Toxicity of aerosols of propylene glycol, vegetable glycerin and nicotine in Sprague-Dawley rats in a 90d OECD 413 sub-chronic inhalation study


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Introduction

Several experts have concluded that electronic cigarettes (EC) should be considered as a reduced-risk alternative to conventional combustible cigarettes (Szwarcz et al., 2014; Benowitz & Goren, 2015; Tashkin et al., 2015, Royal College of Physicians, 2016). Nicotine, propylene glycol (PG) and vegetable glycerin (VG) together, with distilled water and flavors are the main constituents of the liquids used to generate aerosols in EC. Two simulation studies have been published using EC as the test item (Banerjee et al., 1999; Wethy et al., 2011) both showing relatable minor effects after exposures even up to 30 mg/l PG for 20 days. Two inhalation studies with EC have been performed, also showing only minor effects on the base of the agglutinin epithelial layering at the base of the agglutinin exposure concentration up to 3.6 mg/L, considered as adaptive response to the exposure (Bene et al., 1992).

While toxicities of nicotine, PG, and VG have been individually assessed previously, the present study assessed their toxicity when combusted (with or without nicotine). The animals were exposed to mixtures of PG and VG nebulized to a target concentration of 1.22 mg/L PG and 0.023 mg/L VG with or without nicotine (delivered to a target concentration of 0.023 mg/L). The exposure period of 90 days was performed according to the OECD TG101 guidelines, including the full evaluation of systemic and histopathological responses to the test aerosols.

Study design

Test system: Sprague-Dawley rats, 10 males and 10 female animals per group

Test item: Mixture of PG and VG at 3 concentrations, with fixed nicotine (L023 mg/l) (see panel A and B for the concentrations).

Exposure: Aerosols were generated from the solution using jet impaction nebulizers. The aerosol was directed to the target concentrations using fans. The aerosol was delivered to the animals in flow rate nose-only exposure chambers, type IPP 122 (panel C).

Test atmosphere: PG, VG, nicotine, temperature, relative humidity (±5%), droplet size distribution.


Systemic and post-test at the end of study: hematology, clinical chemistry, respiratory physiology, pulmonary inflammation (cells in broncho-alveolar lavage fluid, BAL), histopathology of the respiratory tract, non-respiratory tract organs.

Statistics: Statistical comparison to the sham group is indicated as follows: *p≤0.05, **p≤0.01 relative to sham.

Results

Exposure and uptake

Conduct of exposure and aerosol uptake. Aerosol samples were collected four times per day from the breathing zone of each of the exposure chambers. Evaluation of the same important parameters over the course of the study, target concentrations for each constituents were met in all chambers (A – C). Uptake of nicotine (Nic) and propylene glycol (PG) was determined by measuring concentrations in plasma and showed plasma levels in proportion to the chamber aerosol concentrations as expected. Plasma measurement of glycerin was not possible due to high endogenous levels of the compound.

Organ Weights

Two-tailed t-tests on the organ weights. Mainly nicotine-dependent organ weight effects were seen in the animals. This included increased absolute weight of the adrenals (A and B) and lower absolute weight of the thymus (not shown) in both sexes relative to the sham or vehicle groups. These findings have been observed previously in intranasal cigarette-exposed animals and attributed to a stress response following exposure to high nicotine or TPM concentrations. In addition, a nicotine-dependent increase in liver weight was observed in the females (C and D), as also previously observed (Phillips et al., 2015) and considered as a metabolic (but not pathologic) effect in the animals in response to nicotine exposure.

Clinical Chemistry

Blood chemistry changes in animals exposed to test aerosol. Several clinical chemistry parameters were mainly limited to liver enzyme activities, alanine aminotransferase (ALT), glutamate dehydrogenase (GDP), increased in animals exposed to the nicotine-containing test aerosol (panel A – D). These changes correspond to mild vascularization observed in the livers from nicotine-exposed animals during the histopathological evaluation (panel E and F). A reduction in certain metabolic parameters was also observed in the animals exposed to the test item (decreased total cholesterol and glucose, data not shown). These changes, the clinical chemistry parameters have been observed previously in animals exposed to similar concentrations of nicotine (Phillips et al., 2015). Other clinical chemistry parameters, including protein levels, and salt concentrations were generally similar between the exposure groups.

Histopathology of the respiratory tract

Histopathological findings in test item-exposed animals. A histopathological assessment of the lungs and respiratory tract of the animals was performed at the end of the study both before and after staining with hematoxylin and eosin. Several parameters were assessed for each organ and tissue. Histopathological changes in the non-respiratory tract organs were limited to a mild increase in the liver of the test item-exposed animals. In the respiratory tract and its associated organs, the evaluation of histopathological changes led to the following findings:

Conclusions

• Readministration of a mixture of propylene glycol and vegetable glycerin (with or without nicotine) effectively delivered the constituent molecules to the animals through the inhalation route.

• Inhaled aerosols were well tolerated by the animals, with minimal effects on the breathing parameters (respiratory physiology).

• Systemic effects indicated low levels of toxicity which was only observed following exposure to the nicotine-containing aerosols. These effects included increased liver enzyme activity in the animals, and slight weight gains in the nicotine-exposed groups.

• Mild histopathological effects were observed mainly in the larynx and in the nicotine-containing test item aerosols.

• Histopathological changes in the non-respiratory tract organs were limited to a mild increase in the liver of the test item-exposed animals.

• Propylene glycol and vegetable glycerin (without nicotine) had very little effect in all parameters relative to the sham or vehicle-exposed animals.


Verbeeck, J. and Dulewska, M.P. (2013). Clinical chemistry, respiratory physiology, pulmonary inflammation (cells in broncho-alveolar lavage fluid, BAL), histopathology of the respiratory tract, non-respiratory tract organs. Statistical comparison to the sham group is indicated as follows: *p≤0.05, **p≤0.01 relative to sham.

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