

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

Oviedo, A. *, Lebrun, S. #, Ho, J. *, Krishnan, S. *, Tan, W.T. *, Vuillaume, G. #, Leroy, P. #, Peitsch, M.C. #, Hoeng, J. #, Vanscheuwijk, P.

* Philip Morris International Research Laboratories, 50 Science Park road, 117406, Singapore. #Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland (part of PMI group of companies)

Background

The toxicity of aerosol from the mentholated variant of the tobacco stick used with the Tobacco Heating System (THS2.2M), a candidate modified risk tobacco product (cM RTP), was characterized in Sprague-Dawley rats in a 90-day subchronic inhalation study in accordance with the OECD Test Guideline 413, and compared to the toxicity of mainstream smoke (MS) from mentholated reference cigarettes (MRC).

Study Design & Endpoints



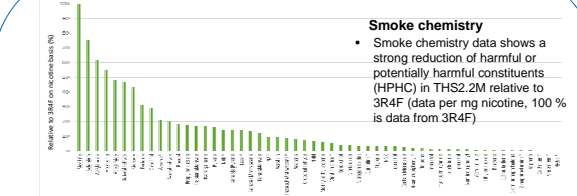
OECD TG 413 Endpoints – Standard Toxicology

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

In addition to OECD endpoints, multi-analyte profiling of pulmonary inflammation was performed.

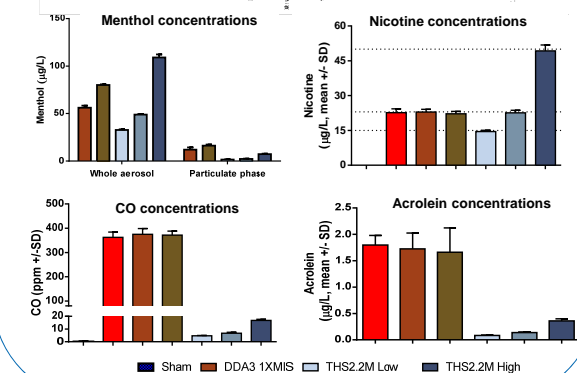
Sprague-Dawley rats were nose-only exposed for 6 hours per day, 5 days per week for 90 days to either filtered air (Sham), to mainstream smoke (MS) from MRC to a target nicotine concentration of 23 µg/L, to a single concentration of MS from the 3R4F reference cigarette (23 µg nicotine/l) or to aerosol from THS2.2M to 3 different target nicotine concentrations (15, 23 and 50 µg/L). DDA3 1XMIS and DDA3 2XMIS were designed to match the nicotine, TPM, and CO level of 3R4F with a menthol yield in smoke condensate of 2.04 mg/cig and 2.58 mg/cig respectively when smoked according to ISO 3308 standard. An additional 42 days of post-inhalation period was included to assess recovery or delayed occurrence of findings.

Test Atmosphere Characterization

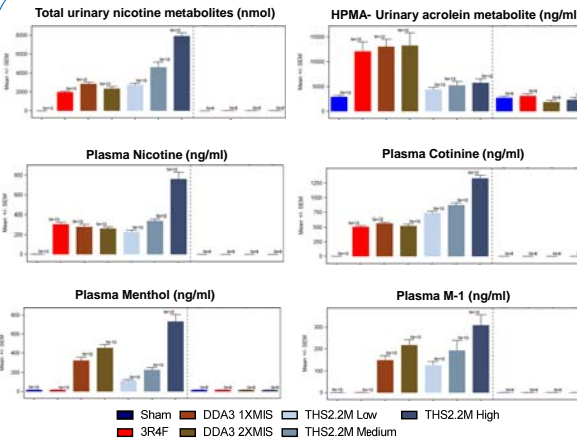


Smoke chemistry

- Smoke chemistry data shows a strong reduction of harmful or potentially harmful constituents (HPHC) in THS2.2M relative to 3R4F (data per mg nicotine, 100% is data from 3R4F)

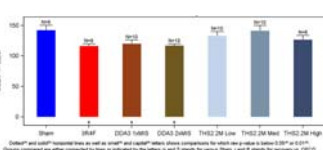


Biomarkers of Exposure



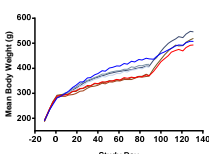
- Aerosols were reproducibly taken up by the animals as indicated by the measured urinary nicotine metabolites and metabolites of selected aerosol constituents analyzed in urine and blood. Bars after the dotted line indicates values obtained during the post-inhalation recovery period.

