A Seven Month Systems Toxicology Inhalation Study in C57BL/6 Mice Demonstrates Reduced Pulmonary Inflammation and Emphysema Following Smoking Cessation or Switching to E-vapor Aerosol Exposures

Ashutosh Kumar,[1] Ulrike Kogel,[2] Marja Talikka,[2] Anthony Skowronek,[3] Julia Hoeng,[2] Manuel Peitsch,[2] and K. Monica Lee[1]

[1]Altria Client Services LLC, Richmond, VA
[2]Philip Morris International R&D, Neuchâtel, Switzerland;
[3]Battelle, West Jefferson, OH

73rd TSRC - Tobacco Science Research Conference
September 15-18, 2019, Leesburg, VA, USA

WWW.ALTRIA.COM/ALCS-SCIENCE

ABSTRACT

Cigarette smoking causes lung cancer, heart disease, emphysema, and other serious diseases. While cessation remains the most effective approach to minimize smoking-related diseases, alternative nicotine delivery products that limit the generation of combustion by-products may offer reduced risk to those who would otherwise continue to smoke. E-vapor products are one set of such promising nicotine-delivery products. E-vapor aerosols typically contain significantly reduced levels of smoking-related harmful and potentially harmful chemicals; however, the health risks of long-term inhalation exposures are unknown. We designed a chronic inhalation(4 h/day, 5 d/week, 7 months) study in C57BL/6 mice to evaluate the long-term respiratory toxicity of MarkTen® e-vapor aerosols in comparison to the reference 3R4F cigarette smoke (CS). Additional groups were added to explore the impact of CS cessation or switching to e-vapor exposures after 3 months of CS exposure. There were no significant changes in in-life (body weights, clinical signs) observations in e-vapor groups compared to the sham control. The CS group had lower body weight and showed transient signs of distress post-exposure and reduced respiratory function during exposure. Following 7 months of exposure, e-vapor resulted in no or minimal increase in pulmonary inflammation, while the CS induced consistently elevated pulmonary inflammation (infiltration of activated macrophages, T and B cells, neutrophils) and emphysema. Biological changes in the switching group were similar to those in the cessation group. Transcriptomics analysis showed that the CS exposure elicited a large number of differentially expressed genes (DEG) in the lung and nasal epithelium, while drastically reduced gene expression changes were observed in response to e-vapor exposure. Compared with the CS group, the number of DEGs was much smaller in the lungs of the switching or cessation groups, while the reduction in nasal epithelium was less pronounced in the switching or cessation groups. The liver showed (data not shown) transcriptomic changes (related to steroid biosynthesis) in response to e-vapor aerosol exposure at Month 4 but not at Month 7. The majority of these genes were also differentially expressed in the liver of the CS group. In conclusion, chronic exposure to e-vapor aerosol in the context of this study resulted in minimal pathophysiological and largely reduced gene expression changes in mouse respiratory tissues, which were significantly lower than changes induced by CS exposure. In conclusion, this in vivo model system demonstrates reduced pulmonary inflammation and emphysema following smoking cessation or switching to e-vapor aerosol exposure.

METHODS

11-week-old female C57BL/6 mice were exposed to cigarette smoke (3R4F; 550 µg/L TPM (Total particulate matter) or e-vapor aerosol (MarkTen® Test Red; 1100 µg/L TPM) via nose-only inhalation for 4 hours/day, 5 days/week, for up to 7 months. After 3 months of exposure 2 groups of 3R4F mice were exposed to 1) Test Red ("Switching") or 2) filtered air ("Cessation"), while a control group was maintained on 3R4F (Study Design: see Table 1).

Mice were examined for in-life observations (clinical signs, body weights). At 3, 4, and 7 months cohorts were evaluated for various biological endpoints including respiratory physiology, biomarkers of exposure (plasma nicotine and cotinine), histopathology, bronchoalveolar lavage (BAL) fluid analysis, and transcriptomics (lung, nasal epithelium, liver, and heart).

