A 7-Month Inhalation Study in C57BL/6 Mice to Investigate Potential Toxicity of E-vapor Aerosols Compared to Cigarette Smoke Using Cessation and Switching Study Design

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Abstract

Chronic exposure to cigarette smoke is a risk factor for the development of various diseases including chronic obstructive pulmonary disease. Smoking cessation remains the most effective approach to minimize smoking related diseases. It is generally regarded that switching from combustible cigarette to e-cigarettes (e-vapors), can offer a potential alternative for tobacco-related harm reduction. Though various variants of e-vapor products are available, studies evaluating long-term toxicity and biological implications of e-vapors alone and in the context of switching from cigarettes to e-vapors are not available. We designed a 7-month nose only inhalation (4 hours/day, 5 days/week, for 7 months) study to evaluate chronic toxicity of MarkTen® e-vapor aerosols (4% nicotine by weight) and compared it to responses from exposure to the 3R4F reference cigarette (CS). Additional groups of mice were added to explore the impact of switching or cessation after first 3-months of exposure to CS (followed by 4 months of cessation or e-vapor exposures).

Over the 7 months of exposure, there were no notable in-life observations from the e-vapor groups. Their body weights were comparable to the sham control, whereas the CS group showed consistently lower body weight. Upon cessation or switching to e-vapor exposure, body weights gradually increased. In contrast to e-vapor, the 3R4F group showed transient clinical signs of distress post-exposure, and, in part because of markedly reduced respiratory volume during exposure, they had lower plasma nicotine and cotinine levels compared to the e-vapor group. In addition, the CS exposure induced significant changes in organ weights (e.g., increases in lung; decreases in kidney, thymus, and liver weights). Following 7 months of exposure, e-vapor resulted in no or minimal increase in inflammatory cellular responses in bronchoalveolar lavage fluid, while the CS group showed consistently elevated responses (activated macrophages, CD4+T cells, B cells, neutrophils and eosinophils). Also, the extent of changes observed in switching group was overall similar to changes observed in the cessation group. Altogether, the pathophysiological changes induced by e-vapor exposures were significantly lower than changes induced by CS exposure. This study suggests that complete switching from CS to e-vapor products could significantly reduce biological changes associated with cigarette smoke.

Methods

- Female C57BL/6 mice, 11 weeks, were exposed to cigarette smoke (3R4F; 550 µg/L TPM [Total particulate matter]) or e-vapor aerosols (MarkTen® Test Red; 1100 µg/L TPM) via nose-only inhalation for 4 hours/day, 5 days/week, for up to 7 months. After the first 3 months of exposure, groups of 3R4F mice were subjected to different exposures of: 1) Test Red ("Switching") or 2) filtered air ("Cessation"), while a group remained on 3R4F exposures (Study Design: see Table 1).
- Mice were examined for in-life observations (clinical signs, body weights) throughout the study. At selected timepoints (Months 3, 4, and 7), cohorts were evaluated for various biological endpoints including: respiratory physiology; blood and urine sampling for biomarkers of exposure (plasma nicotine and cotinine, blood carboxyhemoglobin, urinary metabolites), anatomy (organ weights, gross findings) and histopathology, bronchoalveolar lavage (BAL) fluids (cytology), and genotoxicity (bone marrow micronucleus).

TABLE 1. Study Design

	Group	Exposure (µg/L WTPM)	Total Number of Animals
1	Sham	0	161
2	3R4F*	550	161
3	Test Red#	1100	161
4	Switching	550/1100	102
5	Cessation	550/0	102

*Exposure regimen for 3R4F, CIR regimen (55/30/2; a 55±0.3 mL/puff, a puff every 30 sec, a 2-sec puff duration) for 8 puffs/cigarette
#Exposure regimen for Test Red, modified CRM81 regimen (55/30/5; a 55±0.3 mL/puff, a puff every 30 sec, a 5-sec puff duration) for 130 puffs/cartridge

Exposure	Month 1	2	3	4	5	6	7
Sham	Air						
	Air						
	Air						
3R4F	3R4F						
	3R4F						
	3R4F						
Test Red (TA)	TA						
	TA						
	TA						
Switching	3R4F			TA			
	3R4F			TA			
Cessation	3R4F			Air			
	3R4F			Air			

Results

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
Acrolein [µg/L]	Month 1	BLOQ	3.306954	0.007610
	Month 3	0.000298	3.455845	0.009886
	Month 4	0.000759	3.375584	0.009971
	Month 6	BLOQ	3.165442	0.009687
	Month 7	0.000951	4.813747	0.012874
Acetaldehyde [µg/L]	Month 1	BFB	34.604537	0.041258
	Month 3	0.000849	35.258584	0.052991
	Month 4	0.001054	32.268813	0.057191
	Month 6	0.000713	33.540513	0.055081
	Month 7	0.001071	32.389975	0.061445
Formaldehyde [µg/L]	Month 1	0.012687	0.898244	0.037161
	Month 3	0.003940	0.923797	0.032049
	Month 4	0.003070	0.827892	0.043567
	Month 6	0.009656	0.803764	0.037130
	Month 7	0.002963	0.489221	0.036943
Propionaldehyde [µg/L]	Month 1	0.007430	9.420541	0.014357
	Month 3	0.011064	9.185413	0.020443
	Month 4	0.000799	3.480762	0.005311
	Month 6	0.003885	8.564756	0.010677
	Month 7	0.004308	6.145158	0.009134
Crotonaldehyde [µg/L]	Month 1	ND	0.846773	ND
	Month 3	BLOQ	0.918520	BLOQ
	Month 4	ND	0.914780	ND
	Month 6	BLOQ	0.582760	BLOQ
	Month 7	BLOQ	0.533726	BLOQ

NM = Not Measured; ND = Not Detected; BFB = Below field blank concentration; BLOQ = Below lower limit of quantification

RESPIRATORY PHYSIOLOGY

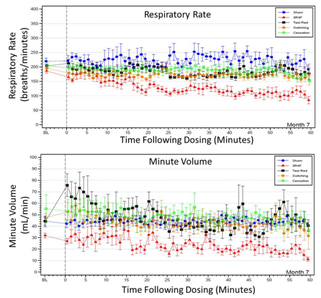

FIG 1. Respiratory rate (RR: top) and minute volume (MV: bottom) during the first hour of exposure at 7 month. The 3R4F group had the largest reduction in RR (and MV), while the e-vapor group showed comparable values to the sham control.

