Chronic Toxicity and Lung Carcinogenicity in A/J Mice Following Lifetime Exposure to Aerosol from a Candidate Modified Risk Tobacco Product and Mainstream Cigarette Smoke

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Poster ID P144

Introduction and Objective

Background: Chronic exposure to cigarette smoke (CS) is the leading cause of chronic obstructive pulmonary disease (COPD) and lung cancer. The chronic inflammatory state observed in COPD is one contributing factor linking COPD and lung cancer development. Emphysematous changes in the peripheral lung, which arise in the context of chronic inflammation and an impaired protease/anti-protease balance, may create a susceptible microenvironment and confer a growth advantage to lung epithelial cells. Impaired clearance of carcinogenic substances resulting from chronic airflow obstruction can also contribute to lung carcinogenesis.

Candidate modified risk tobacco products (cMRTP) have been developed with the aim of reducing the level of harmful constituents in the smoke from conventional cigarettes. However, the impact of cMRTPs on disease development and the mechanisms linking chronic lung inflammation, COPD, and lung cancer development are largely unknown.

The A/J mouse is highly susceptible to lung tumor development and has been widely used as a screening system in carcinogenicity testing, including that of CS (Stinn et al., 2013).

Objective: This study was performed to evaluate and compare the impact of lifetime exposure to CS from the 3R4F reference cigarette and aerosol from the Tobacco Heating System (THS) 2.2, a cMRTP, on lung tumor incidence and multiplicity, the extent of lung inflammation and emphysematous changes, and systemic toxicity in A/J mice.

Study Design and Endpoints

Female A/J mice were exposed to filtered air (Sham), to three concentrations of THS 2.2 aerosol (6.7, 13.4, 26.8 µg/L nicotine), and to one concentration of 3R4F CS (13.4 µg/L nicotine) for six hours/day, five days/week, for 18 months (Figure 1). Additional male mice were exposed to Sham and THS 2.2 aerosol (26.8 µg/L nicotine) for 15 months. Care and use of the mice were in accordance with the National Advisory Committee for Laboratory Animal Research Guideline (2004). All animal experiments were approved by the Institutional Animal Care and Use Committee. Interim dissections of subgroups of female mice were performed after one, five, and 10 months of exposure. Terminal dissections were performed at Months 15 and 18 for the male and female mice, respectively. At selected time points, animals were allocated for the analysis of the following endpoints: bronchoalveolar lavage fluid (BALF) analysis by flow cytometry and multi-analyte (cytokines/chemokines) profiling, histopathological evaluation of the lungs, lung function tests, lung morphometry, lung tumor analysis, and an extensive systems toxicological analysis (transcriptomics, proteomics, DNA sequencing).

