A Six-Month Inhalation Study in ApoE$^{-/-}$ Mice to Investigate Cardiovascular and Respiratory Exposure Effects of E-Vapor Aerosols Compared with Cigarette Smoke

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Introduction

Chronic exposure to cigarette smoke is a risk factor for the development and progression of cardiovascular disease and chronic obstructive pulmonary disease. Electronic cigarettes are gaining popularity as a potential alternative to conventional cigarettes. Most e-cigarette formulations contain vehicle (propylene glycol (PG) and/or vegetable glycerin (VG)), nicotine and flavor ingredients [1]. In contrast to 3R4F cigarette smoke (CS), e-cigarettes deliver nicotine without smoke constituents that arise from the combustion of tobacco. Currently, there are limited data on the safety profile of e-cigarette usage in terms of safety toxicology or disease risk assessment as compared with that of conventional cigarette use [2-4]. We previously conducted various inhalation studies to assess the impact of CS exposure on respiratory and cardiovascular system using apolipoprotein E-knockout (Apoe$^{-/-}$) mice [5-6]. To support comprehensive assessment of exposure effects, the impact of PG/VG, nicotine as well as flavor constituents will be evaluated on the respiratory and cardiovascular systems of Apoe$^{-/-}$ mice.
In a similar way as previously described in this study we used the whole body exposure system to expose mice to e-cigarette aerosol and analyze the impact, compared with exposure to CS from the reference cigarette 3R4F.

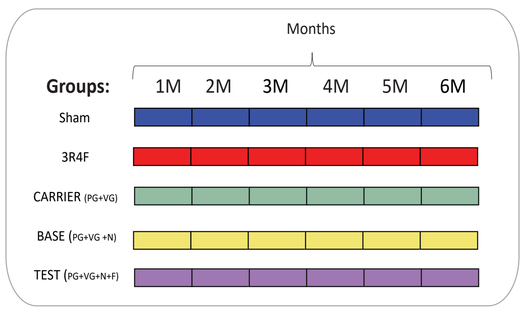
Materials & Methods

Experimental Design

In vivo ApoE$^{-/-}$ mice

Female ApoE$^{-/-}$ mice (12-14 weeks at initial dosing) were exposed to air (Sham), 3R4F cigarette smoke (CS), or e-vapor aerosols generated from CARRIER (PG/VG/water), BASE (CARRIER plus 4% nicotine), and TEST (BASE plus flavors).

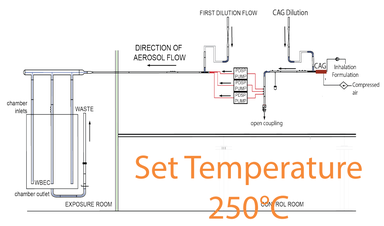
ApoE$^{-/-}$ mice were exposed via whole body inhalation system for up 3 hours/day, 5 days/week for 6 months. Following the last exposure mice were subjected to the sample collection and analyses.



Quantification of pulmonary inflammation, lung function tests, histopathological assessments, measurement of atherosclerotic plaque areas and serum cholesterol concentrations were performed.

	1M	2M	3M	4M	5M	6M
Atherosclerosis plaque progression			x			x
Blood lipids			x			x
Lung function and weight			x			x
Lung inflammation			x			x
Histopathology			x			x

Capillary aerosol generator (CAG) was used to generate e-vapor aerosols



CAG was used to generate e-vapor from various e-liquids: “CARRIER” containing humectants alone, “BASE” containing humectants and 4% nicotine, and “TEST” containing humectants, 4% nicotine and flavors.

Endpoint Name	CARRIER (PG/VG)	BASE (PG/VG/N)	TEST (PG/VG/N/F)
CAG solution consumption (g)	173.88 (+/-) 22.51	168.16 (+/-) 14.52	172.72 (+/-) 19.81
CAG temperature (°C)	250.03 (+/-) 1.76	249.19 (+/-) 2.47	249.74 (+/-) 1.65

Data are represented as MEAN +/- SD.

