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.

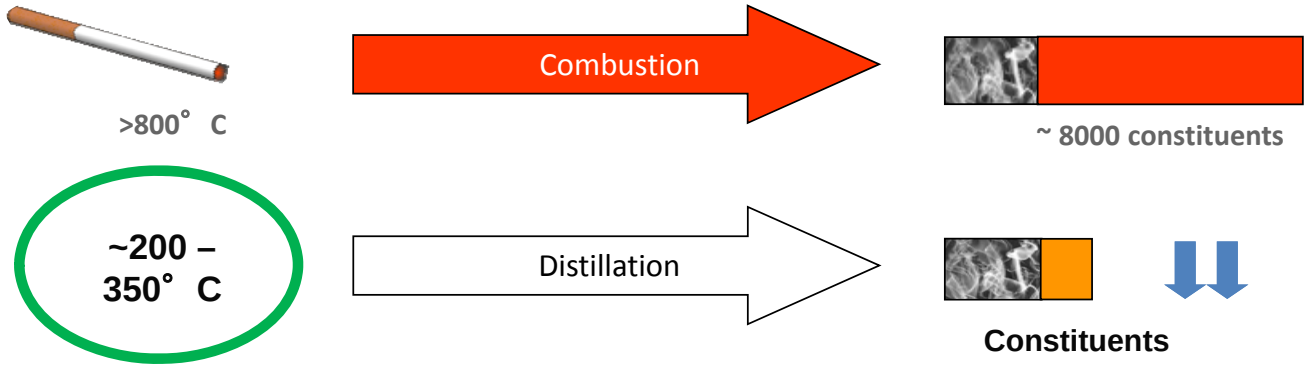
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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)

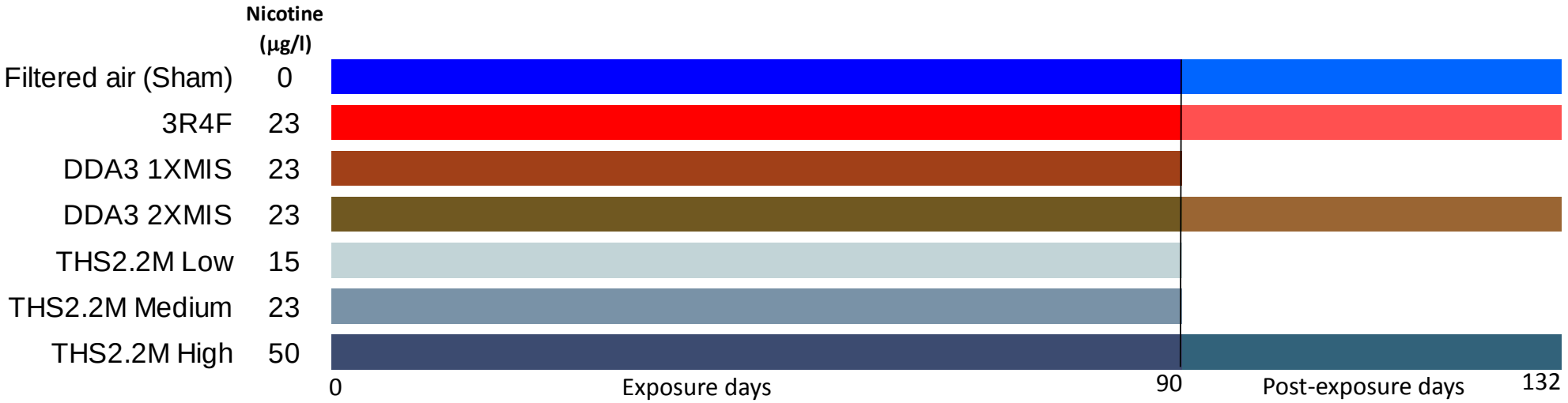
- DDA3 1XMIS with a menthol yield of 2.08 mg/cigarette*
- DDA3 2XMIS with a menthol yield of 2.58 mg/cigarette*

+ 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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Study Design

