Evaluation of chronic inhalation toxicity of e-vapor aerosols in 18-month study in A/J mice

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Poster P180

Introduction and Objectives

There is limited information on the inhalation toxicity especially after repeated exposures of the flavor ingredients in e-liquid formulations. Based on structural grouping, we selected 38 flavor group representatives from a total of 245 flavor chemicals used by PMI and ALCS, combined as prototype e-liquid formulations and tested in an 18-month inhalation study in AJ mice. The objectives were to characterize and compare the potential impact of flavor containing e-vapor aerosols to the chronic inhalation toxicity of cigarette smoke.

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 (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 aerosol nicotine concentration was 15 µg/L. Mice were evaluated for systemic toxicity (clinical signs, body weight, survival). Lung inflammation was evaluated by bronchoalveolar lavage fluid (BALF) analysis and histopathology evaluation. Blood samples were evaluated by FACS and Sysmex XN-1000 automated cell counter. Serum clinical chemistry was obtained using UniCel® DXC 600 clinical analyzer. Care and use of the mice was in accordance with the National Advisory Committee for Laboratory Animal Research Guidelines (2004). All animal experiments were approved by the Institutional Animal Care and Use Committee (P15055).

	Short Name	Target nicotine in test atmosphere	Flavor in formulation
SHAM	SHAM	--	--
3R4F	3R4F	15 µg/L	--
PG/VG	PG/VG	--	--
PG/VG/F-H	F-H	--	18.6%
PG/VG/N	Nic	15 µg/L	--
PG/VG/N/F-L	N-F(L)	15 µg/L	1.2%
PG/VG/N/F-M	N-F(M)	15 µg/L	4.6%
PG/VG/N/F-H	N-F(H)	15 µg/L	18.6%

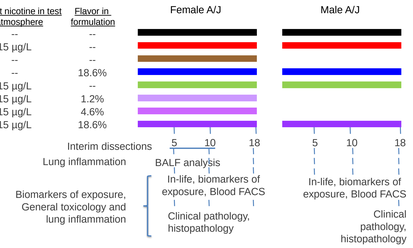


Figure 1. *Schematic overview of study design and endpoints.*

Test Atmosphere Characterization

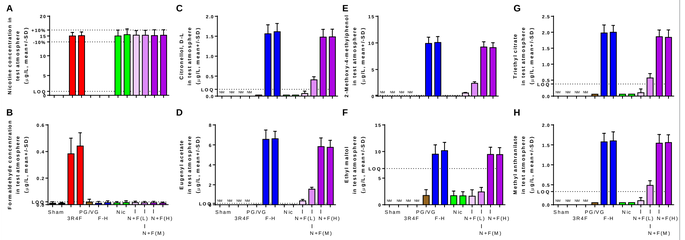


Figure 2*: Test atmosphere characterization*

Concentrations of (A) Nicotine, (B) formaldehyde, and selected flavor chemicals: (C) citronellol, D.L., (D) eugenyl acetate, (E) 2-methoxy-4-methylphenol, (F) ethyl maltol, (G) triethyl citrate and (H) methyl anthranilate in the test atmosphere. Data are presented as means [µg/L] ± SD. The target nicotine concentration was +/-10% of 15 µg/L. LOQ: Limit of quantification; NM: Not measured, PG: propylene glycol; VG, vegetable glycerol; N, nicotine; F-X, flavor-(concentration); L, low; M, medium; H, high.