Controlled aerosol delivery

-Target TPM 600 µg/L, for the 3R4F group.

-PG/VG/N “BASE” and PG/VG/N/F “TEST” at matching nicotine concentration to 3R4F 36 µg/L.

-PG/VG “CARRIER” at matching TPM concentration as PG/VG/N “BASE” group.

ATHEROSCLEROTIC PLAQUE AREA MEASUREMENTS -- Aortic arches were dissected and opened longitudinally for planimetry analysis.

LUNG FUNCTION -- Lung function was measured in selected animals 18–24 hours after exposure using the SCIREQ flexiVent™ system.

INFLAMMATORY CELLS IN BALF -- Number and subtype of inflammatory cells in BALF were measured by flow cytometry.

INFLAMMATORY MEDIATOR CONCENTRATIONS -- Concentration of inflammatory mediators were measured from BALF samples using Luminex technology and commercialy available assay according to manufacturer’s instructions.

HISTOPATHOLOGY -- Lung and respiratory nasal epithelium (RNE) tissue was sectioned and stained with H&E, and the abundance of inflammatory cells and type were evaluated with a scoring method (0-5 score).

Results

Characterization of aerosol constituents

Endpoint Name	Sham	3R4F	CARRIER (PG/VG)	BASE (PG/VG/N)	TEST (PG/VG/N/F)
Total particulate matter (TPM), (µg/L)	-5.93 (+/-) 7.2	562.43 (+/-) 64.8	1 093.11 (+/-) 150.9	1 103.23 (+/-) 161.4	1 083.40 (+/-) 176.7
Nicotine (µg/L)	0.02 (+/-) 0.0	35.15 (+/-) 4.8	0.02 (+/-) 0.0	35.53 (+/-) 4.9	35.73 (+/-) 5.6
Mass median aerodynamic diameter (MMAD) (µM +/- GSD)		0.82 (+/-) 0.1	0.96 (+/-) 0.1	0.92 (+/-) 0.1	1.01 (+/-) 0.2

Data are represented as MEAN +/- SD.

CAG system was successfully set up to generate and consistently deliver respirable e-vapor aerosols to whole body mouse exposure system.

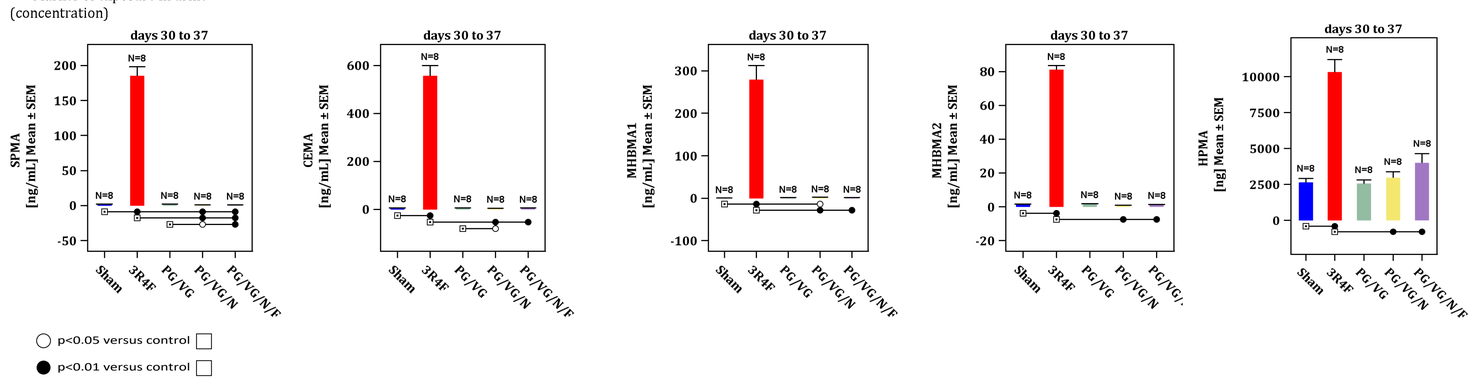
Endpoint Name	Sham	3R4F	CARRIER (PG/VG)	BASE (PG/VG/N)	TEST (PG/VG/N/F)
Carbon monoxide, CO (ppm)	0.18 (+/-) 0.27	596.41 (+/-) 66.61			
Propylene glycol (µg/L)	0.38 (+/-) 0.00	0.36 (+/-) 0.00	179.38 (+/-) 21.31	171.33 (+/-) 16.65	173.03 (+/-) 19.39
Vegetable glycerin (µg/L)	12.94 (+/-) 63.87	53.56 (+/-) 6.35	576.92 (+/-) 65.59	543.52 (+/-) 68.42	546.21 (+/-) 74.21
Acrolein (µg/L)	0.00 (+/-) 0.00	3.98 (+/-) 0.45	0.00 (+/-) 0.00	0.00 (+/-) 0.00	0.00 (+/-) 0.00
Acetaldehyde (µg/L)	0.01 (+/-) 0.00	30.69 (+/-) 1.90	0.01 (+/-) 0.00	0.02 (+/-) 0.01	0.02 (+/-) 0.01
Formaldehyde (µg/L)	0.02 (+/-) 0.01	0.67 (+/-) 0.08	0.03 (+/-) 0.01	0.04 (+/-) 0.03	0.03 (+/-) 0.01
Propionaldehyde (µg/L)	0.00 (+/-) 0.00	3.47 (+/-) 0.62	0.01 (+/-) 0.00	0.01 (+/-) 0.00	0.01 (+/-) 0.00
Crotonaldehyde (µg/L)	0.00 (+/-) 0.00	2.84 (+/-) 0.75	0.00 (+/-) 0.00	0.00 (+/-) 0.00	0.00 (+/-) 0.00