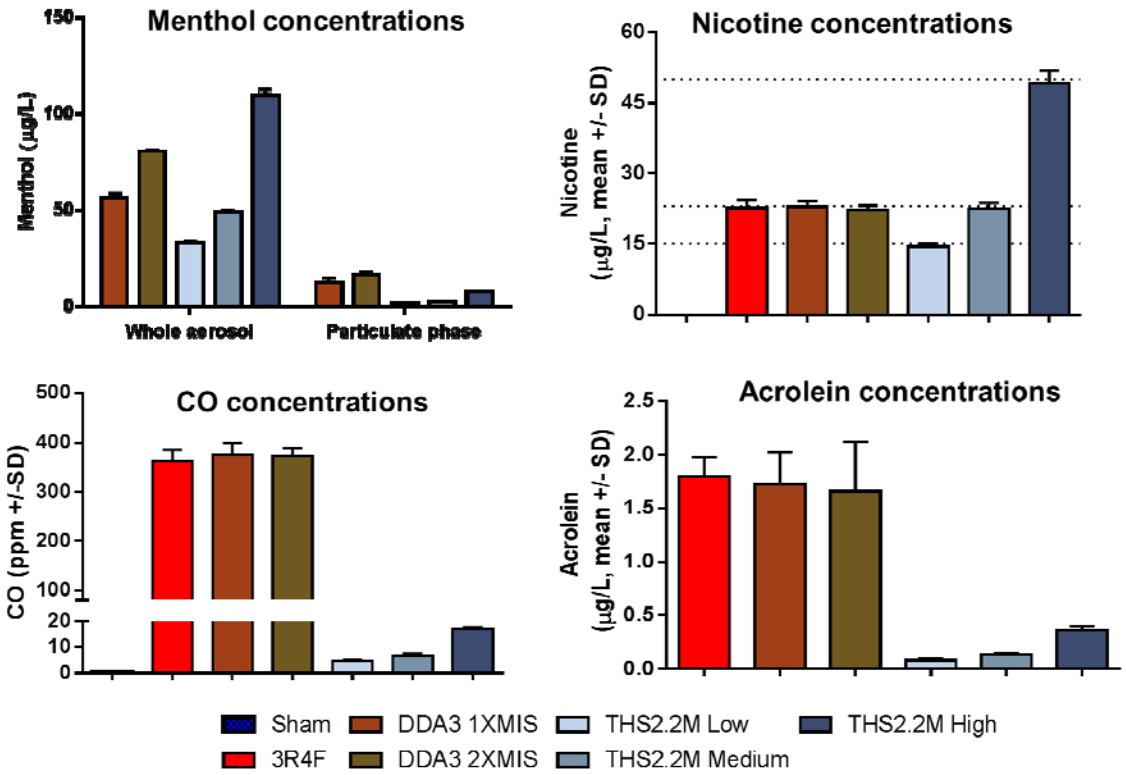


- Target nicotine concentrations were selected based on a dose-range finding study previously conducted.
- Animals were exposed 6h per day during 5 days per week for a total of 13 weeks (90 days).
- An additional 42 days of post-exposure recovery period was included to assess recovery or delayed occurrence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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Results

