Evaluation of respiratory tract organ toxicity and carcinogenicity of e-vapor aerosols in an 18-month inhalation study in A/J mice

E. Wong*, K. Luettich#, L.R. Demenescu#, A. Kondylis#, J. Zhang@, D Sciuscio#, M. Peitsch#, K. M. Lee@, P. Vanscheeuwijck#.
* PMI R&D, Philip Morris International Research Laboratories Pte Ltd, Singapore
# PMI R&D, Philip Morris Products S.A., Neuchâtel, Switzerland
@ Altria Client Services LLC, Richmond, VA, U.S.A.

Poster P534

Introduction and Objectives

There is limited toxicological information on the long-term inhalation exposures of e-vapor aerosols containing various flavors, humectants, and nicotine. Based on structural grouping [2, 3], we selected 38 flavor group representatives from a total of 245 flavor chemicals, combined to prepare a prototype e-liquid formulation and tested in an 18-month inhalation study in A/J mice. The objectives were to evaluate respiratory tract organ toxicity as well as lung tumor incidence and multiplicity upon life-time exposure to cigarette smoke or to aerosols from the prototype e-liquid formulation containing 38 selected flavors [2].

Study Design & Endpoints

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 (a total flavor load of 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. Urine and plasma analytes (nicotine, cotinine and selected flavors) were measured by chromatographic quantification. The study design generally followed the OECD TG453 [1], with histopathological evaluation, lung function (month 5) and morphometric measurements as the key endpoints to evaluate respiratory tract toxicity and carcinogenicity. Care and use of the mice was in accordance with the National Advisory Committee for Laboratory Animal Research Guidelines and approved by the Institutional Animal Care and Use Committee.

	Short Name	Chamber Name	Target nicotine in test atmosphere	Flavor in formulation
SHAM	SHAM	1A/B	--	--
3R4F	3R4F	2A/B/C	15 µg/L	--
PG/VG	PG/VG	3	--	--
PG/VG/F-H	F-H	4A/B	--	18.6%
PG/VG/N	Nic	5A/B/C	15 µg/L	--
PG/VG/N/F-L	N-F(L)	6	15 µg/L	1.2%
PG/VG/N/F-M	N-F(M)	7	15 µg/L	4.6%
PG/VG/N/F-H	N-F(H)	8A/B/C	15 µg/L	18.6%

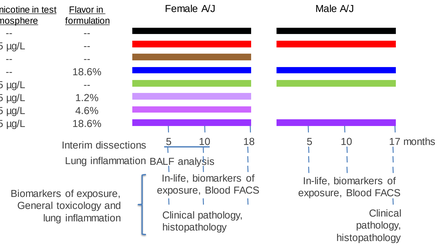

Figure 1. *Schematic overview of study design and endpoints.*

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.

Results
Test Atmosphere and Biomarkers of Exposure

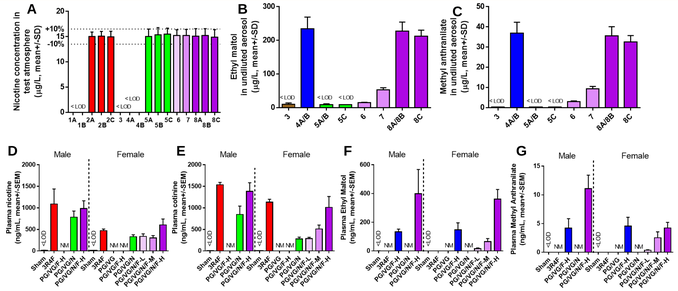

Figure 2*: Test atmosphere characterization and biomarkers of exposure*

Upper panel: *Aerosol concentrations of (A) nicotine, (B) ethyl maltol, and (C) methyl anthranilate. Data are presented as mean ± SD. Dotted lines indicate ±10% of target nicotine concentration. N=89-442 per chamber for nicotine and N=5-20 per chamber for flavor compounds.*

Lower panel*: Plasma concentrations of (D) nicotine, (E) cotinine, (F) ethyl maltol, and (G) methyl anthranilate. Data are presented as mean ± SEM. N=6-8 per study group for plasma biomarkers. LOQ: Limit of quantification; LOD, Limit of detection; NM: Not measured.*

Results (Lung Evaluation)