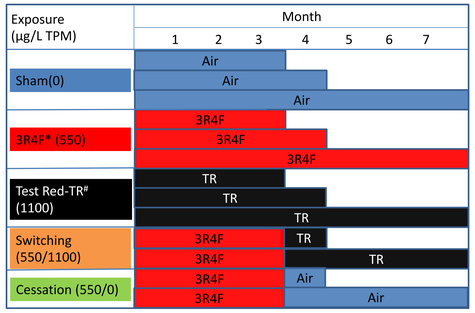

Table 1. Study Design

*Exposure regimen for 3R4F (Health Canada Intense):55±0.3 mL/puff, 30-sec interpuff interval, 2-sec puff duration for 8 puffs/cigarette; #Exposure regimen for Test Red (modified CRM81): 55±0.3 mL/puff, 30-sec interpuff interval, 5-sec puff duration for 130 puffs/cartridge

Table 2. Smoke/Aerosol Characterization

Exposure Parameter (mean ± SD)	GROUPS		
	Sham (Air) Control (0 µg TPM/L)	3R4F Cigarette (550 µg TPM/L)	Test Red (1100 µg TPM/L)
Total Particulate Matter [µg/L]	0±0	540±15	1091±38
Carbon Monoxide [ppm]	NM	643±21	NM
Nicotine [µg/L]	ND	42.1±2.9	29.4±2.3
Propylene Glycol [µg/L]	ND	BLOQ	205±22
Glycerol [µg/L]	ND	59.7±3.6	788±40
Particle Size (MMAD [µM] ± GSD)	NM	0.66±1.4	1.1±1.5

NM = Not Measured; ND = Not Detected; BLOQ = Below lower limit of quantification

RESULTS

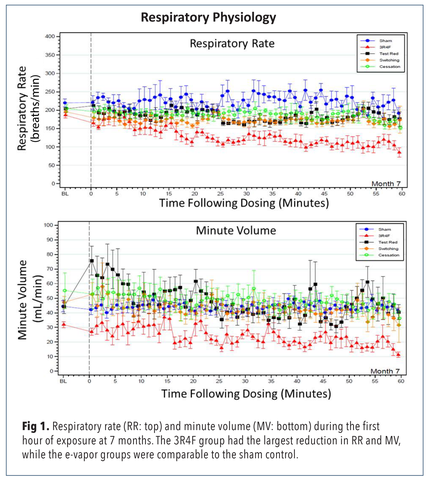

Fig 1. Respiratory rate (RR: top) and minute volume (MV: bottom) during the first hour of exposure at 7 months. The 3R4F group had the largest reduction in RR and MV, while the e-vapor groups were comparable to the sham control.

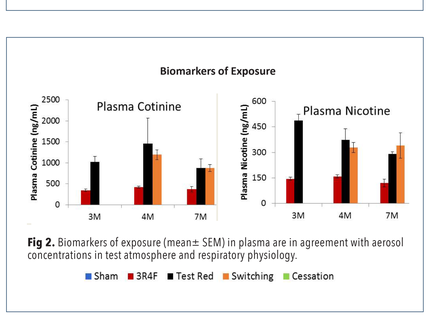

Fig 2. Biomarkers of exposure (mean± SEM) in plasma are in agreement with aerosol concentrations in test atmosphere and respiratory physiology.

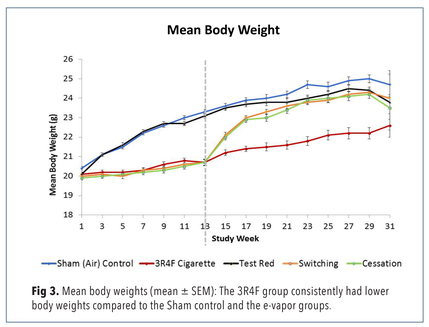

Fig 3. Mean body weights (mean ± SEM): The 3R4F group consistently had lower body weights compared to the Sham control and the e-vapor groups.