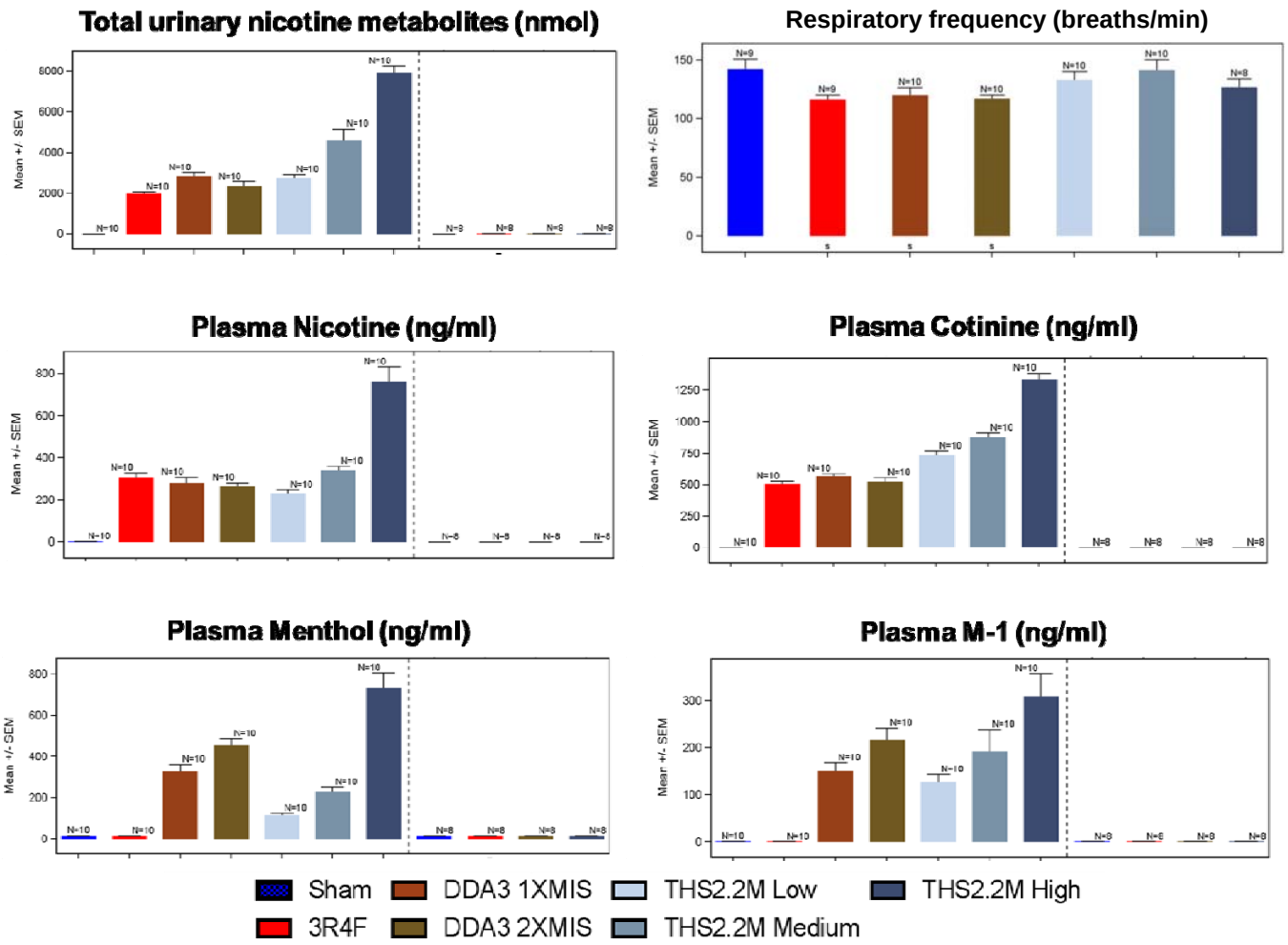
Test atmosphere characterization



- 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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