Results

1. Test Atmosphere Uptake (Biomarkers of Exposure)

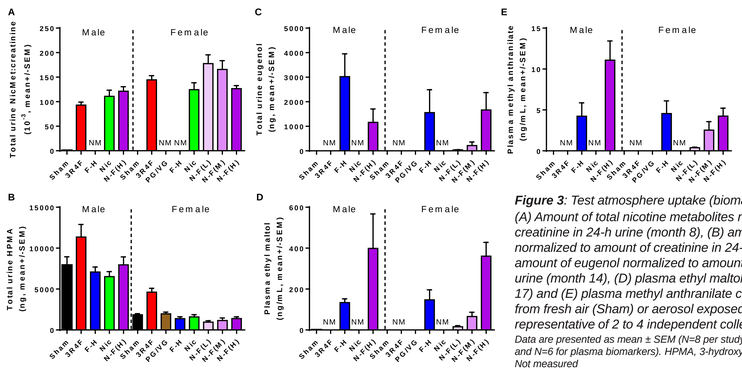
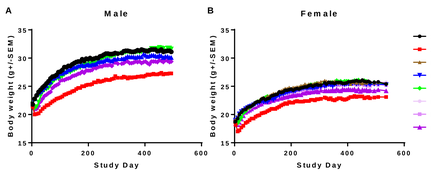


Figure 3*: Test atmosphere uptake (biomarkers of exposure)*

(A) Amount of total nicotine metabolites normalized to amount of creatinine in 24-h urine (month 8), (B) amount of HPMA normalized to amount of creatinine in 24-h urine (month 3), (C) amount of eugenol normalized to amount of creatinine in 24-h urine (month 14), (D) plasma ethyl maltol concentration (month 17) and (E) plasma methyl anthranilate concentrations (month 17) from fresh air (Sham) or aerosol exposed A/J mice. Urine data are representative of 2 to 4 independent collections in the study.

Data are presented as mean ± SEM (N=8 per study group for urine biomarkers and N=6 for plasma biomarkers). HPMA, 3-hydroxypropylmercapturic acid, NM: Not measured

2. Bodyweight



3. Survival

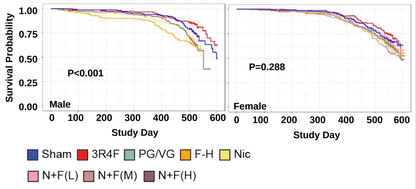


Figure 4*: In-life body weight progression in 18-month study*

(A) Male and (B) female mice were exposed to fresh air (Sham) or aerosol exposed groups. Body weights were taken once per week and data are presented as mean body weights for each group.

Figure 5*: Kaplan-Meier survival curves*

Survival fractions in 18-month study were determined for male (left) and female (right) mice. Statistical significance and P-values were determined using the log-rank test.

4. Lung Inflammation (female)

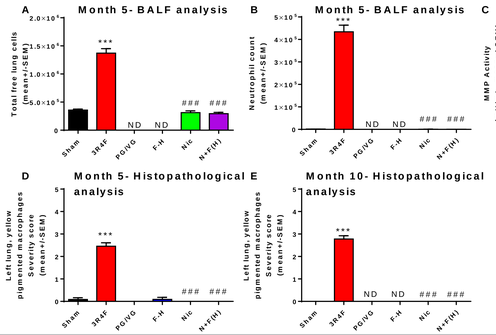


Figure 6: *Lung inflammation*

BALF analysis included (A) total free lung cell counts, (B) neutrophil counts, and (C) matrix metalloproteinase (MMP) activity performed at month 5. Histopathology evaluation of the left lung (hematoxylin–eosin stained slides) was performed at (D-E) months 5 and 10, with severity scored 0 to 5. Only female groups were included in months 5 and 10 interim dissection.

*Data are presented as means ± SEM (N=9-12 per study group. *** p<0.001 vs sham; ### p<0.001 vs 3R4F. BALF, bronchoalveolar lavage fluid; ND: Not determined.*

Results (continued)