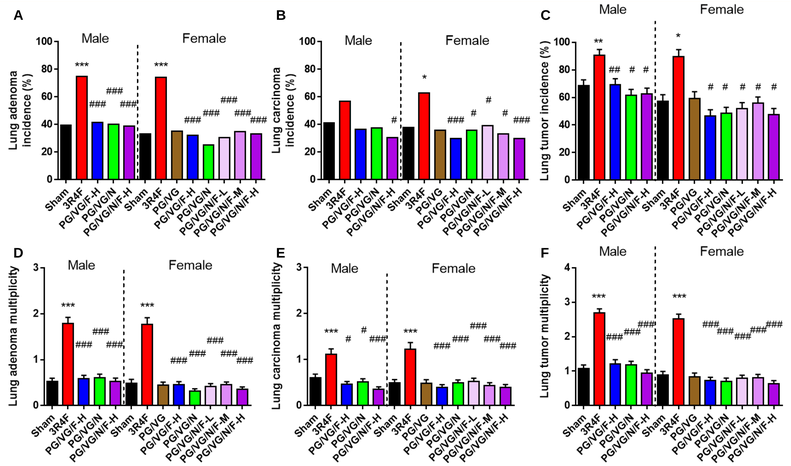

Figure 3*: Histopathological assessment of preneoplastic and neoplastic lung lesions*

Survival-adjusted incidences of (A) lung adenoma, (B) lung carcinoma, (C) lung tumors (adenoma or carcinoma), and survival-adjusted multiplicity of (D) lung adenoma, (E) lung carcinoma, and (F) lung tumors at terminal timepoints (months 17-18).

*Hematoxylin & eosin-stained step-serial lung sections were evaluated. Data are presented as mean ± SEM. N=97–128/group. *, **, *** p<0.05, p<0.01, p<0.001 vs sham; #, ##, ### p<0.05, p<0.01, p<0.001 vs 3R4F.*

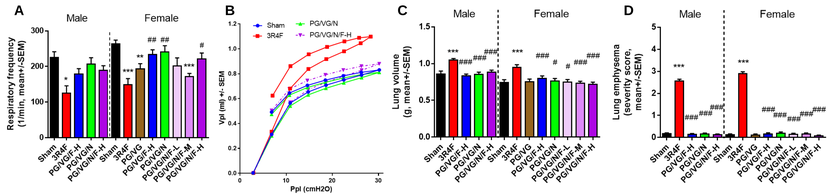

Figure 4*: Assessment of lung function and emphysema*

(A) Respiratory frequency, and (B) pressure-volume loop lung function test (month 5), (C) lung volume of whole lung and (D) histopathology assessment of emphysema in left lungs at terminal timepoints (months 17-18).

*Histopathology assessment was with severity scores 0 to 5, with 0 indicating findings within normal limits; 1, minimal changes; 2, mild changes; 3, moderate changes; 4, marked changes; and 5, severe changes. Data are presented as mean ± SEM. N=9–12 per study group for lung function tests, N=14–27 for lung volume measurement and N=61-97 for histopathology assessment. *, **, *** p<0.05, p<0.01, p<0.001 vs sham; #, ##, ### p<0.05, p<0.01, p<0.001 vs 3R4F.*

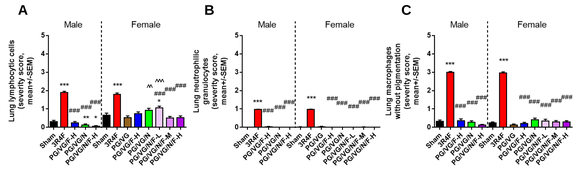

Figure 5*: Histopathological assessment of lung inflammation (left lung)*

Severity scores for histopathological findings of (A) lymphocytic cells in lung interstitium, (B) neutrophilic granulocytes and (C) macrophages in the lumens of alveoli at terminal timepoints (months 17-18).

*Data are presented as mean ± SEM. N=61–97 per study group. *, **, *** p<0.05, p<0.01, p<0.001 vs sham; ### p<0.001 vs 3R4F; ^^, ^^^ p<0.01 and p<0.001 vs PG/VG.*

Results (Larynx and Nose evaluation)