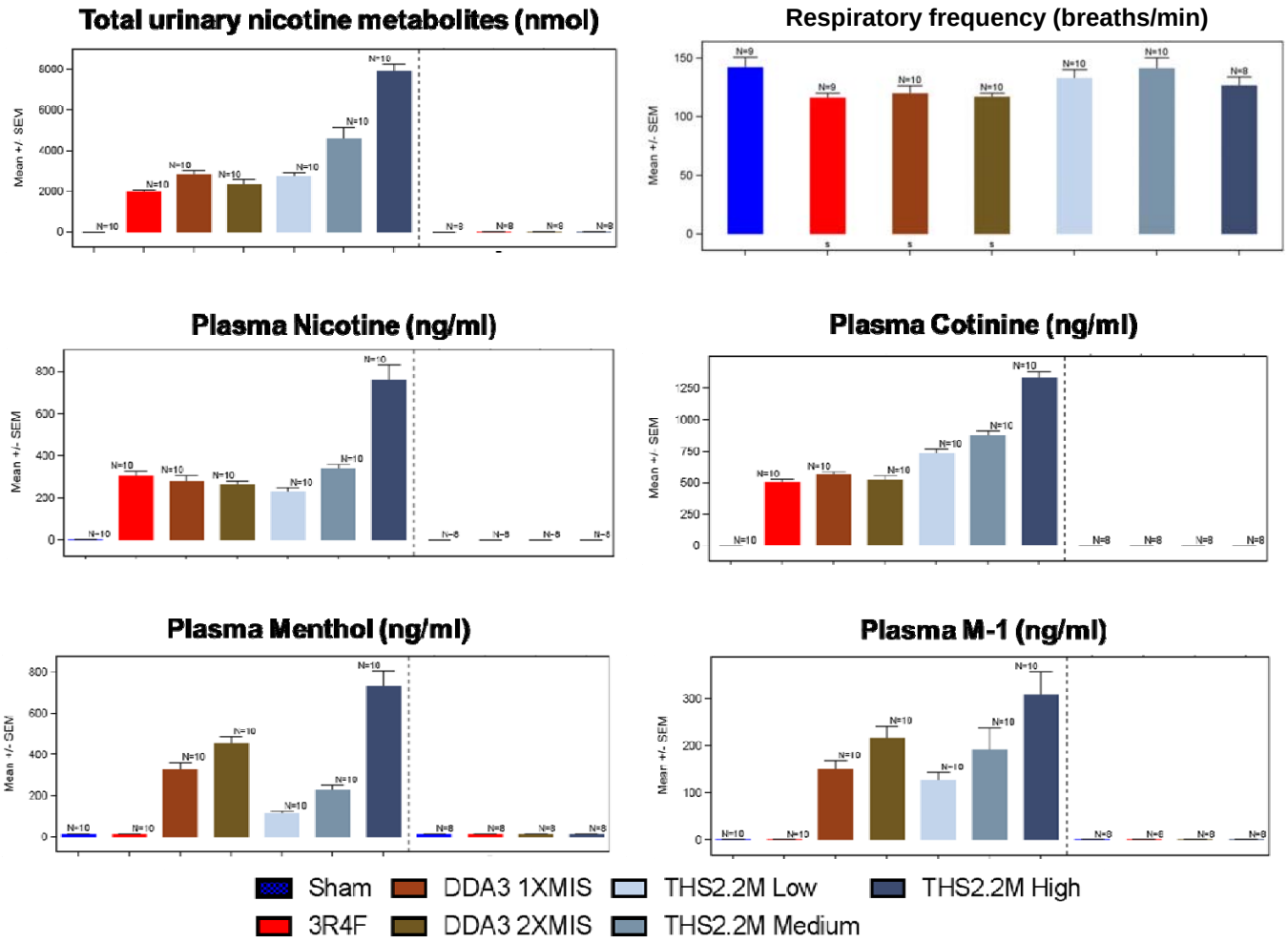
Biomarkers of exposure



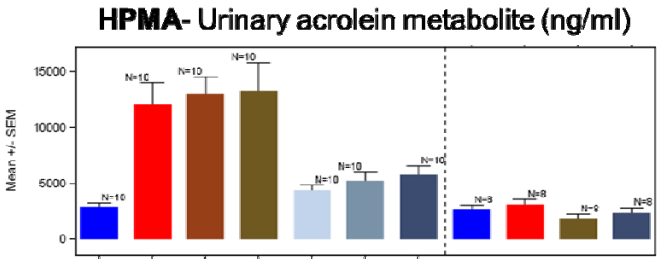
- Total amount of nicotine metabolites excreted higher in THS2.2M-exposed animals
- The plasma nicotine/cotinine and menthol/M-1 concentrations showed a dose dependent increase

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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

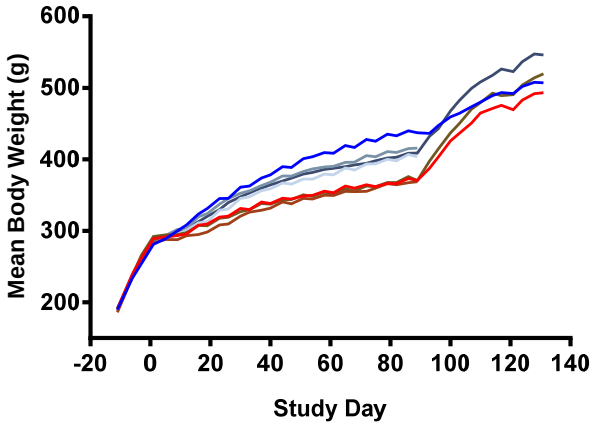


- Concentration of these biomarkers (e.g. HPMA) remained low in all THS2.2M groups

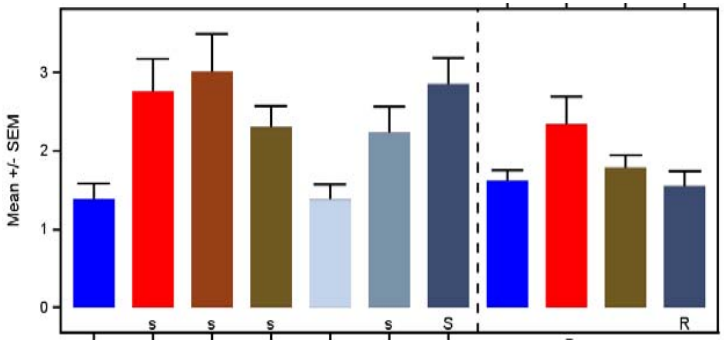
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Systemic toxicity

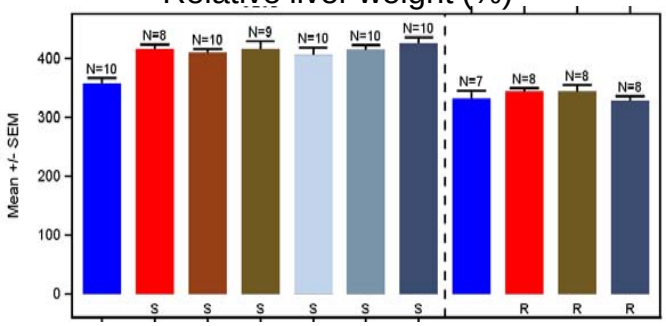
Bodyweight progression



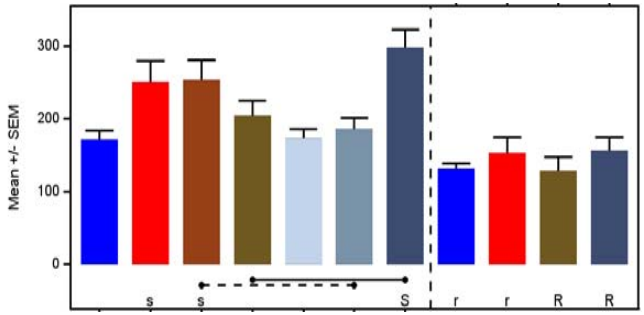
Neutrophil counts (10^3/mL)