Data are represented as MEAN +/- SD.

The e-vapor aerosol (CARRIER, BASE and TEST) present a reduced level of harmful smoke constituents in the atmosphere as compared with conventional burning tobacco products (3R4F). The concentration of acrolein, crotonaldehyde, formaldehyde, propionaldehyde and acetaldehyde is sharply reduced in CARRIER, BASE and TEST test atmosphere in comparison to 3R4F.

e-Vapor aerosol contain reduced levels of selected harmful and potentially harmful constituents (HPHCs)

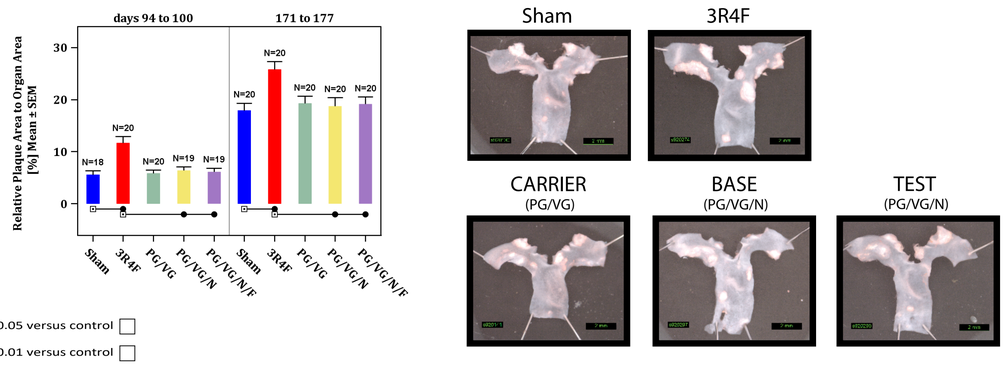
► Aerosol uptake and exposure
» Marker of exposure in urine (concentration)



Exposure to 3R4F significantly increases the level of specific smoke metabolites in urine after exposure. The e-vapor aerosol containing aerosol CARRIER, BASE and TEST induced lower levels of harmful smoke metabolites such as SPMA (exposure marker of benzene), CEMA (exposure marker of acrylonitrile), MHBMA1, MHBMA2 (exposure markers of 1,3-butadiene) and HPMA (exposure marker of acrolein) as compared with conventional burning tobacco products (3R4F).