5. Hematology and Organ Weights (at terminal dissection months 17 to 18)

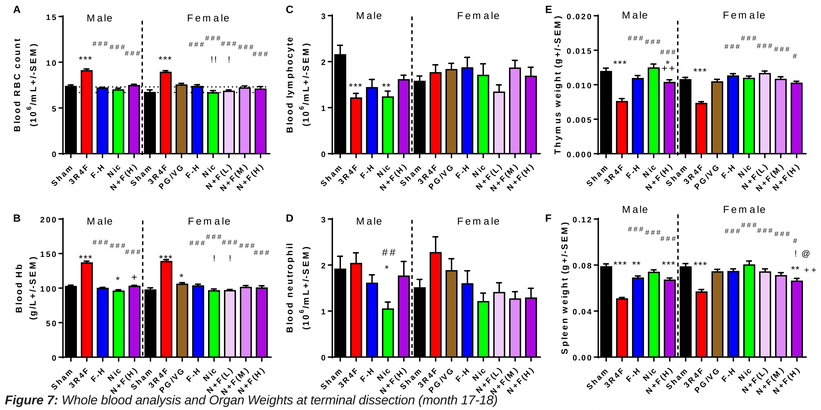


Figure 7: *Whole blood analysis and Organ Weights at terminal dissection (month 17-18)*

Whole blood analysis using Sysmex automated cell counter included (A) erythrocyte counts, (B) hemoglobin concentration, (C) total lymphocyte counts and (D) neutrophil counts. Selected organ weights included (E) thymus and (F) spleen weights.

*Group size was at least N=13 per study group. * p<0.05 vs sham; ** p<0.01 vs sham; *** p<0.001 vs sham; # p<0.05 vs 3R4F; ## p<0.01 vs 3R4F; ### p<0.001 vs 3R4F; @ p<0.05 vs PG/VG/F-H; + p<0.05 vs PG/VG/N; ++ p<0.01 vs PG/VG/N; ! p<0.05 vs PG/VG; !! p<0.01 vs PG/VG.*

6. Serum Clinical Chemistry (at month 10 and terminal dissection)

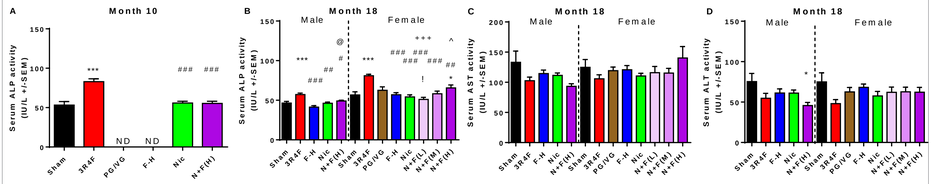


Figure 8: *Serum alkaline phosphatase (ALP) activity was determined at months (A) 10 and (B) 18, while (C) serum aspartate aminotransferase (AST) and (D) alanine aminotransferase (ALT) were from month 18. Only female groups were included in month 10 interim dissection.*

Group size was at least N=9 per study group. p<0.05 vs sham; *** p<0.001 vs sham; # p<0.05 vs 3R4F; ## p<0.01 vs 3R4F; ### p<0.001 vs 3R4F; +++ p<0.001 vs PG/VG/N/F-H; ^ p<0.05 vs PG/VG/N; @ p<0.05 vs PG/VG/F-H; ! p<0.05 vs PG/VG.*

Conclusions

Aerosol was reproducibly generated and delivered to the animals. Body weights before terminal dissection (month 17-18) were lower in the male PG/VG/F-High and male and female PG/VG/N/F-High groups compared to the Sham group; however, all the e-vapor aerosol groups showed consistently higher body weights compared to the 3R4F group. Exposures were well tolerated with minimal clinical signs, mainly transient tremor observed post-exposure in a few nicotine with high flavor aerosol exposed mice. There were no exposure-related differences in survival rates amongst the female groups, but the survival rate was lower in the male PG/VG/F-High group. In contrast to 3R4F exposure, exposure to the e-vapor aerosols did not cause notable lung inflammation. Typical 3R4F-induced changes in serum liver function parameters (Fig 8) were either less pronounced or no difference in the e-vapor aerosol groups as compared to the 3R4F groups. Reduction in thymus or spleen weights in the high flavor groups were less pronounced as compared to the 3R4F groups. In summary, overall systemic toxicity are less pronounced (compared to 3R4F) or absent in the flavor aerosol groups. Furthermore, chronic exposure to flavor e-vapor aerosols under the tested condition did not result in notable lung inflammation (pending confirmation of terminal histopathology results in Q2 2022).

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