Relative liver weight (%)



Alkaline phosphatase activity (IU/L)



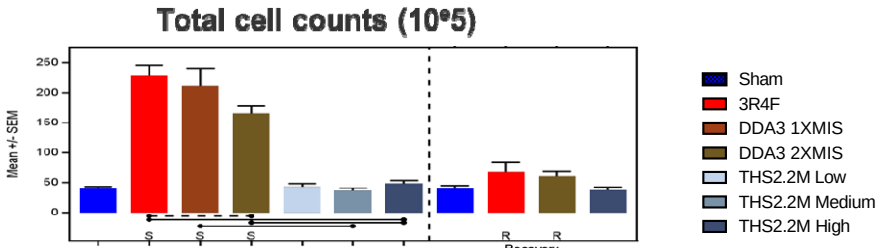
s. p< 0.05 S. p< 0.01
r. p< 0.05 R. p< 0.01
- - - p< 0.05 ---p< 0.01

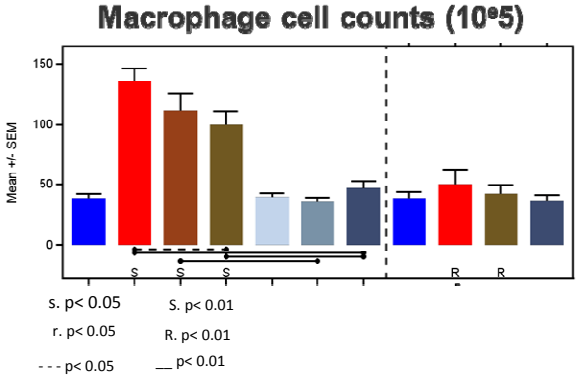
Sham DDA3 1XMIS THS2.2M Low THS2.2M High
3R4F DDA3 2XMIS THS2.2M Medium

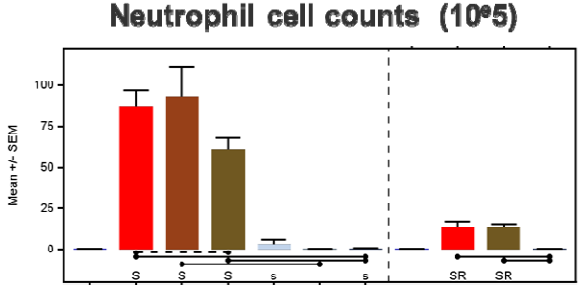
- Higher body weight gained over in THS2.2M exposed groups as compared to reference groups
- No remarkable toxic effects in THS2.2M exposed animals
- Nicotine concentration-related increases in:
  i. Neutrophil count in blood
  ii. Relative weight of liver
  iii. Liver enzyme activity

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Lung inflammation







Cytokine expression of BALF

	OECD						3R4F	THS2.2M High	DDA3 2xMIS
vWF	2.0	1.5	1.5	1.1	1.0	1.4	1.3	1.0	1.2
VEGF-A	1.7	1.3	1.3	0.7	0.7	1.0	0.7	0.7	0.7
VCAM-1	3.6	2.7	2.9	0.7	0.9	1.4	2.7	1.3	2.2
TIMP-1 Mouse	1.8	1.8	1.6	1.0	1.3	1.6	1.0	1.2	1.0
SCF	2.0	1.8	1.6	1.3	1.3	1.5	0.7	0.8	1.2
Resistin	2.5	1.5	1.8	0.9	0.9	1.4	1.2	1.0	1.1
PAI-1	5.6	3.0	4.4	1.0	1.1	1.5	2.3	1.1	1.7
MCP-3	37	21	33	0.6	1.0	3.5	3.4	1.9	3.8
MCP-1	147	93	112	0.8	0.8	3.3	9.3	1.4	9.3
MMP-9	24	17	21	1.6	1.1	1.1	18	2.3	19
MIP-2	1.0	1.3	1.2	1.5	1.5	1.7	0.9	1.4	1.0
MIP-1 beta	6.7	4.4	5.5	1.1	1.0	1.7	1.7	0.9	1.9
MDC	12	6.5	6.9	0.9	0.9	0.9	2.2	1.4	2.7
M-CSF-1	5.0	4.6	4.9	1.1	1.0	1.4	2.6	0.9	2.2
Insulin	1.1	1.0	1.2	1.1	1.0	1.1	1.1	0.8	1.0
Haptoglobin	1.1	1.1	1.1	1.0	1.0	1.1	1.0	1.0	1.0
GCP-2 Rat	1.1	1.0	1.0	1.0	1.0	1.0	2.9	1.0	5.1
Fibrinogen	2.0	1.5	1.9	0.4	0.7	1.0	1.3	1.4	1.1
Eotaxin	1.3	1.5	1.4	0.7	0.7	1.0	0.6	0.6	1.1
CRP Rat	1.2	0.9	0.9	0.6	0.7	1.1	1.0	1.1	1.1
Apo A-I	0.6	0.5	0.9	0.8	0.7	0.6	1.0	1.1	1.2
	DDA3 1xMIS	DDA3 2xMIS	3R4F	THS2.2M Low	THS2.2M Medium	THS2.2M High	Recovery		

