Evaluation of Chronic Toxicity and Carcinogenicity of Flavored E-Vapor Aerosols in an 18-Month Inhalation Study in A/J Mice

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Introduction and Objectives

There is limited toxicological information to evaluate the chronic inhalation toxicity of e-vapor aerosols containing flavors, humectants, and nicotine. In this study, A/J mice were tested to evaluate and compare the local and systemic toxicity upon life-time exposure between cigarette smoke (CS) and aerosols from prototype e-liquid formulations containing 38 selected flavors, as well as nicotine and carriers.

Study Design

A/J mice (Jackson Laboratory, Bar Harbor, ME, USA) were whole-body exposed to air (Sham), aerosol from carriers propylene glycol (PG) and vegetable glycerol (VG), PG/VG with nicotine (N; 2% [w/w]), PG/VG/N with flavors (F) at low, medium and high concentrations (1.2 to 18.6% [w/w]), PG/VG/F-High or to mainstream smoke (MS) from the 3R4F reference cigarette for 6 h/day, 5 days/week for up to 18 months. The target nicotine aerosol concentration was 15 µg/L. The study design (Figure 1) generally followed the OECD TG453, with in-life measurements, histopathological evaluation, and clinical pathology as the key endpoints to evaluate respiratory tract and systemic toxicity and carcinogenicity. Care and use of mice was 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%

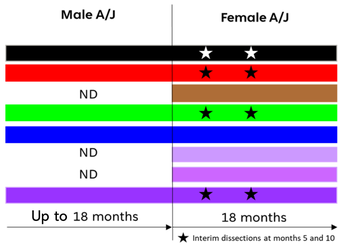

Figure 1. Schematic overview of study design.
To maintain a minimum number of mice for terminal dissection, the male mice were dissected beginning month 17, while female mice were dissected beginning month 18 of the study. PG: propylene glycol; VG, vegetable glycerol; N, nicotine; F-X, flavor-(concentration);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

Results – In-Life Measures

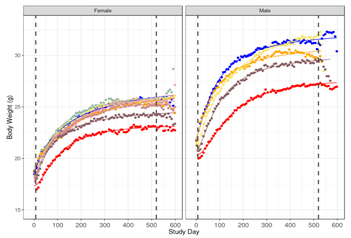

Figure 2: Bodyweight progression over study duration in female (left) and male (right) mice.
Data shown are average body weights recorded once a week during the study. Colored lines represent the fitted growth curves for each group. Dotted lines represent the start (day 1) and end (day 520) of the study 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.

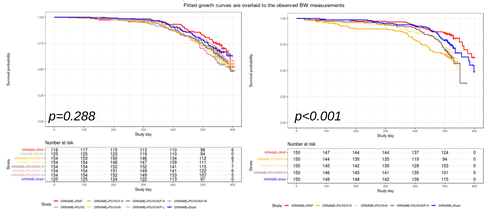

Figure 3: Survival probability analysis.
Data shown are survival fractions/probability for female (left) and male mice (right) using the Kaplan-Meier method. P-values were obtained by log-rank tests.

NB: Higher mortality rate in male PG/VG/F-H group relative to Sham group, driven mainly by euthanasia of moribund mice.

Results – Local Toxicity

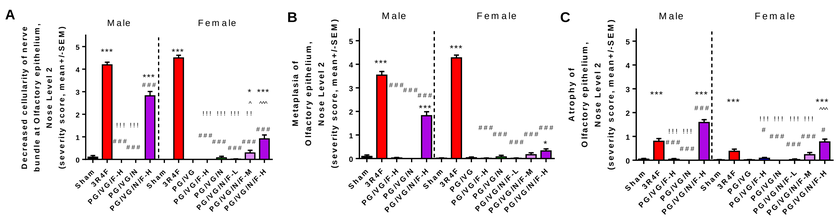

Figure 4: Histopathological evaluation at month 18: Changes in the nasal olfactory epithelium. Decreased cellularity of nerve bundles (A), metaplasia (B), and atrophy (C).
*Data shown are mean severity scores (grade 1: minimal, grade 2: slight, grade 3: moderate, grade 4: marked, grade 5: severe); error bars represent standard error of the mean. N= 74–98 per male groups; 62–78 per female groups. * p<0.05 vs sham; *** p<0.001 vs sham; # p<0.05 vs 3R4F; ### p<0.001 vs 3R4F; ^ p<0.05 vs PG/VG; ^^^ p<0.001 vs PG/VG; !! p<0.01 vs PG/VG/N/F-H; !!! p<0.001 vs PG/VG/N/F-H.*