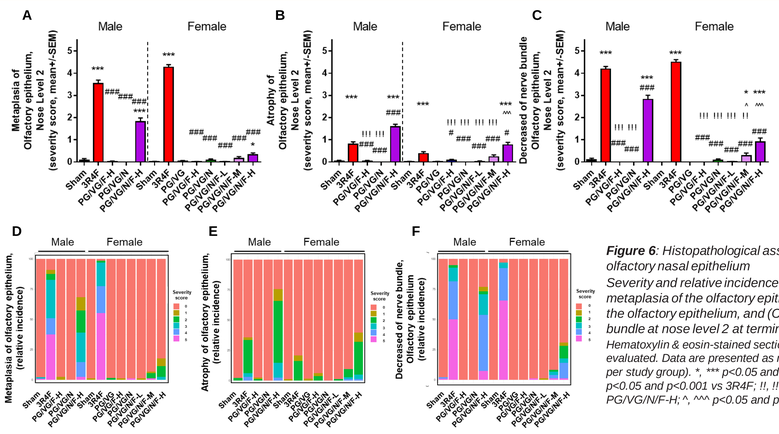

Figure 6*: Histopathological assessment of the olfactory nasal epithelium*

Severity and relative incidence of (A, D) squamous metaplasia of the olfactory epithelium, (B, E) atrophy of the olfactory epithelium, and (C, F) decrease in nerve bundle at nose level 2 at terminal timepoints.

*Hematoxylin & eosin-stained sections of the nose were evaluated. Data are presented as mean ± SEM. (N=62–98 per study group). *, *** p<0.05 and p<0.001 vs sham; #, ### p<0.05 and p<0.001 vs 3R4F; !!, !!! p<0.01, p<0.001 vs PG/VG/N/F-H; ^, ^^^ p<0.05 and p<0.001 vs PG/VG..*

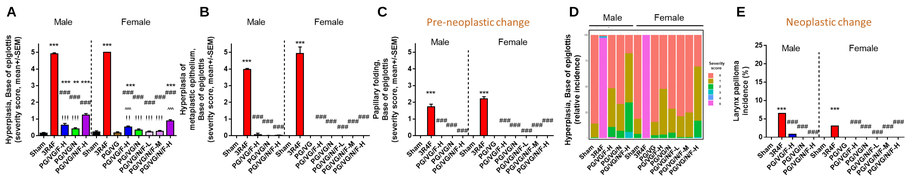

Figure 7*: Histopathological assessment of the larynx*

Severity of (A) hyperplasia, (B) hyperplasia of the metaplastic epithelium, and (C) papillary folding at the base of the epiglottis; (D) incidence of hyperplasia at the base of epiglottis, and (E) incidence of papilloma at the floor of the arytenoid projections at terminal timepoints.

*Hematoxylin & eosin-stained sections of the larynx were evaluated. Data are presented as mean ± SEM. N=62–98 per study group. **, *** p<0.01, p<0.001 vs sham; ### p<0.001 vs 3R4F; !!, !!! p<0.01, p<0.001 vs PG/VG/N/F-H; ^^^ p<0.001 vs PG/VG.*

Conclusions

Aerosol atmosphere was stable throughout the study and animals were consistently exposed based on biomarkers of exposure. Exposure to e-vapor aerosols resulted in minimal or no changes in respiratory rate, lung function, lung inflammatory and emphysema-related parameters, as well as lung tumor incidence and multiplicity compared to the Sham group. In contrast, exposure to cigarette smoke (CS) suppressed the respiratory rate, decreased lung function, led to pulmonary inflammation and emphysematous changes, and increased lung tumor incidence and multiplicity compared to the Sham group. Histopathological evaluation of the respiratory tract also showed severe changes and papilloma development in the larynx in the CS group. In contrast, laryngeal histopathological changes were only observed in the high flavor e-vapor groups, with significantly lower severity scores and incidence compared to the CS groups. Nasal and pulmonary changes—when present in the e-vapor groups — were also less severe and less frequent than in the CS groups. In addition, there appears to be some effects of high-flavors and nicotine among e-vapor aerosols. In summary, chronic exposure to e-vapor aerosols under the tested conditions showed consistently reduced toxicity and carcinogenicity responses in the respiratory tract compared to CS exposure, supporting their potential role in tobacco harm reduction. To use a read-across approach based on the 38 selected flavors tested in the current study needs to be further validated.

References

1. OECD 2018, OECD Test Guideline 453: Combined chronic toxicity/ carcinogenicity studies.
2. Sciuscio *et al* 2022. Toxicological assessment of flavor ingredients in E-Vapor products. Front Toxicol 2022, 4: 1-15.
3. Wong et al, 2022. Assessment of inhalation toxicity of cigarette smoke and aerosols from flavor mixtures: 5-week study in A/J mice. J Appl Toxicol 2022, 42(10): 1701-1722.

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