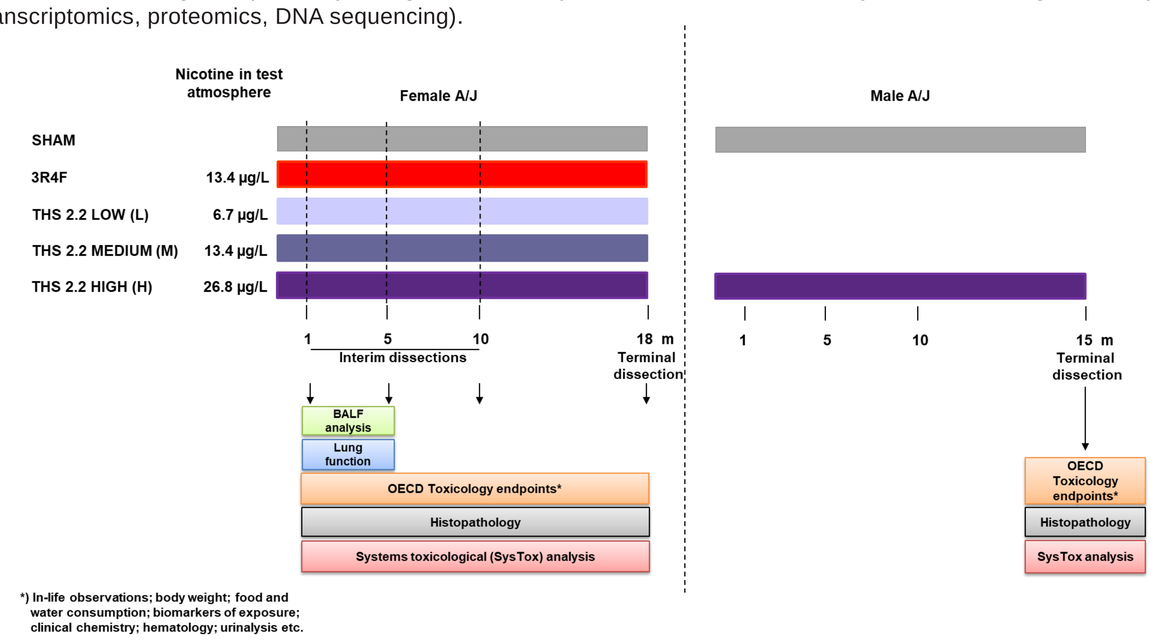


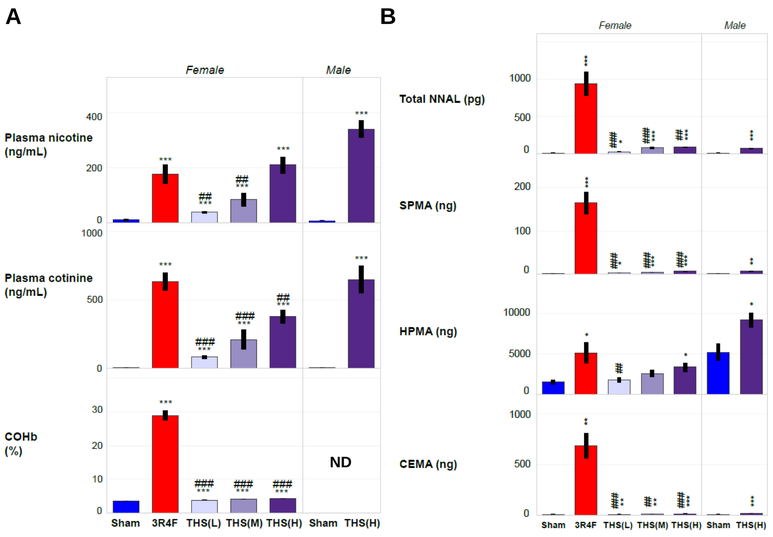
Figure 1: *Schematic overview of study design, dissection time points, and study endpoints.*

Aerosol Exposure and Uptake

The daily monitoring of aerosol components indicated that the aerosol/CS was generated and delivered to the inhalation chambers in a consistent manner, with mean nicotine test atmosphere concentrations very close to the target concentrations. Aerosol generated from THS 2.2 sticks contained lower concentration of total particulate matter, carbon monoxide, and carbonyls compared with 3R4F CS at the same nicotine concentration (Table 1).

Table 1: *Test atmosphere characterization. Data are presented as mean ± SD. The number of daily average measurements are shown in parentheses. F: female; M: male; TPM: total particulate matter; CO: carbon monoxide; NA: not available.*