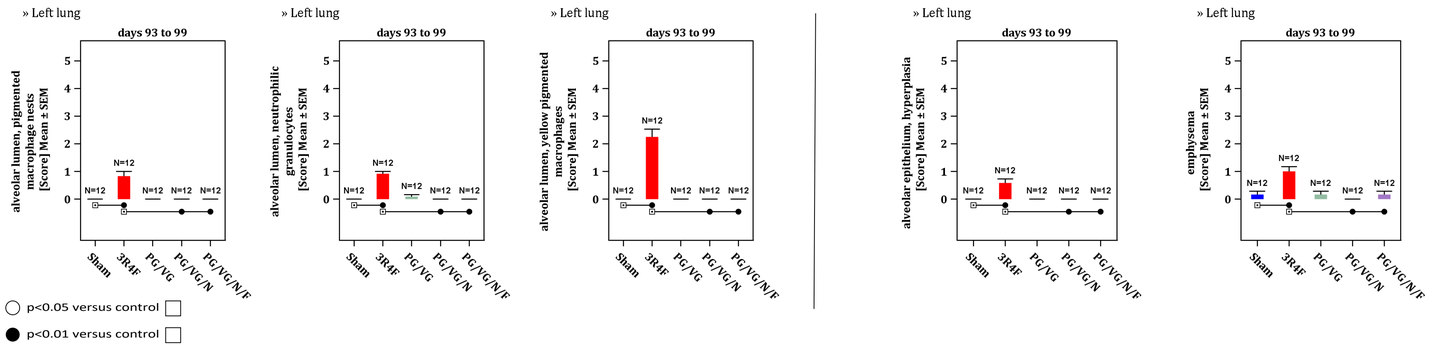
E-cigs aerosol exposure results in reduced development of atherosclerotic plaque

Aortic arch planimetry images (Month 6)



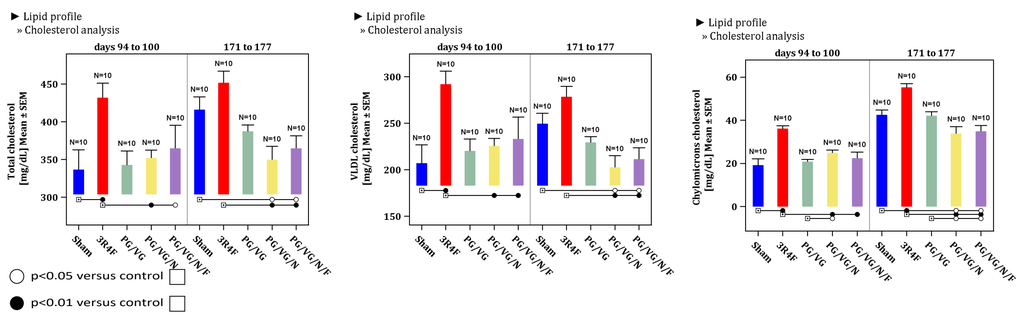
Exposure to 3R4F resulted in increased atherosclerotic plaque formation in the aortic arch of ApoE$^{-/-}$ mice compared with the sham exposure, observed at 3 and 6 months of exposure. Exposure to e-vapor aerosol (CARRIER, BASE and TEST) resulted in a slowing of the plaque formation, as the plaque area in these groups was significantly lower than the continuous 3R4F-exposed group. There was no difference in plaque area in animals exposed to CARRIER, BASE and TEST aerosol for six months compared to the fresh air-treated animals.

e-Vapor aerosol exposure results in lower inflammatory cell influx and lung injury



Histopathological evaluation performed at month three after exposure demonstrated that inflammatory infiltration in the lung was mainly restricted to the 3R4F CS group: increased numbers of neutrophils and macrophages were observed in the alveolar spaces of 3R4F CS-exposed mice at Month 3 onward. Histopathological semi-quantitative scoring of alveolar emphysema and epithelium hyperplasia showed a significant increase in 3R4F-exposed animals compared with Sham-exposed animals.No significant differences to the Sham group were seen in response to e-vapor (CARRIER, BASE AND TEST) aerosol exposure.

