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Abstract
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Characterization of Chronic Toxicity and Carcinogenicity of E-vapor Aerosol and Cigarette Smoke in A/J Mice

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Introduction and Objectives

Information on the toxicological profiles and potential carcinogenic properties of many chemicals used in e-vapor products is currently limited. In addition, while nicotine (N), a major component in nicotine-containing e-vapor products, is not considered carcinogenic, its oncogenic potential and relationship to cancer progression have been debated for years. In this study, we evaluated the chronic toxicity and carcinogenicity of select e-vapor flavor mixtures including carriers, with and without N.

Study Design

A/J mice (Jackson Laboratory, Bar Harbor, ME, USA) were whole-body exposed to filtered air (Sham), aerosol from the carriers propylene glycol (PG) and vegetable glycerol (VG), PG/VG with nicotine (PG/VG/N, 2% [w/w]), PG/VG/N with flavors at low, medium, and high concentrations ((PG/VG/N/F - 1.2 to 18.6% [w/w]), PG/VG/F-H, and to mainstream smoke from the 3R4F reference cigarette (3R4F) for 6 h/day, 5 days/week for up to 18 months. The target nicotine concentration in the test atmosphere was 15 µg/L. Based on structural grouping (Sciuscio *et al.*, Front. Toxicol. 2022, 4:1-15), we selected 38 flavor group representatives from a total of 245 flavor chemicals and combined them to prepare prototype e-liquid formulations. The study design (Figure 1) generally followed the OECD Test Guideline 453, with in-life measurements, histopathological evaluation, and clinical pathology as the key endpoints to evaluate respiratory tract and systemic toxicity and carcinogenicity. The care and use of mice were in accordance with the National Advisory Committee for Laboratory Animal Research Guidelines and approved by the Institutional Animal Care and Use Committee.

	Nicotine in test atmosphere	Flavor in formulation
SHAM	—	—
3R4F	15 µg/L	—
PG/VG	—	—
PG/VG/N	15 µg/L	—
PG/VG/F-H	—	18.6%
PG/VG/N/F-L	15 µg/L	1.2%
PG/VG/N/F-M	15 µg/L	4.6%
PG/VG/N/F-H	15 µg/L	18.6%

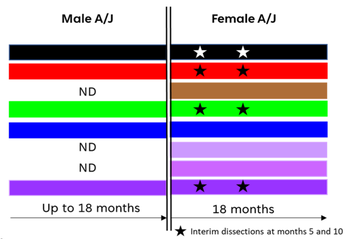


Figure 1. Schematic overview of the study design.
To maintain a sufficient number of mice at terminal dissection to adequately power the primary endpoints (N ≥ 50), the male mice were dissected beginning month 17, while female mice were dissected beginning month 18 of the study. PG: propylene glycol; VG, vegetable glycerol; N, nicotine; F-X, flavor-(concentration); L, low; M, medium; H, high; ND, not done.

Results – Survival and Bodyweights

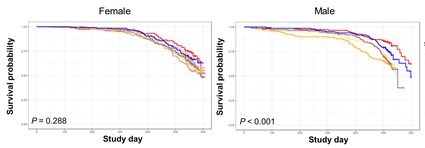


Figure 2: Survival probability analysis. *Data shown are survival fractions/probability for female (left) and male (right) mice using the Kaplan-Meier method. P-values were obtained by log-rank tests vs Sham.*

Note: Lower survival rate in the male PG/VG/F-H group compared to the Sham group was not completely attributed to flavor exposure because lower survival rates relative to the control groups were not observed in either the male PG/VG/N/F-H group or the female flavor e-vapor exposed groups.

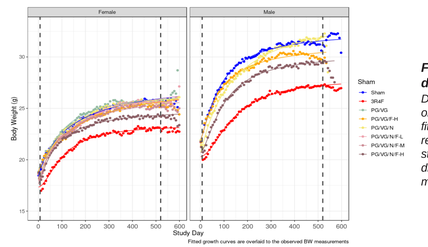
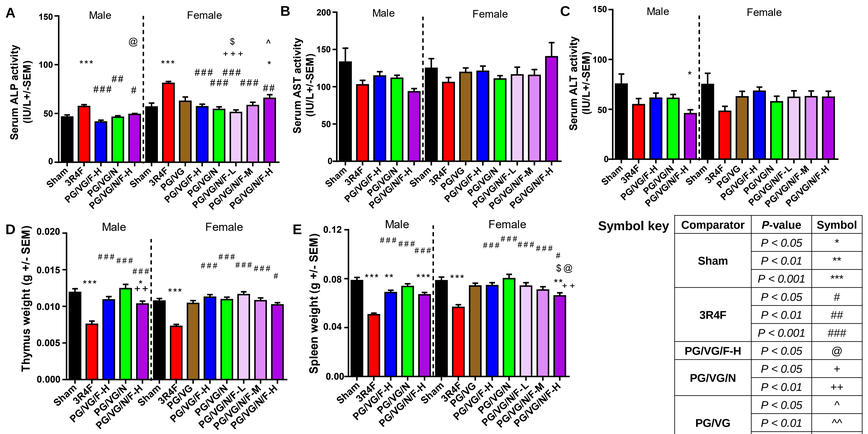