Chamber name	Sham_F	3R4F_F	THS 2.2 Low_F	THS 2.2 Med_F	THS 2.2 High_F	Sham_M	THS 2.2 High_M
Target nicotine concentration (µg/L)	0	13.4	6.7	13.4	26.8	0	26.8
Achieved nicotine concentration (µg/L)	0.0 ± 0.0 (92)	13.0 ± 1.4 (397)	6.7 ± 0.8 (397)	13.0 ± 1.3 (397)	26.7 ± 2.0 (397)	0.0 ± 0.0 (82)	26.1 ± 2.8 (332)
Achieved TPM concentration (µg/L)	−2.3 ± 1.7 (397)	295.7 ± 19.0 (397)	61.4 ± 6.4 (397)	138.7 ± 16.8 (397)	272.4 ± 27.2 (397)	−2.3 ± 2.0 (335)	291.0 ± 41.2 (334)
Achieved CO concentration (ppm)	0.2 ± 0.5 (391)	389.3 ± 26.3 (394)	3.6 ± 0.5 (390)	6.4 ± 1.2 (392)	11.0 ± 1.4 (373)	0.5 ± 0.3 (330)	12.9 ± 1.7 (332)
Achieved formaldehyde concentration (µg/L)	NA	0.40 ± 0.06 (80)	0.04 ± 0.01 (80)	0.06 ± 0.02 (79)	0.10 ± 0.02 (80)	NA	0.09 ± 0.02 (67)



Analysis of biomarkers of aerosol uptake, measured in plasma or urine, confirmed aerosol uptake consistent with the exposure groups and the chemical composition of the CS/aerosol (Figure 2).

Figure 2: *Biomarkers of exposure in (A) plasma and (B) urine. Plasma biomarker data from Months 12 (male mice; no COHb at this time point; N=8-10) and 16 (female mice; N=9-10) are presented as mean ± SEM; urinary biomarker data from Months 14 (male mice; N=7-11) and 16 (female mice; N=8-14) are presented as mean ± SEM. *: p<0.05; **: p<0.01; ***: p<0.001 vs. Sham (fresh air); ##: p<0.01; ###: p<0.001 vs. 3R4F (t-tests).*

Results

1. Chronic Toxicity

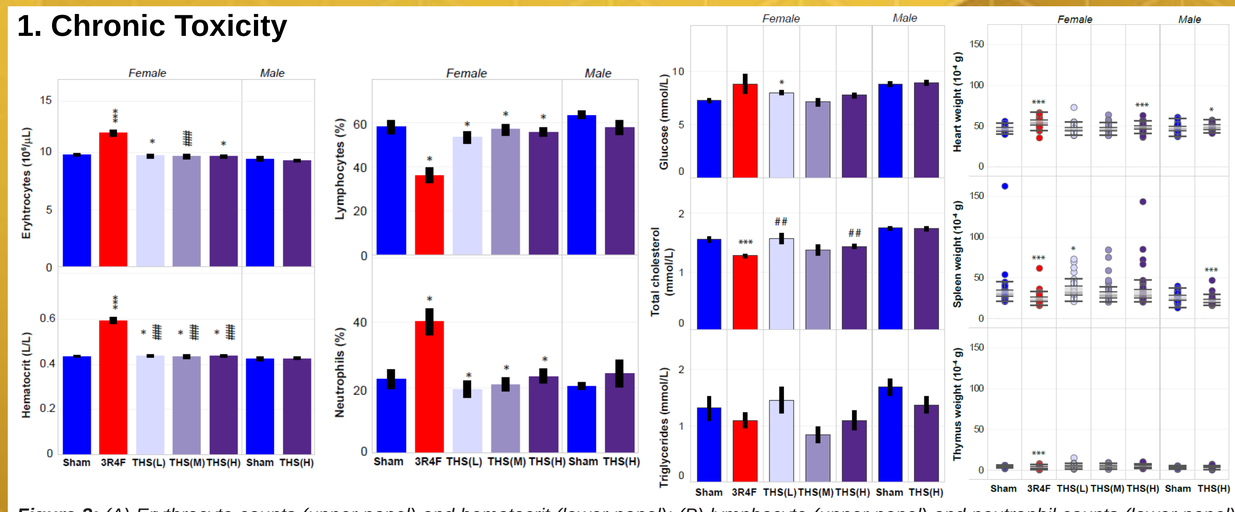


Figure 3: *(A) Erythrocyte counts (upper panel) and hematocrit (lower panel); (B) lymphocyte (upper panel) and neutrophil counts (lower panel); (C) serum glucose (upper panel), total cholesterol (middle panel), and triglycerides (lower panel); and (D) heart (upper panel), spleen (middle panel), and thymus (lower panel) weights relative to body weight after exsanguination. Data are from terminal dissection and presented as means ± SEM. *: p<0.05; ***: p<0.001 vs. Sham; ###: p<0.001 vs. 3R4F (t-tests).*