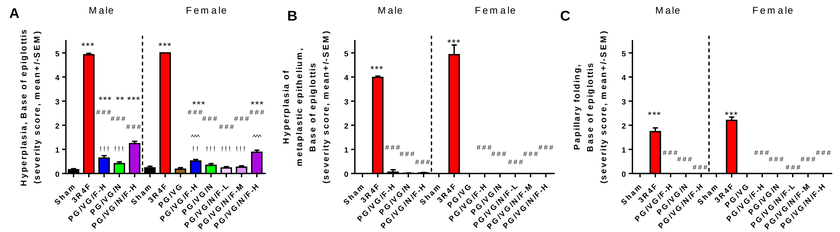

Figure 5: Histopathological evaluation at month 18: Epithelial changes at the mid-base of the epiglottis. Hyperplasia (A), hyperplasia of the metaplastic epithelium (B), and papillary folding (C).
*Data shown are mean severity scores (grade 1: minimal, grade 2: slight, grade 3: moderate, grade 4: marked, grade 5: severe); error bars represent standard error of the mean. N= 72–98 per male groups; 62–76 per female groups. ** p<0.01 vs sham; *** p<0.001 vs sham; ### p<0.001 vs 3R4F. *** p<0.001 vs sham; ^^^ p<0.001 vs PG/VG; !! p<0.01 vs PG/VG/N/F-H; !!! p<0.001 vs PG/VG/N/F-H.*

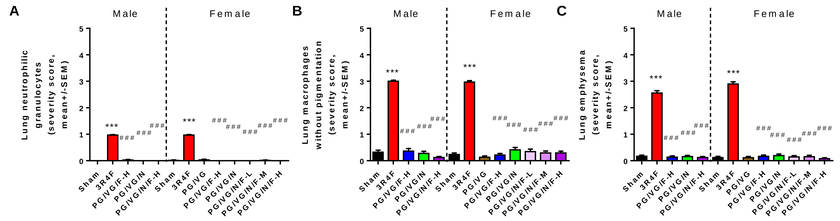

Figure 6: Histopathological evaluation at month 18: Alveolar neutrophilic granulocytes (A) and non-pigmented macrophage (B) infiltrates, and alveolar emphysema (C).
*Data shown are mean severity scores (grade 1: minimal, grade 2: slight, grade 3: moderate, grade 4: marked, grade 5: severe); error bars represent standard error of the mean. N= 74–97 per male groups; 61–79 per female groups. *** p<0.001 vs sham; ### p<0.001 vs 3R4F.*

Results – Carcinogenicity

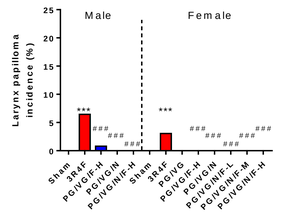

Figure 7: Histopathological evaluation at month 18: Larynx papilloma.
*Data shown are mean incidences. N= 74–98 per male groups; 62–78 per female groups. *** p<0.001 vs sham; ### p<0.001 vs 3R4F*

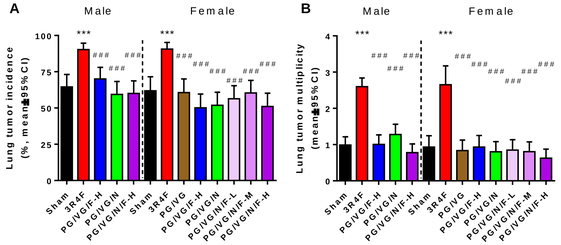

Figure 8: Histopathological evaluation at month 18: Lung tumor incidence (A) and multiplicity (B).
*Data shown are survival-adjusted means of incidences (left) and multiplicities (right) of bronchiolo-alveolar adenomas and bronchiolo-alveolar carcinomas; error bars represent 95% of confidence interval. N= 123–125 per male groups; 97–128 per female groups. *** p<0.001 vs sham; ### p<0.001 vs 3R4F.*

