % confluent mass cultures and were seeded at a density of 20.000 cells/well into 96-well plates (200 µl/well). Seeded cell densities were chosen that cell cultures did not reach confluency during the total experimental and exposure period. 24 hours after seeding, cells were completely attached and spread to the bottom of the wells. Then, culture medium was discarded and replaced by fresh culture medium containing the primary extract of tobacco smoke or e-liquid vapour to yield the following concentrations of the primary extract in the test: 0 – 10 – 25 – 50 – 100 vol% with 0 vol% as control (= only culture medium without primary extract) und 100 vol% as undiluted primary extract. The exposure time to the different concentrations of the primary extracts was 24 hours.

Thereafter, culture medium was discarded and replaced by 190 µl/well of culture medium and 10 µl/well of WST-1 (Roche Diagnostics, Mannheim). 96-well plates were incubated for another hour at 37 °C in the incubator and the optical density of each well was examined by a difference measurement at ΔOD = 450 minus 690 nm using a double-wavelength elisa reader (BioTEK Elx 808). The red tetrazolium dye WST-1 is cleaved by the metabolic activity of the cells to yield yellow formazan crystals which are soluble in aqueous solutions. The intensity of the resulting yellow solution is directly correlated with cell vitality and metabolic activity. The results were expressed graphically as relative values in comparison to untreated controls. Experiments were done in triplicate.

Results and conclusions

The alterations of lung cell morphology were dramatic after exposure to the primary extract of tobacco smoke and caused a marked rounding, detachment and death of the cells within 24 hours (not shown). Even the lowest test concentration of 10 vol% caused a loss in cell vitality by more than 30 %; the maximum reduction of cell vitality by approximately 97 % was achieved with the undiluted primary extract of tobacco smoke (= 100 vol%; Figure 2).

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In contrast, human lung cells which were exposed to the extracts of e-liquid vapour did not show any morphological signs of toxicity at all concentrations tested (not shown). Examination of the cell vitality by the enzymatic method did not show any statistical significant differences of the values from those obtained for the controls (Wilcoxon-Mann-Whitney-Test; Figure 2).

The results of the present investigation have shown that the smoke of tobacco cigarettes has a markedly stronger acute toxic effect when compared with the vapour of the e-liquids tested here. Within an exposure period of 24 hours, the vast majority of human lung cells exposed to tobacco smoke extracts lost their vitality. In contrast, the vapour of the e-liquids of the brand My-eLiquid did not show any acute toxic effects – even at concentrations about 10x higher than tobacco smoke extracts.

Investigator and responsible for the correctness of the presented experiments and results.

Schongau, April 28, 2015

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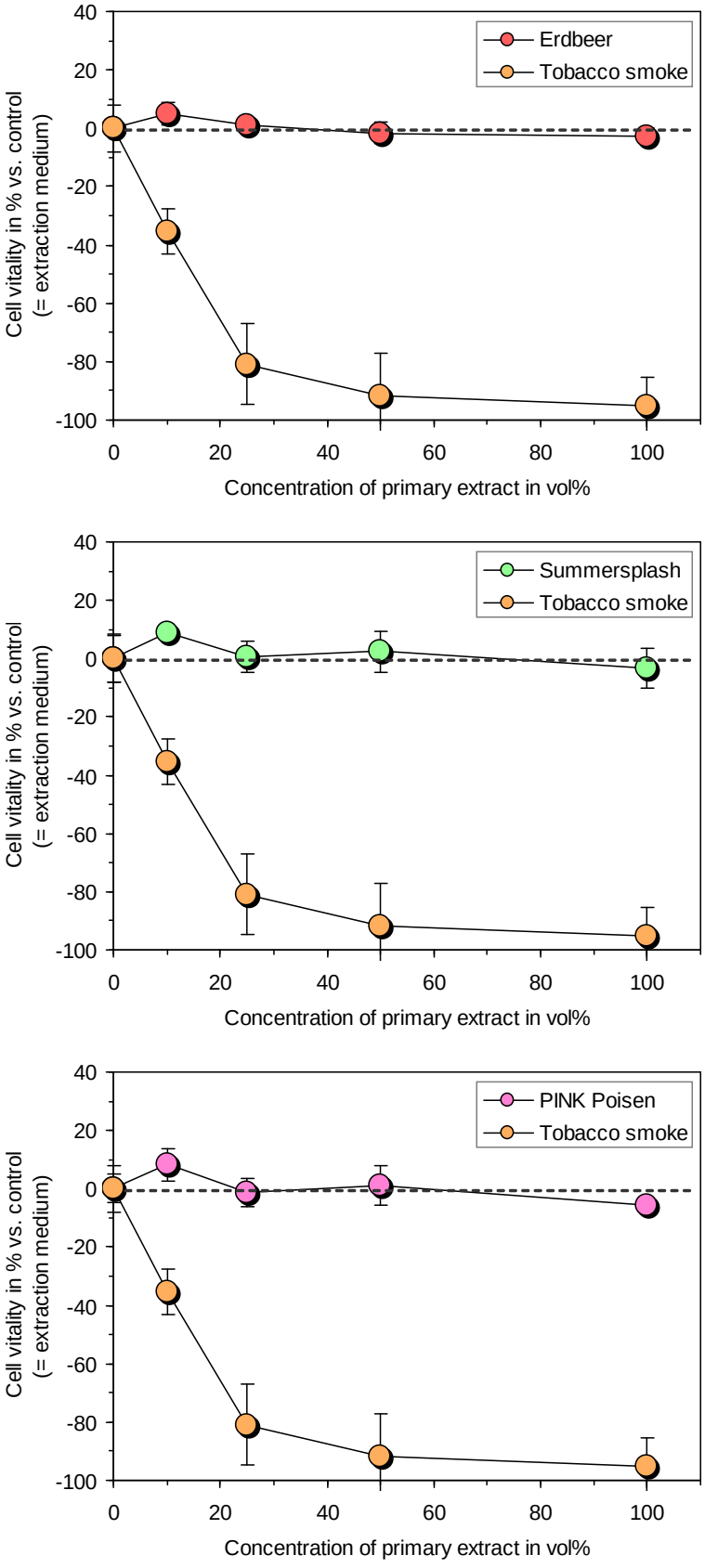

Figure 2: Graphical presentation of the results on acute toxicity of tobacco smoke in comparison to the vapour of the three different e-liquids. While the primary extract of tobacco smoke causes a marked loss in lung cell vitality at only 10 vol%, the e-liquids show no significant difference to controls even at 100 vol% (= undiluted primary extracts). Data represent mean values ± standard deviations of three experiments.