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 (E-cigarettes) should be considered as a reduced risk alternative to conventional combustible cigarettes, (Goniewicz et al., 2014; Benowitz and Goniewicz, 2013; McNeill et al., 2015, Royal College of Physicians, 2016). Nicotine, propylene glycol (PG) and vegetable glycerin (VG) together with distilled water and flavours are the main constituents of the liquids used to generate aerosols in E-cigarettes. Two inhalation studies have been published using PG as the test item (Suber et al., 1989; Werley et al., 2011), both showing relatively minor effects after exposures even up to 30 mg/l PG for 28 days. Two inhalation studies with glycerin have been performed, also showing only minor effects on the base of the epiglottis epithelium lining at the base of the epiglottis (exposure concentration up to 3.91 mg/l) concentration, considered as adaptive response to the exposure (Renne et al., 1992).

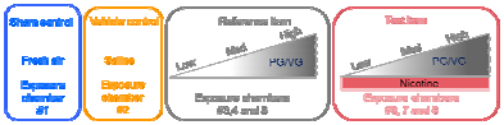
While toxicities of nicotine, PG, and VG have been individually assessed previously, the present study assessed their toxicity when combined (with or without nicotine). The animals were exposed to mixtures of PG and VG nebulized to a target concentration of 1.52 mg/l PG and 1.89 mg/l VG with and without nicotine (delivered to a target concentration of 0.023 mg/l). The exposure period of 90 days was performed according to the OECD TG413 guidelines, including the full evaluation of systemic and histopathological responses to the test aerosols.

Study design

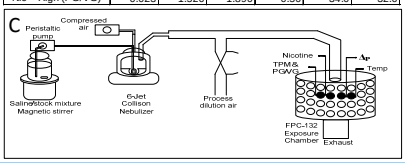
A

Stock solutions	Target concentrations of aerosol constituents (mg/l)			Relative proportions of stock solution constituents (% volume)		
	Nicotine	PG	VG	Nicotine	PG	VG
Sham (fresh air)	NA	NA	NA	NA	NA	NA
Vehicle (saline)	NA	NA	NA	NA	NA	NA
Low (PG/VG)	NA	0.174	0.210	NA	3.8	3.6
Med (PG/VG)	NA	0.520	0.630	NA	11.3	10.7
High (PG/VG)	NA	1.520	1.890	NA	34.0	32.0
Nic + Low (PG/VG)	0.023	0.174	0.210	0.50	3.8	3.6
Nic + Med (PG/VG)	0.023	0.520	0.630	0.50	11.3	10.7
Nic + High (PG/VG)	0.023	1.520	1.890	0.50	34.0	32.0

B



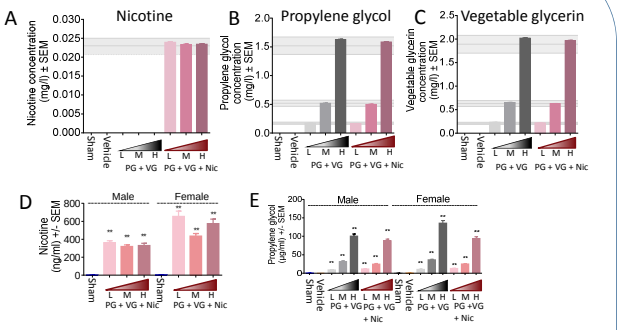
C



Test system: Sprague-Dawley rats, 10 male and 10 female animals per group
Test item: Mixture of PG and VG at 3 concentrations, with fixed nicotine (0.023 mg/l) (see panel A and B for concentrations and test groups).
Exposure: Aerosols were generated from the solutions using 6 jet collison nebulizers. The aerosol was diluted to the target concentrations using fresh air. The aerosol was delivered to the animals in flow past nose-only exposure chambers, type FPC-132 (panel C).
Test atmosphere monitoring: PG, VG, nicotine, temperature, relative humidity (air supply), droplet size distribution.
Bio-monitoring: Respiratory physiology, urinary nicotine metabolites, ophthalmoscopy.
In-life monitoring (exposure phase): clinical observations, body weight, food consumption.
Systemic end points at the end of the study: hematology, clinical chemistry, pulmonary inflammation (cells in broncho-alveolar lavage fluid, BALF), histopathology of the respiratory tract, non-respiratory tract organs
Statistics: Statistical comparison to the sham group are indicated as follows:
* p<0.05, ** p<0.01 relative to sham