Respiratory Frequency

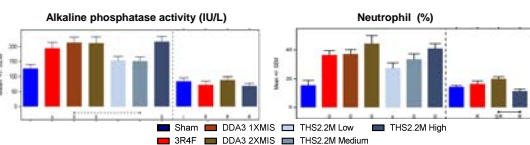


- Lower respiratory frequency in reference groups suggest a reduction in respiratory minute volume (with no obvious effect due to the added menthol). The respiratory minute volumes of rats exposed to aerosol from THS2.2M remained unaffected.

Systemic toxicity



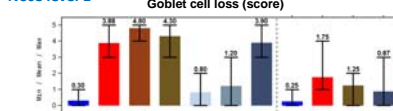
- Aerosol exposure was well tolerated by the animals. Animals in all groups gained weight progressively over time.



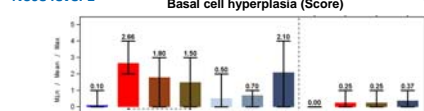
- Nicotine concentration-related increases in:
 - Neutrophil count in blood
 - Relative weight of liver (m/f)
 - Liver enzyme activity

Histopathology of respiratory track organs

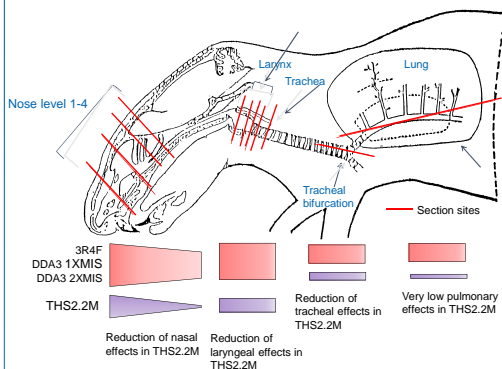
Nose level 1



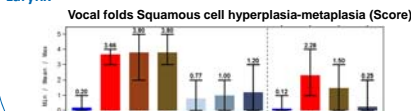
Nose level 1



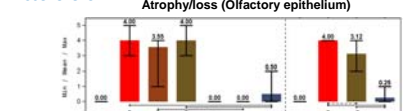
- Increased goblet cell loss and basal cell hyperplasia in respiratory epithelium in MRC-exposed groups.



Larynx

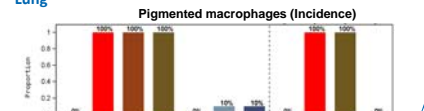


Nose level 3



- 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

Lung

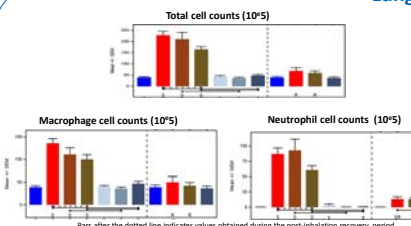


- Very low pulmonary effects in THS2.2M-exposed groups

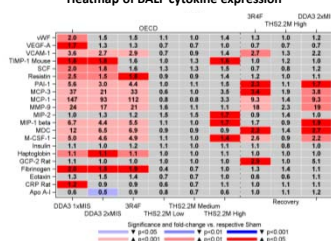
- Reduced severity in the findings observed in THS2.2M-exposed groups

Bars after the dotted line indicates values obtained during the post-inhalation recovery period

Lung inflammation



Heatmap of BALF cytokine expression



Conclusions

- Test atmosphere of the mainstream aerosol from THS2.2M analysis demonstrated a reduction of up to 90% and 98% in aldehydes and CO, respectively when compared to mainstream smoke (MS) from reference cigarettes.
- Respiratory frequency across THS2.2M-exposed groups was similar to that of Sham-exposed animals (even with the test atmosphere containing double nicotine concentration). In addition, THS2.2M-exposed groups had higher nicotine and cotinine levels recovered in blood and urine reflecting the lower irritant capacity of the MS from MRC.
- Systemic toxicity was significantly lower in THS2.2M exposed rats, observed effects were mainly attributed to nicotine concentration-related toxicity.
- Consistent microscopic changes were noted in animals exposed to MS from MRC.
- Reduction of toxicological relevant constituents in the test atmospheres, resulted in notable and significant lower alterations in the respiratory tract in THS2.2M-exposed animals.
- Histopathological changes observed in THS2.2M-exposed animals were statistically significantly lower when compared to animals exposed to MS from MRC and mainly restricted to nose level 1. Moreover, THS2.2M low and THS2.2M medium exposure-related effects were comparable to sham-exposed animals at most levels of the respiratory tract.
- No additional menthol-related effects were observed in the MRC-exposed animals when compared to 3R4F-exposed animals

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PMI RESEARCH & DEVELOPMENT

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