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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Conflict of Interest Statement

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Aim

Assessment of Cardiovascular Effects following E-vapor and Conventional Cigarette Smoke Exposure in the ApoE-/- Mouse Model
What Is the Objective of Harm Reduction?

- Smoking is addictive and causes a number of serious diseases
- Worldwide, it is estimated that more than 1 billion people will continue to smoke in the foreseeable future*
- Offering smoke-free alternatives to adult smokers is a sensible, complementary addition to existing tobacco control strategies

Successful harm reduction requires that current adult smokers be offered a range of Reduced-Risk Products they can fully switched to, should they decide not to quit.

Figure adapted from Clive Bates presentation to E-Cigarette Summit (19 Nov 2013)
Note: Reduced Risk Products ("RRPs") is the term PMI uses to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switched to these products versus continued smoking.
PMI’s Scientific Assessment Approach

The assessment framework integrates what is known about combustible cigarette (CC) smoking and incorporates both epidemiological and mechanistic evidence to define the assessment approach.

http://dx.doi.org/10.1016/j.yrtph.2016.07.006

These assessment steps are designed to provide five levels of evidence as the assessment program is completed.
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.

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.

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<sup>-/-</sup> mice.
A Six-Month Inhalation Study in ApoE−/− Mice to Investigate Cardiovascular and Respiratory Exposure Effects of E-Vapor Aerosols Compared with Cigarette Smoke

**EXPERIMENTAL DESIGN**

- 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) using CAG (capillary aerosol generator) system.

- ApoE−/− mice were exposed via whole body inhalation system for up to 3 hours/day, 5 days/week for 6 months.

- Fresh air breaks in-between 1h exposure

* Cardiorespiratory tissue collected for analysis
A Six-Month Inhalation Study in ApoE<sup>−/−</sup> Mice to Investigate Cardiovascular and Respiratory Exposure Effects of E-Vapor Aerosols Compared with Cigarette Smoke

The 3R4F cigarettes were smoked according to the Health Canada Intensive Smoking Protocol (Health Canada, 1999).

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

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

- Target TPM 600 µg/L, for the 3R4F group.
- PG/VG/N and PG/VG/N/F at matching nicotine concentration to 3R4F 35 µg/L.

<table>
<thead>
<tr>
<th>Nicotine µg/L</th>
<th>3R4F</th>
<th>CARRIER (PG/VG)</th>
<th>BASE (PG/VG/N)</th>
<th>TEST (PG/VG/N/F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3R4F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrier (PG/VG)</td>
<td>35.15</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>35.73</td>
</tr>
<tr>
<td>Base (PG/VG/N)</td>
<td>35.65</td>
<td>1.931</td>
<td>1.033</td>
<td>1.053</td>
</tr>
<tr>
<td>Test (PG/VG/N/F)</td>
<td>35.73</td>
<td>1.0834</td>
<td>1.033</td>
<td>1.053</td>
</tr>
</tbody>
</table>

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**AEROSOL CONSTITUENTS IN TEST ATMOSTHERE**

- **Acetaldehyde** (µg/L)
- **Acrolein** (µg/L)
- **Crotonaldehyde** (µg/L)
- **Formaldehyde** (µg/L)
- **Propionaldehyde** (µg/L)

Compared with cigarette smoke, the E-Vapor aerosol (CARRIER, BASE and TEST) present a lower level of harmful smoke constituents in the atmosphere.

**Scientific studies have shown that as the temperature of tobacco increases, the levels of potentially harmful chemicals formed increases.**

**COMBUSTION IS A KEY**

**Residual Solid and PAH Formation, Food and Chemical Toxicology, 45,6,1039**

**Polycyclic Aromatic Hydrocarbons from Tobacco: the "Link" between Low Temperature Combustion and Potentially Harmful Chemicals**

**Hajaligol, V., 2007, Formation of Polycyclic Aromatic Hydrocarbons from Tobacco ("Link" between Low Temperature Combustion and Potentially Harmful Chemicals), Food and Chemical Toxicology, 45, 1039-1050**
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Assessment of E-Vapor Aerosols in 6-Month ApoE−/− Mouse Study -- Lung Effects

**LUNG INFLAMMATION**

- Cell-free BALF supernatants were analyzed using a multiplexed bead array

**LUNG STRUCTURAL DAMAGE**

- Compared with cigarette smoke, histopathological semi-quantitative scoring shows that exposure to E-Vapor aerosols resulted in lower emphysematous changes in lung.