2. Lung inflammation, emphysema

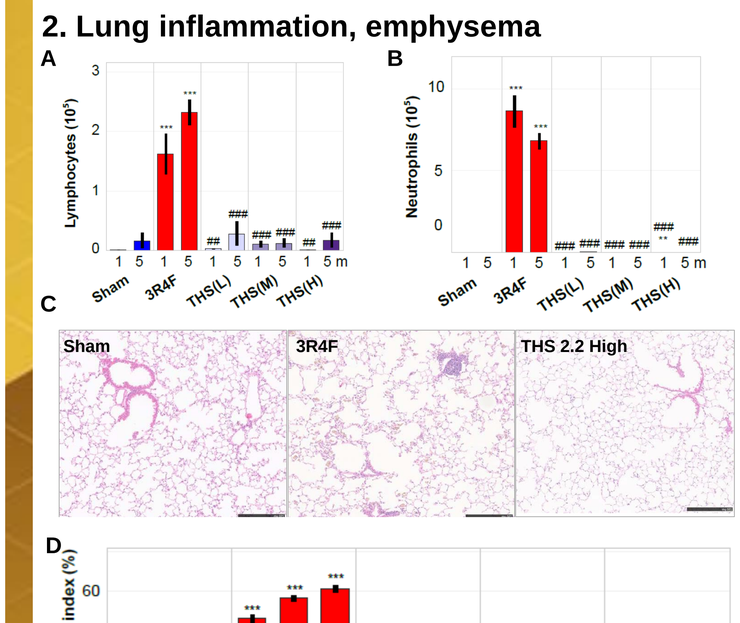


Figure 4: *(A) Lymphocyte and (B) neutrophil counts in BALF following one and five months (m) of exposure. (C) Representative images of hematoxylin/eosin-stained cross-section of lungs from mice exposed for 10 months (scale bar: 250 µm). (D) Destructive index in lungs from mice exposed for 5, 10, or 18 months. Data are from female mice and presented as means ± SEM (N=10). **: p<0.01; ***: p<0.001 vs. Sham; #: p<0.05, ##: p<0.01; ###: p<0.001 vs. 3R4F (t-tests).*

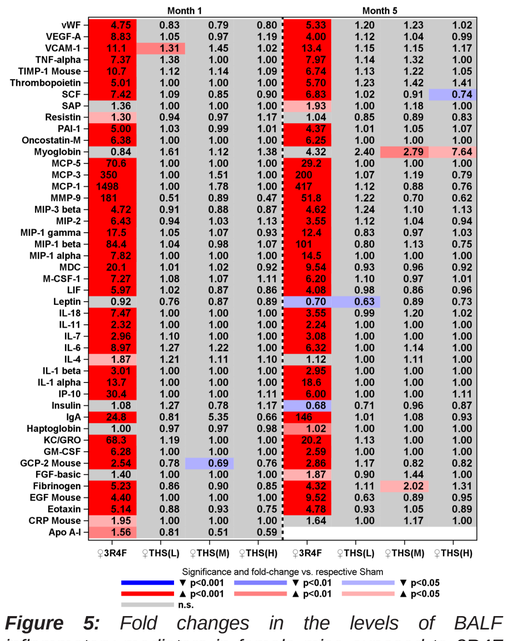


Figure 5: *Fold changes in the levels of BALF inflammatory mediators in female mice exposed to 3R4F CS or THS 2.2 aerosol for one or five months relative to Sham (N=10 per study group).*

