Toxicological characterization of the mentholated heat-not-burn product THS2.2M in a 90-day OECD inhalation study

17 March, 2016
Society of Toxicology
55th Annual Meeting and ToxExpo, New Orleans, U.S.A

Alberto Oviedo
Philip Morris International R&D
Philip Morris Products S.A. (part of PMI group of companies)

This research was funded by Philip Morris International
PMI R&D - Background

• Smoking causes serious diseases such as cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease.
• Philip Morris International is developing novel products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes.
• To determine whether such potentially reduced-risk products (RRP) have the potential to reduce individual risk, we are conducting extensive and rigorous scientific studies comparing their biological impact with that of cigarettes.
Heat-not-Burn
Cigarette smoke vs. Heat-not-Burn

Underlying Principles
- Approximately 8000 constituents identified in cigarette smoke
- Some of these constituents are categorized as harmful and potentially harmful (HPHCs)
- Many of the HPHCs are formed during combustion (burning) of the tobacco
- It is not known which HPHCs are responsible for tobacco-related diseases – selective reduction not an effective approach

Lower temperatures reduce constituents in the aerosol
THS2.2 – Operating Principles

Key Principles

• Electrically heated tobacco system version 2.2 (THS2.2)
  – Tobacco plug which generates visible aerosol
  – Tobacco blends and flavor systems developed to suit lower operating temperature (< 350° C)

• Heating engine precisely controlled using built-in software
  – Heater maintains tobacco temperature in the distillation range
  – Heater also acts as a temperature sensor
Study objectives and methods
Study Objective

To characterize the toxicity of the THS2.2-menthol aerosol delivered by a repeated daily inhalation in a 90-day study following the OECD Test Guideline 413 (OECD TG 413) and to compare the results to the toxicity inherent to cigarette smoke (CS) from mentholated reference cigarettes.

OECD TG 413 Endpoints – Standard Toxicology

1. In-life observations
2. Hematology
3. Clinical chemistry
4. Organ weights
5. Inflammatory cells in BALF
6. Histopathology
Test item and reference cigarettes

**TEST ITEM: THS2.2-M sticks**
- Mentholated version of the THS2.2 with a menthol yield of 2.09 mg/cigarette*

**REFERENCE CIGARETTES: 3R4F-like cigarettes**
Mentholated combustible cigarettes designed for reference purposes (DDA3)
- DDA3 1XMIS with a menthol yield of 2.08 mg/cigarette*
- DDA3 2XMIS with a menthol yield of 2.58 mg/cigarette*

+ In addition to mentholated items, 3R4F cigarettes (standard Reference Cigarette from University of Kentucky) were included in the study for reference purposes

* When smoked according to ISO 3308 standard
**Study Design**

<table>
<thead>
<tr>
<th>Nicotine (µg/l)</th>
<th>Exposure days</th>
<th>Post-exposure days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered air (Sham)</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>3R4F</td>
<td>23</td>
<td>90</td>
</tr>
<tr>
<td>DDA3 1XMIS</td>
<td>23</td>
<td>90</td>
</tr>
<tr>
<td>DDA3 2XMIS</td>
<td>23</td>
<td>90</td>
</tr>
<tr>
<td>THS2.2M Low</td>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>THS2.2M Medium</td>
<td>23</td>
<td>90</td>
</tr>
<tr>
<td>THS2.2M High</td>
<td>50</td>
<td>90</td>
</tr>
</tbody>
</table>

- Target nicotine concentrations were selected based on a dose-range finding study previously conducted.
- Animals were exposed 6h per day during 5 days per week for a total of 13 weeks (90 days).
- An additional 42 days of post-exposure recovery period was included to assess recovery or delayed occurrence of findings.
Results
Test atmosphere characterization

- Data showed that most of the menthol is carried in the gas-vapor phase
- Nicotine levels were highly reproducible throughout the entire exposure period
- The aerosol generated from THS2.2M contained 53-fold less CO compared to aerosol from reference cigarettes at the same nicotine concentration.
- The amount of acrolein present in the aerosol generated from THS2.2M were approximately 12-fold less as compared to aerosol generated from reference cigarettes at equal nicotine concentration.
Biomarkers of exposure

- Total amount of nicotine metabolites excreted higher in THS2.2M-exposed animals
- The plasma nicotine/cotinine and menthol/M-1 concentrations showed a dose dependent increase
Biomarkers of exposure

- Total amount of nicotine metabolites excreted higher in THS2.2M-exposed animals
- The plasma nicotine/cotinine and menthol/M-1 concentrations showed a dose dependent increase
- Concentration of these biomarkers (e.g. HPMA) remained low in all THS2.2M groups
Systemic toxicity

- Higher body weight gained over in THS2.2M exposed groups as compared to reference groups.