- 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**

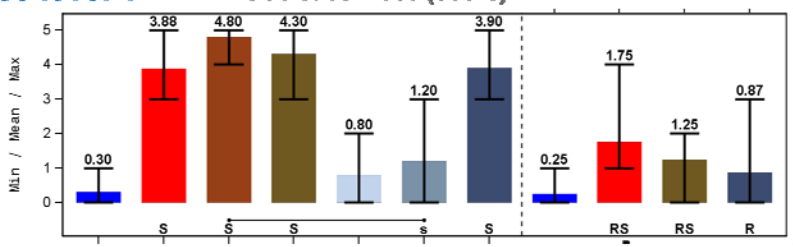
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Histopathology

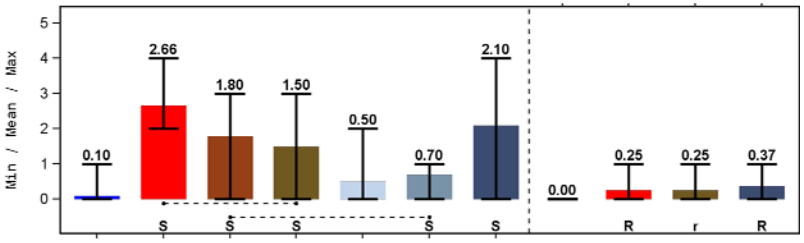
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Histopathology of respiratory tract organs – Nose

Nose level 1 Goblet cell loss (score)



Nose level 1 Basal cell hyperplasia (Score)

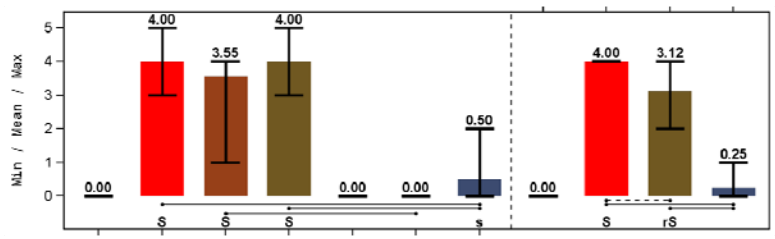


- Increased goblet cell loss and basal cell hyperplasia in respiratory epithelium in animals exposed to reference cigarettes

Sham
3R4F
DDA3 1XMIS
DDA3 2XMIS
THS2.2M Low
THS2.2M Medium
THS2.2M High

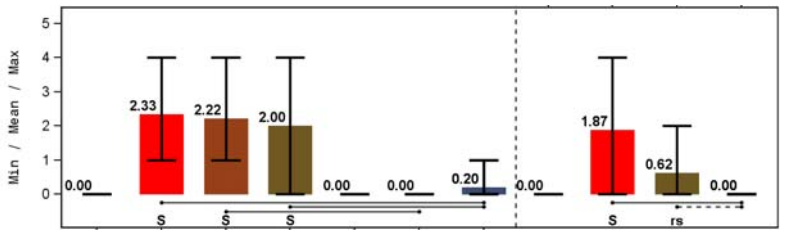
s. p< 0.05 S. p< 0.01
r. p< 0.05 R. p< 0.01
- - - p< 0.05 ___ p< 0.01

Nose level 3 Atrophy/loss (Olfactory epithelium)



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

Nose level 4 Atrophy/loss (Olfactory epithelium)

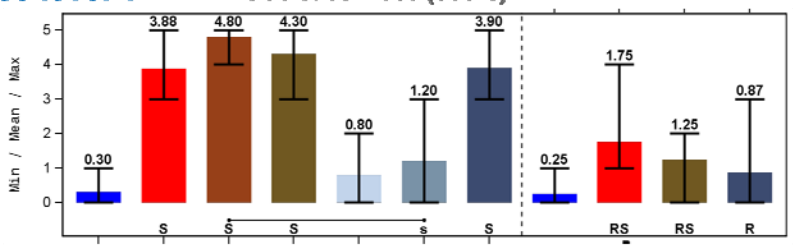


- No significant findings were observed in nose level 4 in THS2.2M-exposed groups