**Compared with cigarette smoke, exposure to E-Vapor aerosols resulted in lower number of inflammatory cells in lung BALF.**

**Compared with cigarette smoke, exposure to E-Vapor aerosols resulted in lower level of inflammatory mediators.**
A Six-Month Inhalation Study in ApoE^{-/-} Mice to Investigate Cardiovascular and Respiratory Exposure Effects of E-Vapor Aerosols Compared with Cigarette Smoke

**Assessment of E-Vapor Aerosols in 6-Month ApoE^{-/-} Mouse Study -- Lung Effects**

**LUNG MOLECULAR CHANGES**

Compared with cigarette smoke, exposure to E-Vapor aerosols resulted in lower molecular changes in lung tissue.

Compared with cigarette smoke, exposure to E-Vapor aerosols induced significantly less molecular changes related to stress responses, cell proliferation and inflammation in lung tissue.
A Six-Month Inhalation Study in ApoE\(^{-/-}\) Mice to Investigate Cardiovascular and Respiratory Exposure Effects of E-Vapor Aerosols Compared with Cigarette Smoke

Cardiovascular Disease

- Atherosclerosis is an inflammatory disease characterized by the accumulation of lipoprotein and leucocytes as plaque in the arterial layer. Uncontrolled, it can lead to coronary heart disease (CHD) and underlying clinical events such as heart attack or angina.

- Development of CHD is accelerated by a variety of risk factors, including male gender, smoking, dyslipidemia, elevated blood pressure, physical inactivity, obesity and diabetes.

- Patients with COPD have increased cardiovascular morbidity and mortality.


https://intervals.science
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The effect of 3R4F CS and E-Vapor aerosols on atherosclerotic plaque formation

Compared with cigarette smoke, exposure to E-Vapor aerosols (CARRIER, BASE and TEST) induced lower atherosclerotic plaque formation.

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.
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The effect of 3R4F CS and E-Vapor aerosols on heart ventricle

MOLECULAR ANALYSIS

Compared with cigarette smoke, exposure to E-Vapor aerosols (CARRIER, BASE and TEST) lower molecular changes in aorta and heart tissue (e.g., including mechanisms reflecting stress responses and those linked to the extracellular matrix).
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The effect of 3R4F CS and E-Vapor aerosols on carotid and abdominal artery

ASSESSMENT OF ARTERIES FUNCTION

PWV = Distance/Time

ULTRASOUND VEVO 3100 system

Pulse Wave Velocity (PWV) is depending on physical properties of the vessels, and especially on their STIFFNESS.

The nicotine-containing liquid aerosols induce significantly less arterial stiffness than cigarette smoke.
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The effect of 3R4F CS and E-Vapor aerosols on heart

**ASSESSMENT OF HEART FUNCTION**

**Myocardial performance index**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>3R4F</th>
<th>CARRIER (PG/VG)</th>
<th>BASE (PG/VG/N)</th>
<th>TEST (PG/VG/N/F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>0.5</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td>0.55</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6M</td>
<td>0.55</td>
<td>0.65</td>
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</tbody>
</table>

+ p<0.05 significant versus Sham
# p<0.05 significant versus 3R4F
& p<0.05 significant versus PG/VG

**Compared with cigarette smoke, exposure to E-Vapor aerosols resulted in less effects on Myocardial Performance Index, which detects early alterations of systo-diastolic performance of left ventricle**
Compared with cigarette smoke, exposure to E-Vapor aerosols resulted in:

**LUNG INFLAMMATION**

- Lower the level of inflammatory cells and mediators
- Lower atherosclerotic plaque formation
- Lower emphysematous changes in lung

**CARDIOVASCULAR**

**LUNG FUNCTION**

**LUNG HISTOPATHOLOGY**

This study suggests that E-Vapor aerosols induce significantly lower biological responses associated with smoking-related cardiovascular and pulmonary diseases.
PMI | TRAINING

MAY 23-24 2019

In Vitro Exposure Systems and Dosimetry Assessment Tools for Aerosol Inhalation Products

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PHILIP MORRIS INTERNATIONAL co-organized by ALTERTEXX academy
Thank you for your attention
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Compared with cigarette smoke, exposure to E-Vapor aerosols resulted in lower effects on:
- EF (Ejection fraction)
- FS (Fractional shortening)
- IVCT (Isovolumic contraction time)
- IVRT (Isovolumic relaxation time)
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Back up slide
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