- No remarkable toxic effects in THS2.2M exposed animals.

- Nicotine concentration-related increases in:
  i. Neutrophil count in blood
  ii. Relative weight of liver
  iii. Liver enzyme activity
Lung inflammation

- Low numbers of inflammatory cells are recruited in the lungs (BALF) of THS2.2M exposed animals

- Changes in cytokine expression to a much lower extent in THS2.2M exposed animals or non-existing

- Data demonstrates a low degree of pulmonary inflammation in THS2.2M exposed animals
Histopathology
Histopathology of respiratory tract organs - Nose

• Increased goblet cell loss and basal cell hyperplasia in respiratory epithelium in animals exposed to reference cigarettes

• Minimum atrophy/loss of olfactory epithelium in THS2.2M-exposed groups

• No significant findings were observed in nose level 4 in THS2.2M-exposed groups
Histopathology of respiratory tract organs - Nose

**Nose level 1**

- Increased goblet cell loss and basal cell hyperplasia in respiratory epithelium in animals exposed to reference cigarettes

**Nose level 3**

- Minimum atrophy/loss of olfactory epithelium in THS2.2M-exposed groups

**Nose level 4**

- No significant findings were observed in nose level 4 in THS2.2M-exposed groups
Histopathology of respiratory tract organs - Larynx & lungs

**Larynx**

- **Base of epiglotis**
  - Squamous cell hyperplasia-metaplasia (Score)
  - Reduced severity in the findings observed in THS2.2M-exposed groups

- **Vocal folds**
  - Squamous cell hyperplasia-metaplasia (Score)

**Lungs**

- **Pigmented macrophages (Incidence)**
  - No significant findings observed in THS2.2M-exposed groups, no obvious lung inflammation
Summary

Section sites

Larynx

Trachea

Tracheal bifurcation

Lung

Nose level 1-4

3R4F

DDA3 1XMIS

DDA3 2XMIS

THS2.2M

Reduction of nasal effects in THS2.2M

Reduction of laryngeal effects in THS2.2M

Reduction of tracheal effects in THS2.2M

Very low pulmonary effects in THS2.2M
Conclusions

Exposure of rats to aerosol from THS2.2M – even at the highest aerosol concentration - results in a dramatically lower biological effects as compared to exposure to reference cigarettes in

- Systemic toxicity; where effects were observed, they are related to nicotine exposure
- Lung inflammation
- Histopathology of respiratory tract organs

No additional menthol-related effects were observed in the mentholated reference cigarettes-exposed animals when compared to 3R4F-exposed animals.

Heat vs. burn reduces toxicity of mentholated product.

Toxicological assessment with integrated molecular toxicology endpoints demonstrates reduced exposure effects for a mentholated heat-not-burn modified risk tobacco product compared with mentholated combustible cigarette. Oviedo, A. et al. (under preparation)
Acknowledgements

Special thanks to the entire team, which has provided a great level support at all stages of this project.

Thanks also to the following departments
- Aerosol generation
- Workshop and exposure set-up
- Treatment team and animal handling
- Barrier support
- Dissection and lavage
- Bioanalytics
- Computational Biology and Statistics
- Histological processing and pathology
THANK YOU!
Reduced-Risk Products ("RRPs") is the term we use to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes.

PMI’s RRPs are in various stages of development and commercialization, and we are conducting extensive and rigorous scientific studies to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when compared to smoking cigarettes.

Before making any such claims, we will rigorously evaluate the full set of data from the relevant scientific studies to determine whether they substantiate reduced exposure or risk. Any such claims may also be subject to government review and authorization, as is the case in the US today.