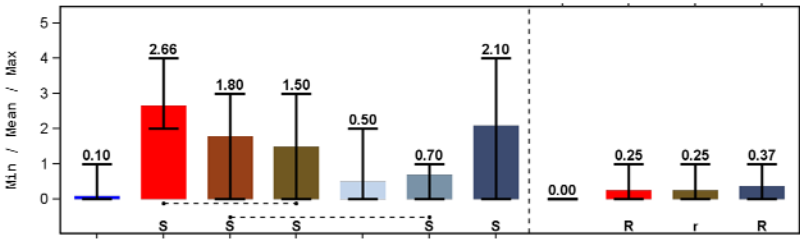
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Histopathology of respiratory tract organs – Nose

Nose level 1 Goblet cell loss (score)



Nose level 1 Basal cell hyperplasia (Score)

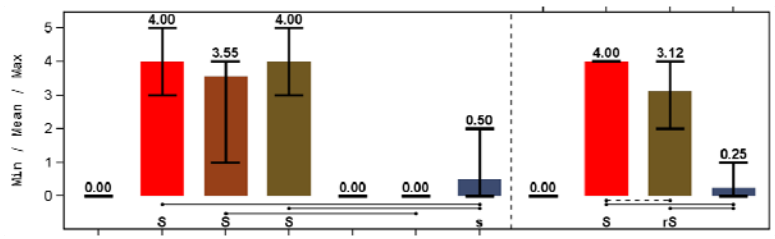


- Increased goblet cell loss and basal cell hyperplasia in respiratory epithelium in animals exposed to reference cigarettes

Sham
3R4F
DDA3 1XMIS
DDA3 2XMIS
THS2.2M Low
THS2.2M Medium
THS2.2M High

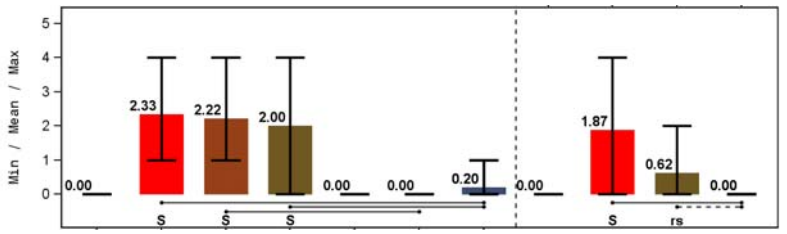
s. p< 0.05 S. p< 0.01
r. p< 0.05 R. p< 0.01
- - - p< 0.05 ___ p< 0.01

Nose level 3 Atrophy/loss (Olfactory epithelium)



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

Nose level 4 Atrophy/loss (Olfactory epithelium)

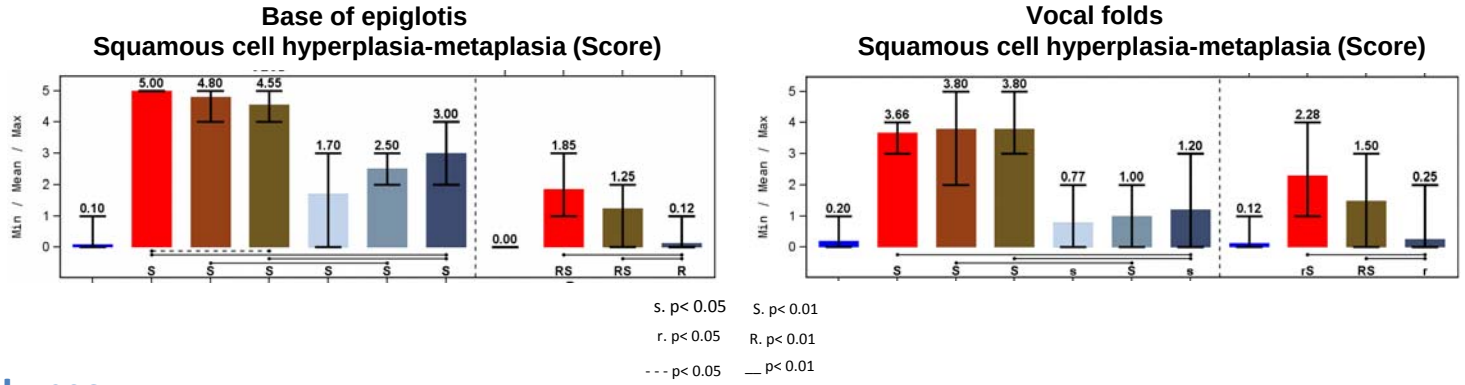


- No significant findings were observed in nose level 4 in THS2.2M-exposed groups