Figure 3: Bodyweight progression over study duration in female (left) and male (right) mice. *Data shown are average bodyweights (BW) recorded once a week during the study. Colored lines represent the fitted growth curves for each group. Dotted vertical lines represent the start (Day 1) and end (Day 520) of the study period used for growth curve analysis. Terminal dissections were performed on study days 499–599 for male mice and study days 536–605 for female mice.*

Results

1. Chronic exposure to e-vapor aerosol elicits markedly smaller systemic effects than cigarette smoke



Symbol key	Comparator	*P*-value	Symbol
	Sham	*P* < 0.05	*
		P < 0.01	**
		P < 0.001	***
	3R4F	*P* < 0.05	#
		P < 0.01	##
		P < 0.001	###
	PG/VG/F-H	*P* < 0.05	@
	PG/VG/N	*P* < 0.05	+
		P < 0.01	++
	PG/VG	*P* < 0.05	^
		P < 0.01	^^
		P < 0.001	^^^
	PG/VG/N/F-H	*P* < 0.05	$
		P < 0.01	$$
		P < 0.001	$$$

Figure 4: Selected systemic toxicity endpoints at month 18—Serum alkaline phosphatase (ALP) activity [A], serum aspartate aminotransferase (AST) activity [B], serum alanine aminotransferase (ALT) activity [C]; thymus [D] and spleen weights [E]. *Data shown are mean values; error bars represent standard error of the mean. N = 72–98 per male group; 62–76 per female group.*

2. Chronic e-vapor aerosol exposure with high flavor concentrations causes less severe nasal and laryngeal epithelial changes than cigarette smoke.

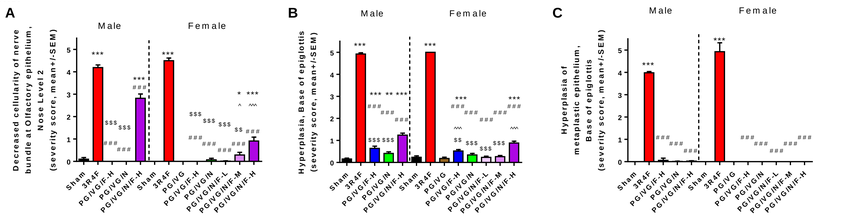


Figure 6: Local toxicity at month 18—Nose and Larynx: Decreased nerve bundles in the olfactory epithelium [A], hyperplasia [B], and squamous cell metaplasia at the base of the epiglottis [C]. *Data shown are mean severity scores, error bars represent standard error of the mean. N = 74–97 per male group; 61–79 per female group.*

3. Contrary to cigarette smoke, chronic e-vapor aerosol exposure does not result in inflammation and emphysematous changes in the lungs.

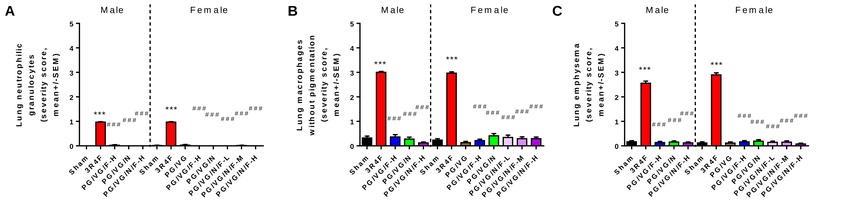


Figure 7: Local toxicity at month 18—Lung: Alveolar neutrophilic granulocytes [A] and non-pigmented macrophage [B] infiltrates, and alveolar emphysema (C). *Data shown are mean severity scores, error bars represent standard error of the mean. N = 74–97 per male group; 61–79 per female group.*

Results – Carcinogenicity

4. Chronic e-vapor aerosol exposure does not increase lung tumor incidences and multiplicities in A/J mice under the test conditions.

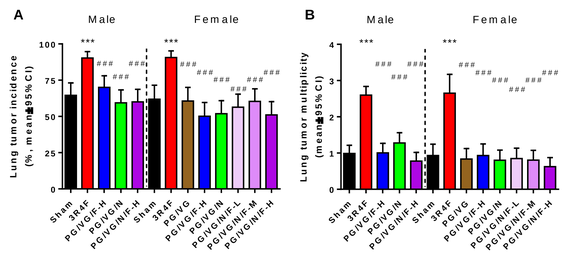


Figure 8: Preneoplastic and Neoplastic lesions at month 18—Lung tumor incidence [A] and multiplicity [B]. *Data shown are survival-adjusted means of incidences (left) and multiplicities (right) of bronchioloalveolar adenomas and bronchioloalveolar carcinomas; error bars represent 95% of confidence interval. N = 123–125 per male group; 97–128 per female group.*

5. Preneoplastic and neoplastic lesions outside the respiratory tract are in line with historical observations in this mouse strain and do not exhibit a clear treatment effect.