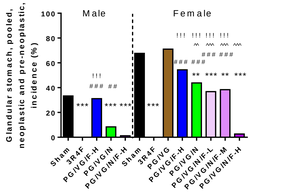

Figure 9: Histopathological evaluation at month 18: Pre-neoplastic and neoplastic lesions of the glandular stomach.
*Data shown are mean incidences of pre-neoplastic (atypical diverticulum, focal hyperplasia) and neoplastic (adenoma) lesions combined. N= 74–98 per male groups; 62–78 per female groups. ** p<0.01 vs sham; *** p<0.001 vs sham; ## p<0.01 vs 3R4F; ### p<0.001 vs 3R4F; ^^ p<0.01 vs PG/VG, ^^^ p<0.001 vs PG/VG; !!! p<0.001 vs PG/VG/N/F-H.*

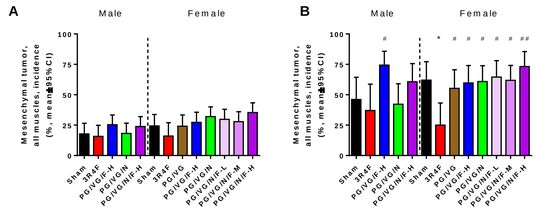

Figure 10: Histopathological evaluation at month 18: Strain-specific pathology.* Mesenchymal tumors of the skeletal system.
*Data shown are survival-adjusted means of incidences in scheduled dissected and early deaths animals (A) and in early death animals (B) of rhabdomyo-, fibro-, osteo- and undifferentiated sarcoma; error bars represent 95% of confidence interval. N= 124–125 per male groups, and 98–128 per female groups (left), and N= 26–51 early deaths per male group, and N= 31–55 early deaths per female group (right). * p<0.05 vs sham; # p<0.05 vs 3R4F; ## p<0.01 vs 3R4F.*

**) A/J mice develop skeletal muscle tumors at advanced age (Sundberg et al. Vet Pathol 28.3 (1991): 200-206.; Sher et al. PLoS One 6.8 (2011): e23498.)*

Summary and Conclusions

Overall, chronic inhalation exposure to PG/VG/N/F e-vapors did not impact the mortality, lung function, clinical pathology or macroscopic findings (shown previously in Wong et al. (2022) P180). Amongst all treated groups, the CS groups showed the most notable lung inflammation, lung emphysema, increased lung tumor and laryngeal papilloma development, moderate to severe respiratory epithelial (nasal) findings; altered lung function, serum liver function, erythrocyte parameters and atrophy of the thymus (shown previously in Wong et al. (2023) P534). Slightly suppressed body weight gain was noted in the male and female PG/VG/N/F-H groups without obvious reduction in food consumption. In contrast to CS-exposed groups, lung histological changes were absent or minimal in the tested e-vapor groups, with notable microscopic findings limited in the larynx and nose. Laryngeal changes were overall minimal to mild in the PG/VG/N/F-High groups, were of lower severity compared to 3R4F groups and considered adaptive changes. Olfactory epithelial (nasal) findings were observed in 3R4F and PG/VG/N/F groups and are considered degenerative changes, potentially related to irritation from repeated exposures. Findings in non-respiratory organs, such as glandular stomach and skeletal system, were strain-specific and not likely treatment effects.
To conclude, the tested e-vapor aerosols (with exaggerated flavor loads up to 18%) were irritating to the nose—the local site of entry; the recommended LOAEL based on (potentially progressive) degenerative changes in the nose is therefore the MEDIUM flavor concentration. However, changes in the rest of the respiratory tract were consistently and significantly less severe compared to CS effects. In the target organ lung, chronic exposure to e-vapor aerosols up to 18-month did not induce notable toxicity or carcinogenicity compared to air controls. The recommended LOAEL based on the combined lung findings is therefore the HIGH flavor concentration. In addition, chronic exposures to carriers or nicotine alone did not induce notable toxicity or carcinogenicity in the lung. The study results support the reduced toxicity and carcinogenicity of e-vapor aerosols compared to combustible CS and the potential role of e-vapor products in tobacco harm reduction.

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