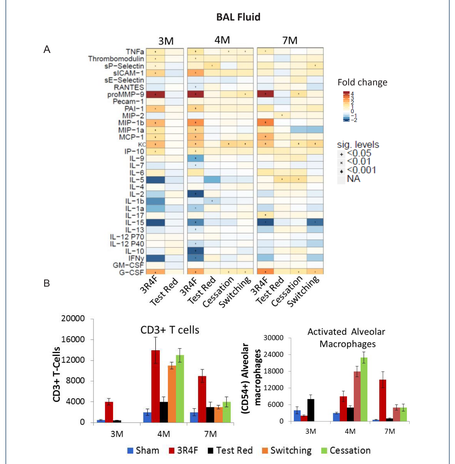

Fig 4. (A) Cytokine profile in BAL Fluid. The color scale reflects the magnitude of the estimated differences between groups (log2 fold change). P values represent significant differences between the group compared with sham. (B) Immuno-phenotyping of BAL cells by Flow Cytometry (absolute cell count/mL).

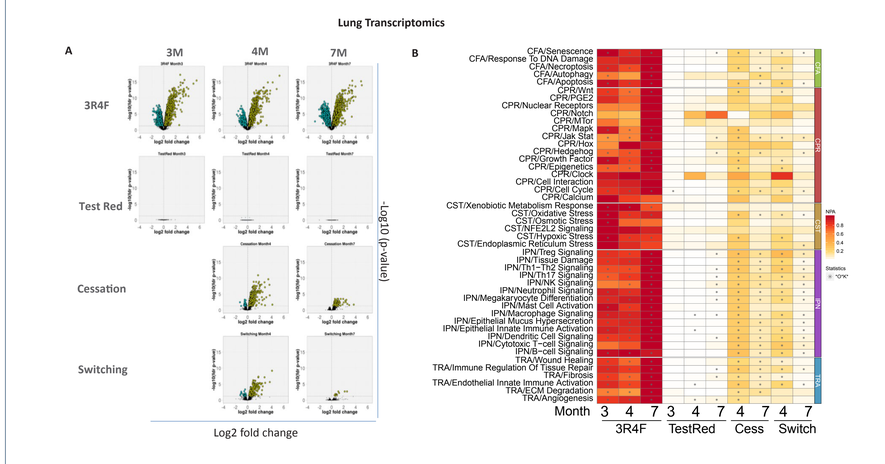

Fig 5. Lung transcriptomics showing (A) differentially expressed genes (DEGs) and (B) a heatmap of network perturbation amplitude (NPA). There were no DEGs at any time point in the lung or heart (not shown) of mice exposed to Test Red compared with the sham groups. While 3R4F exposure impacted the majority of pathways in the lung, the effect was minimal in the Test Red group. The impact in the Cessation and Switching groups at 4 and 7 months was significant but much lower than mice continuously exposed to 3R4F. CFA: Cell fate, CPR: Cell proliferation, CST: Cell stress, IPN: Inflammatory processes network, TRA: Tissue repair and angiogenesis.

Histopathology- Month 7: Incidence (Severity)