Serum cholesterol level



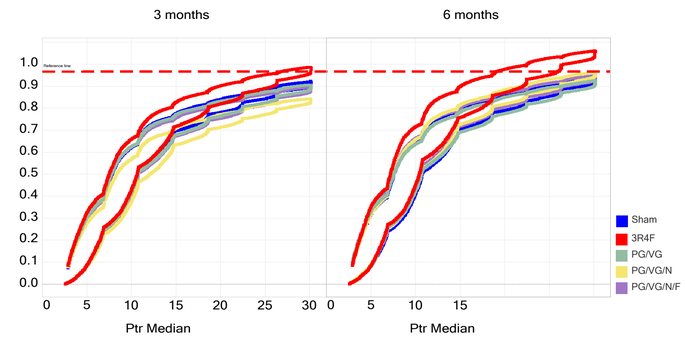
The results indicated an increase in cholesterol, Chylomicron cholesterol and VLDL cholesterol in the 3R4F exposed group (p< 0.05) relative to levels in sham-exposed mice after 3–6 months. E Exposures to e-vapor aerosols showed lower serum total and VLDL/CM cholesterol in BASE and TEST groups compared to 3R4F exposed group.

e-Vapor aerosol exposure results in lower impact on pro-inflammatory mediators

Name	3R4F 3M	3R4F 6M	BASE (PG/VG/N) 3M	BASE (PG/VG/N) 6M	CARRIER (PG/VG) 3M	CARRIER (PG/VG) 6M	TEST (PG/VG/N/F) 3M	TEST (PG/VG/N/F) 6M
G-CSF	3.59	2.72	0.82	0.52	0.74	0.50	0.78	1.09
GM-CSF	2.14	1.57	1.99	0.83	0.98	0.78	2.18	1.06
IFN-g	0.84	1.26	0.95	1.67	0.79	2.89	0.54	1.91
IL-1a	0.54	0.53	0.93	1.32	1.22	1.47	0.89	1.24
IL-1b	1.03	0.94	0.84	0.88	0.75	0.77	1.11	0.95
IL-2	0.49	0.68	0.92	1.11	1.05	1.29	0.79	0.98
IL-4	1.22	0.96	1.07	1.54	0.60	1.52	0.89	1.30
IL-5	1.68	0.87	0.93	0.98	1.30	2.01	1.24	0.83
IL-6	3.88	4.84	0.50	1.72	2.32	2.77	0.82	1.46
IL-7	0.52	0.74	0.92	1.07	0.97	0.69	1.05	0.56
IL-9	0.93	0.84	1.09	1.14	0.98	1.87	0.82	1.14
IL-10	0.32	0.29	0.70	0.94	1.05	1.04	0.63	0.72
IL-12	1.24	3.36	0.57	1.33	0.62	1.93	0.90	0.95
IL-12b	0.60	0.58	0.96	1.16	0.90	1.29	0.69	0.93
IL-13	0.63	0.85	0.96	1.25	0.70	2.06	0.77	1.28
IL-15	0.93	1.03	1.55	0.88	0.87	1.20	0.72	0.68
IL-17	1.79	2.70	0.49	0.65	0.58	1.55	0.56	2.11
IP-10	3.48	3.66	0.88	1.12	0.82	1.47	0.81	1.23
KC	4.99	8.20	0.63	1.02	0.66	1.09	0.61	1.73
MCP-1	6.15	4.77	1.04	0.88	1.05	0.62	1.30	0.60
MIP-1a	2.28	2.70	1.02	1.04	0.86	0.94	0.98	0.71
MIP-1b	10.52	12.82	1.39	1.57	1.02	0.90	0.80	1.48
MIP-2	1.06	0.85	0.95	0.88	0.91	1.25	1.04	1.09
MMP total	1.70	2.19	0.98	1.04	1.10	0.96	0.98	1.00
PECAM-1	1.24	1.07	0.83	1.01	1.09	0.88	0.74	1.04
pro-MMP-9	11.97	17.14	0.98	0.29	1.93	0.50	1.07	0.51
RANTES	0.55	0.79	0.77	0.91	0.65	1.69	0.83	0.96
sE-Selectin	1.04	0.88	0.92	0.91	0.84	2.66	0.89	1.01
sICAM1	2.23	2.13	0.97	0.93	1.08	0.98	1.05	0.89
sP-Selectin	0.94	1.04	0.92	0.98	0.89	1.26	1.18	0.86
Thrombomodulin	1.90	2.34	0.86	0.95	0.91	1.09	0.95	1.06
TNF-a	2.10	2.77	0.82	1.17	0.71	1.45	1.12	0.97
Total PAI-1	2.54	2.60	0.92	1.00	0.97	0.95	1.15	1.14