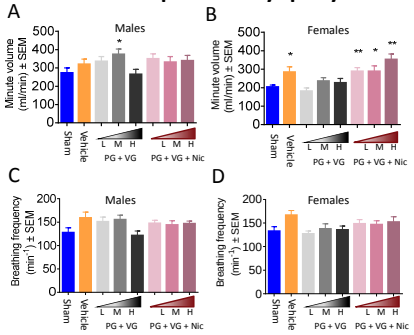
Results

Exposure and uptake



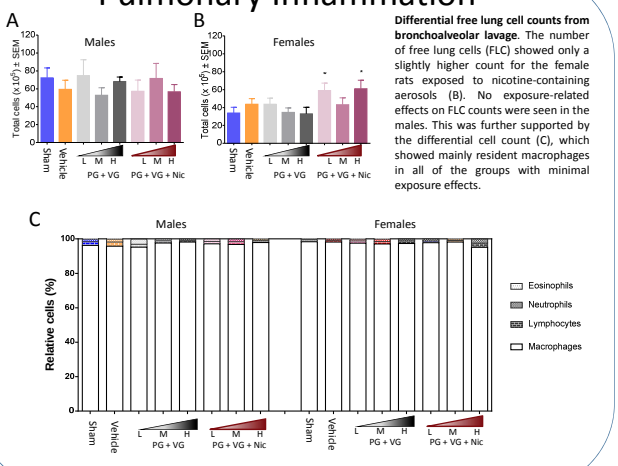
Conduct of exposure and aerosol uptake. Aerosol samples were collected four times per day from the breathing zone of each of the exposure chambers. Evaluation of the samples showed that over the course of the study, the target concentrations for each constituents were met in all chambers (A - C). Uptake of nicotine (D) and propylene glycol (E) was determined by measuring concentrations in plasma samples, 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.

Respiratory physiology



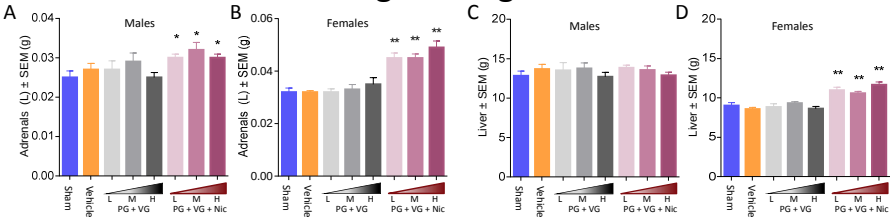
Breathing parameters in the animals during exposure. Respiratory physiology was performed once in the study using head-out plethysmography. The results showed a minimal effect of the nicotine-containing aerosol on the females (higher minute volume compared to the sham, panel B) but little effect on the other breathing parameters or on the male animals.

Pulmonary Inflammation



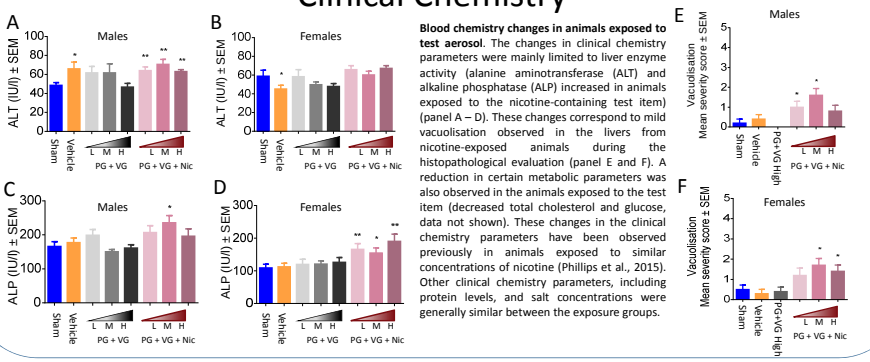
Differential free lung cell counts from bronchoalveolar lavage. The number of free lung cells (FLC) showed only a slightly higher count for the female rats exposed to nicotine-containing aerosols (B). No exposure-related effects on FLC counts were seen in the males. This was further supported by the differential cell count (C), which showed mainly resident macrophages in all of the groups with minimal exposure effects.

Organ Weights



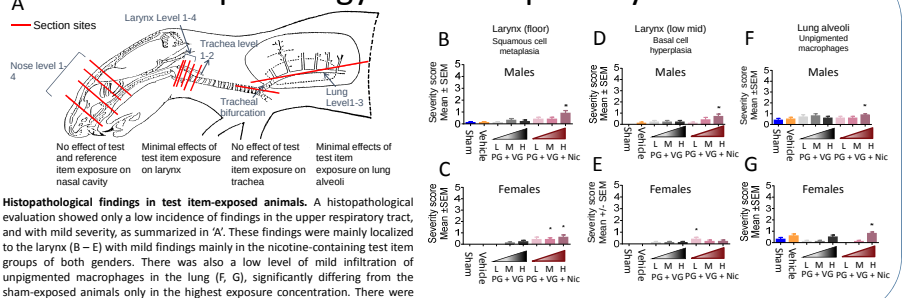
Test-item changes to the organ weights. Mainly nicotine-dependent organ weight effects were seen in the animals. This included increased absolute weight of the adrenals (A and B) and lower absolute weight of the thymus (not shown) in both sexes relative to the sham or vehicle group. These findings have been observed previously in conventional cigarette inhalation studies 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), 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. The changes in clinical chemistry parameters were mainly limited to liver enzyme activity (alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increased in animals exposed to the nicotine-containing test item) (panel A – D). These changes correspond to mild vacuolisation 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 in 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 evaluation showed only a low incidence of findings in the upper respiratory tract, and with mild severity, as summarized in 'A'. These findings were mainly localized to the larynx (B – E) with mild findings mainly in the nicotine-containing test item groups of both genders. There was also a low level of mild infiltration of unpigmented macrophages in the lung (F, G), significantly differing from the sham-exposed animals only in the highest exposure concentration. There were no other consistent or significant findings in the respiratory tract evaluation.

Conclusions

- Nebulization 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.
- All test 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 serum, and slight haematological changes.
- 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 vacuolization in the liver of 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.

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52nd Congress of the European Societies of Toxicology,
4th to the 7th September, 2016, Seville

This work was funded by Philip Morris International