BIOMARKERS OF EXPOSURE

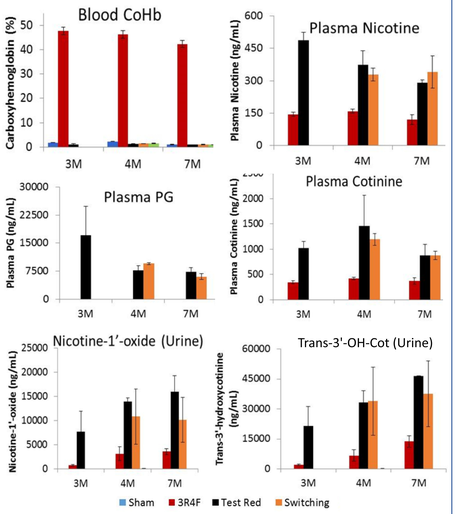

FIG 2. Biomarkers of exposure (mean± SEM) in plasma (top four) and urine (bottom two) are in agreement with aerosol concentrations in test atmosphere and respiratory physiology. Absence of bars for some groups is due to values BLOQ/ND or NM.

MEAN BODY WEIGHT

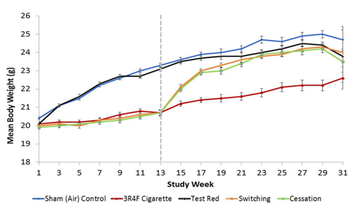

FIG 3. Mean body weights (mean ± SEM): The 3R4F group consistently had lower body weights compared to the Sham control and the e-vapor groups. Dotted vertical line marks first 3 months of exposure.

BAL FLUID (Cytology)

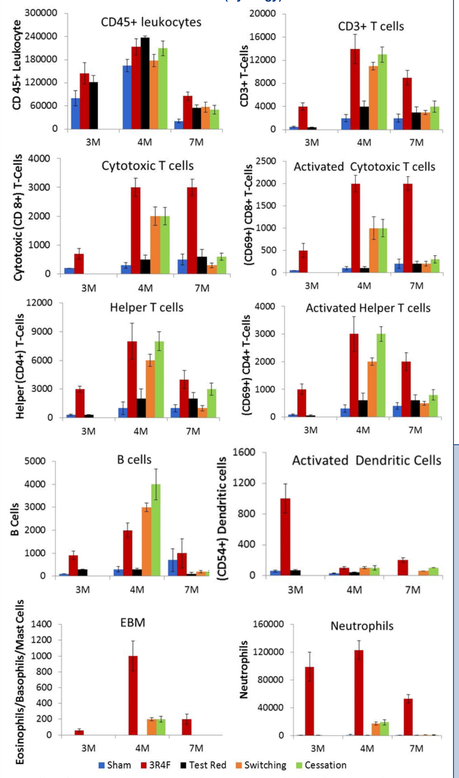

FIG 4. BAL Fluid Immuno-phenotyping by Flow Cytometry (Absolute cell count, per mL): The 3R4F group had multiple changes in the BAL flow cytometry data when compared to the sham control and Test Red groups. At month 4, the Switching group showed relatively higher responses compared to the sham control. Both switching and cessation groups were comparable to the Test-Red and sham control groups at month 7.

RELATIVE ORGAN WEIGHTS (to Body Weight)

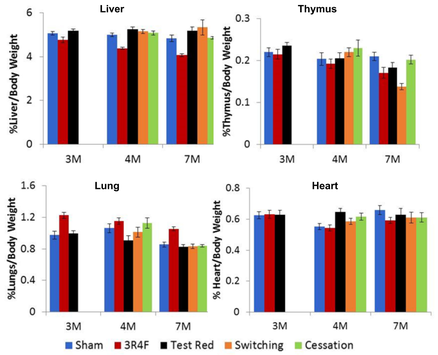

FIG 5. Percent organ weights relative to body weight: A notable finding was consistently decreased liver, kidney (not shown) and thymus weights and increased lung weights in 3R4F group when compared to sham control.

Summary

- An inhalation exposure system successfully generated and 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, decreased mean body weight, changed relative organ weights (e.g., increases in lung; decreases in kidney, thymus, and liver weights) and notable cellular infiltration/changes in BAL fluid.
- Despite higher plasma nicotine, and cotinine levels than the 3R4F, the Test Red (e-vapor) groups had minimal changes in respiratory function, mean body weight, relative organ weights (liver, thymus, lung, kidney), that were similar to the sham control. There were few differences in BAL cytology 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.
- In conclusion, following 7 months of exposure, cigarette smoke induced biological responses in the respiratory tract associated with smoking-related diseases, while e-vapor exposure, even with higher nicotine intake, showed substantially reduced changes in parameters tested.
- This study suggests that complete switching from CS to e-vapor products could significantly reduce biological changes associated with cigarette smoke.

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*Former Altria Client Services employees.