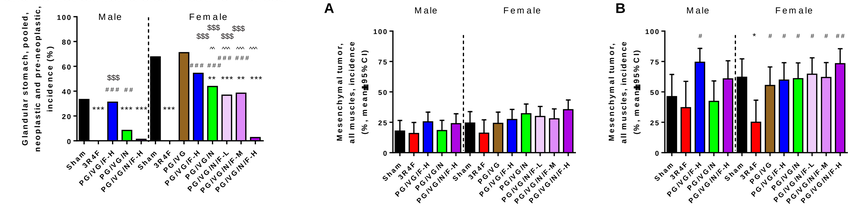


Figure 10: Preneoplastic and Neoplastic lesions at month 18—Glandular stomach*. *Data shown are mean incidences of pre-neoplastic (atypical diverticulum, focal hyperplasia) and neoplastic (adenoma) lesions combined. N = 74–98 per male group; 62–78 per female group.*
** These lesions are frequent features in long-term studies in A/J mice and within historical range.*

Figure 11: Preneoplastic and Neoplastic lesions at month 18—Mesenchymal tumors of the skeletal system*. *Data shown are survival-adjusted means of incidences in scheduled dissected and early death animals [A] and in early death animals [B] of rhabdomyo-, fibro-, osteo- and undifferentiated sarcoma; error bars represent 95% of confidence interval. N = 124–125 per male group, and 98–128 per female group [A], and N = 26–51 early deaths per male group, and N = 31–55 early deaths per female group [B].*

** The A/J mouse strain harbors a null mutation in the dysferlin (Dyfs) gene, therefore lacking Dyfs expression, and develops progressive muscular dystrophy, rhabdomyosarcomas and less differentiated mesenchymal tumors (Sundberg et al., Vet. Pathol. 1991, 28:200-206.; Sher et al., PLoS One 2011, 6:e23498.) Small, not statistically significant changes in scheduled dissected animals are within historical range.*

Summary and Conclusions

Long-term exposure to nicotine-containing e-vapor aerosols was generally well tolerated, with no significant differences in survival rates among the female groups. However, the male PG/VG/F-H group had a lower survival rate compared with the Sham group. Before the final dissection (at 17-18 months), body weights were lower in the male PG/VG/F-H and both male and female PG/VG/N/F-H groups. In contrast, all e-vapor aerosol groups had consistently higher body weights and fewer changes in clinical endpoints compared with the 3R4F groups. The typical changes in serum liver function parameters induced by cigarette smoke (3R4F) exposure were either less pronounced or not observed in any of the e-vapor groups. Reductions in thymus or spleen weights were also less significant in the PG/VG/F-H and PG/VG/N/F-H groups compared to the 3R4F groups (Wong *et al.*, SOT 2022, P180; Wong *et al.*, SOT 2024, P772). **Overall, systemic toxicity was consistently less pronounced or absent in the flavor aerosol groups compared with the 3R4F groups.**

Chronic exposure to PG/VG aerosol with and without nicotine had **no impact on lung tumor endpoints in this mouse strain** under the test conditions, indicating that nicotine did not initiate or promote tumorigenesis **under the test condition**. Neoplastic findings in non-respiratory tract organs, such as glandular stomach and skeletal system, were strain specific and not likely treatment effects (Wong *et al.*, SOT 2024, P772).

More notably, histopathological evaluations showed **no observable lung inflammation, emphysematous changes, or other microscopic changes indicative of local toxicity in the lungs** of animals exposed to e-vapor aerosols with or without nicotine, or with or without flavors. Local respiratory tract effects included the formation of eosinophilic globules and decreased epithelial cell density in the olfactory epithelium, which are common findings in aged rodents from chronic inhalation studies. Basal cell hyperplasia and squamous cell metaplasia were observed at the mid-base of the epiglottis, with slightly higher severity scores in the PG/VG/N and PG/VG/N/F-H groups than in the Sham and/or PG/VG groups, but consistently less severe than the scores from the 3R4F groups. The 3R4F groups showed various changes, including increased white blood cell counts, protein content, and matrix metalloprotease activity in the bronchoalveolar lavage fluid, neutrophil, lymphocyte, and macrophage infiltrates in the lungs, and alveolar tissue destruction indicative of emphysema (Wong *et al.*, SOT 2023, P534; Wong *et al.*, SOT 2024, P772).

Together, the data clearly demonstrate the reduced risk potential of flavored e-vapor products in this mouse model and under the study conditions, including nicotine in PG/VG, compared with combustible cigarettes and the potential role of e-vapor products in tobacco harm reduction.

Competing Financial Interest – The research in this poster was sponsored by Philip Morris Products S.A. and Altria Client Services LLC