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

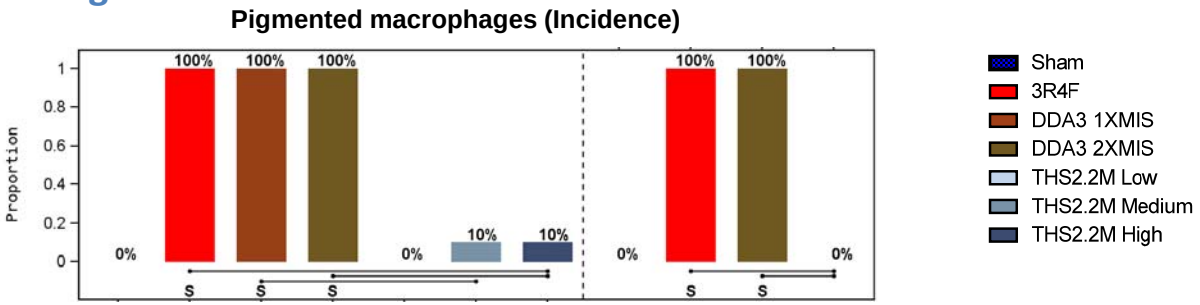
Histopathology of respiratory tract organs – Larynx & lungs

Larynx



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

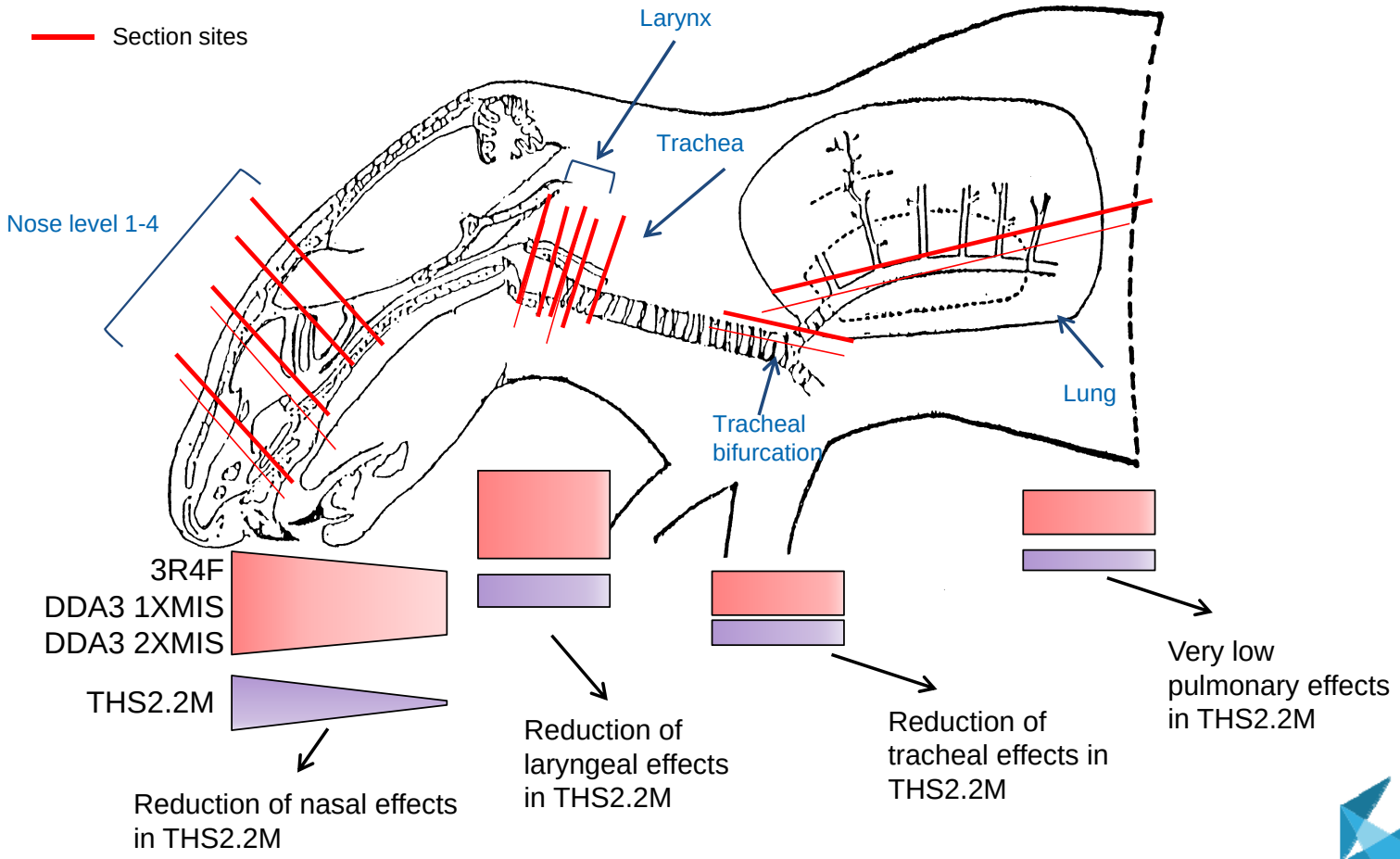
Lungs



- No significant findings observed in THS2.2M-exposed groups, no obvious lung inflammation

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Summary



PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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)*

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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.