	Findings	Sham	3R4F	Test Red	Switching	Cessation
Nose	Atrophy, Olfactory epithelium	0/12	**8/11(1.9)**	0/12	4/11(2.5)	7/12(2.1)
	Hyperplasia, Respiratory epithelium	0/12	**4/11(1.0)**	2/12(1.0)	4/11(1.0)	6/12(1.0)
	Goblet cell hyperplasia, Septum	0/12	**0/11**	0/12	1/11(1.0)	0/12
	Inflammation, Chronic-active	0/12	**8/11(1.4)**	0/12	0/11	0/12
	Respiratory metaplasia of olfactory epithelium	0/12	**5/11(1.2)**	1/12(1.0)	5/11(1.4)	7/12(1.0)
	Squamous metaplasia, Respiratory epithelium	0/12	**5/11(1.4)**	0/12	0/11	0/12
	Epithelium, inclusion body intracytoplasmic	1/12(1.0)	**2/11(1.0)**	12/12(1.8)	11/11(1.6)	8/12(1.3)
Larynx	Squamous metaplasia, Epiglottis	0/12	**11/11(2.5)**	12/12(1.5)	11/11(2.4)	12/12(1.8)
	Hyperplasia, Squamous epithelium, Epiglottis	0/12	**11/11(2.0)**	0/12	6/11(1.0)	0/12
	Hyperkeratosis, Epiglottis	0/12	**11/11(1.6)**	0/12	0/11	1/12(1.0)
Trachea	Squamous metaplasia, Epithelium	0/12	**9/11(1.2)**	0/12	0/11	0/12
Lung	Inflammation, alveolus, mixed	0/12	**11/11(1.9)**	0/12	0/11	0/12
	Infiltrate cellular, Macrophage, Alveolus	0/12	**11/11(2.8)**	0/12	11/11(1.6)	12/12(1.1)
	Infiltrate cellular, Mixed, Interstitium	0/12	**11/11(2.1)**	0/12	11/11(1.2)	6/12(1.0)
	Infiltrate cellular, lymphocytic, perivascular	0/12	**11/11(1.9)**	0/12	9/11(1.6)	9/12(1.1)
	Hyperplasia, Epithelium, Bronchiole	0/12	**11/11(1.0)**	0/12	0/11	0/12
	Dilatation, Alveolus, (Emphysema)	0/12	**11/11(1.0)**	0/12	0/11	0/12

Fig 6. The 3R4F group had the highest incidence of histopathological findings indicating pulmonary inflammation and emphysema. Average severity was calculated by the sum of the severity scores divided by the incidence.

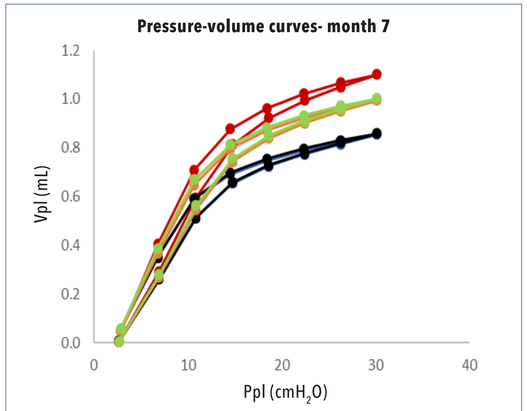

Fig 7. PV curve (FlexiVent) after 7 months of exposure. The larger lung volume in 3R4F exposed mice (compared to sham control) is indicative of tissue destruction as seen in emphysematous changes as a result of cigarette smoke exposure. Ppl: mean value of pressure. Vpl: mean value of volume

SUMMARY AND CONCLUSIONS

- An inhalation exposure system successfully delivered e-vapor aerosols of a respirable size (for 3R4F as well as e-vapor) via nose-only inhalation system for up to 7 months.
- During the exposure, the 3R4F group showed respiratory function depression, increased blood carboxyhemoglobin (not shown), decreased mean body weight, notable cellular infiltration/cytokine profile changes in BAL fluid, increased incidence of histopathological findings (larynx, lung, nose, and trachea) indicating pulmonary inflammation and emphysema, and significant changes in gene expression/biological pathways.
- Despite higher plasma nicotine and cotinine levels than 3R4F, the Test Red group had minimal changes in respiratory function, mean body weight, cytokine profile in BAL fluid, and lung transcriptomics that were similar to the sham control. There were few differences in BAL cytology and histopathology findings in Test Red group compared with the sham control.
- The Switching and Cessation groups often exhibited similar levels of reversibility of 3R4F exposure-related findings after switching to Test Red or air exposure.
- 7 months of exposure to CS induced biological responses in the respiratory tract associated with smoking-related diseases. Conversely, despite higher nicotine exposure via e-vapor aerosol, the e-vapor groups showed substantially fewer changes across the parameters tested.
- This in vivo study in C57BL/6 mice suggests that complete switching from CS to e-vapor products could significantly reduce biological changes associated with cigarette smoking.

Financial Disclosure/Conflict of Interest: The authors are employees of Altria Client Services LLC, Philip Morris International R&D, or Battelle | Acknowledgement: The authors acknowledge Dr. Sam Harbo (while employed at Battelle) for his valuable contribution in study conduct