p<0.05 significant versus Sham

Lung function and weight



Lung weight (g) Mean +/- SEM	Sham	3R4F	CARRIER (PG/VG)	BASE (PG/VG/N)	TEST (PG/VG/N/F)
	0.14609 +/- 0.00821	0.17875 +/- 0.01685 +	0.14525 +/- 0.00659	0.14342 +/- 0.00531 #	0.14392 +/- 0.00516 #

\+ p<0.05 significant versus Sham

\# p<0.05 significant versus 3R4F

Following 3R4F exposure, a significant increase in the abundance of multiple chemokines, cytokines, and interleukins was observed at 3 and 6 months of exposure. Chronic exposure to e-vapor aerosols had only a minimal effect on inflammatory mediators in the free-cell population in BALF. Slight downregulation of KC was observed in BASE, CARRIER and TEST aerosol.

The pressure-volume relationship (PVs-P) demonstrated a typical emphysema-like upward/leftward shift in the Pvs-P loop in CS-exposed mice (Red) compared with measurements in sham-exposed animals (Blue), indicative of CS-induced emphysematous changes in the lung after 6 months. There was no obvious effect of e-vapor aerosol exposure on the PVs-P loop. Lung weight, in 3R4F CS-exposed animals was higher than lung weight in the Sham and e-vapor CARRIER, BASE and TEST exposed groups

Histopathology in RNE

Nose level 1

Endpoint Name	Sham	3R4F	CARRIER (PG/VG)	BASE (PG/VG/N)	TEST (PG/VG/N/F)
respiratory epithelium, eosinophilic globules	1.583 +/- 1.167	0.083 +/- 0.167 +	0.583 +/- 0.386	0.917 +/- 0.520 #	0.917 +/- 0.520 #
respiratory epithelium, hyperplasia	1.000 +/- 1.128	3.583 +/- 0.297 +	0.583 +/- 0.386	0.500 +/- 0.389 #	0.417 +/- 0.297 #
respiratory epithelium, squamous epithelial metaplasia	0.583 +/- 1.167	2.750 +/- 0.261 +	0.000 +/- 0.000	0.000 +/- 0.000 #	0.000 +/- 0.000 #

\+ p<0.05 significant versus Sham # p<0.05 significant versus 3R4F

3R4F CS exposure induced reserve cell hyperplasia, squamous metaplasia and increase eosinophilic globules infiltration in the RNE (nose level 1). The severity of these findings in response to CARRIER, BASE and TEST aerosols was sharply reduced in comparison with 3F4F CS exposure.

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Competing Financial Interest | The research described in this poster was sponsored by Philip Morris International

Conclusions

Exposure to 3R4F CS resulted in significant impact on respiratory and cardiovascular disease parameters: atherosclerotic plaque progression, lung inflammation and lung function. Continuous exposure to the CARRIER, BASE and TEST aerosol resulted in a consistently lower impact on measured parameters related to CVD or COPD. In contrast to CS, exposure to e-vapor aerosol resulted in no increase in leukocyte counts, serum cholesterol concentration, aortic plaque formation, and lung matrix metalloproteinase activity compared with exposure to fresh air. In conclusion, these e-vapor aerosols did not elicit molecular, functional, structural changes that were associated with CS-induced atherosclerosis and COPD in the Apoe mouse model.

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