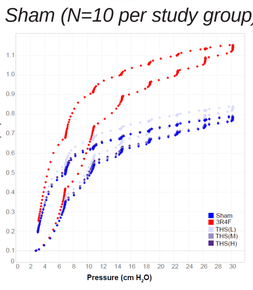


Figure 6: *Pressure-volume curves from lung function measurements in A/J mice exposed for five months. Data are from female mice and presented as means (N=8-10). Error bars were removed for clarity.*

3. Carcinogenicity

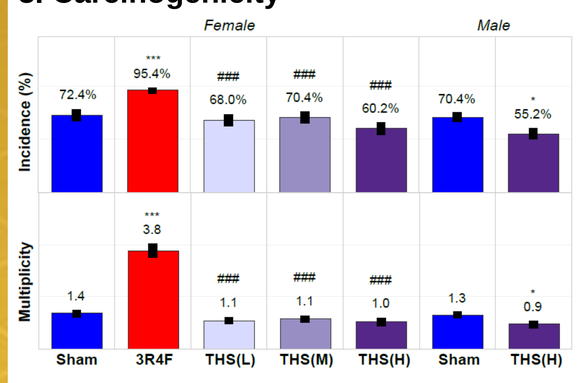


Figure 7: *Lung adenoma and/or carcinoma incidences (upper panel) and multiplicities (lower panel). Data are from terminal dissection and early death animals (study days 74-537) and were adjusted for survival by poly-k test at k=3; multiplicities were also adjusted by study day (day 240 for males, day 400 for females). *: p<0.05; ***: p<0.001 versus Sham (fresh air); ###: p<0.001 versus 3R4F.*

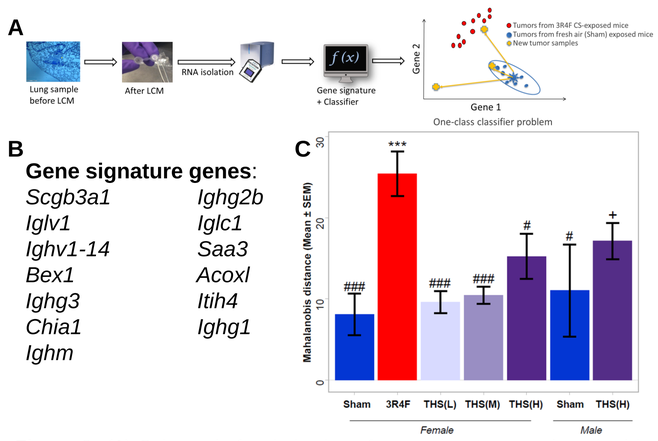


Figure 8: *(A) Schematic for derivation of gene signature as described in Luettich et al. (2014), (B) list of signature genes, and (C) estimates of similarity between lung tumors based on Mahalanobis distance using the gene signature. Data are presented as mean ± SEM.***: p<0.001 vs. Sham; #: p<0.05; ###: p<0.001; +: only two tumor samples in this study group.*

Summary

- Aerosol was reproducibly generated and delivered to the animals.
- Signs of systemic toxicity indicate that stress-related effects and nicotine effects are less pronounced or absent in THS 2.2 aerosol-exposed mice, even at twice the concentration of nicotine in the aerosol.
- No lung inflammation and emphysematous changes were observed in THS 2.2 aerosol-exposed mice, even at twice the concentration of nicotine in the aerosol, whereas clear inflammatory and emphysematous changes were observed upon 3R4F CS exposure.
- Lung tumor incidence and multiplicity were lower in THS 2.2 aerosol than in 3R4F CS-exposed mice, even at twice the concentration of nicotine in the aerosol, whereas 3R4F CS exposure caused significant increases in lung tumor incidence and multiplicity.
- Based on gene expression data, lung tumors in THS 2.2 aerosol-exposed mice are very similar to those developing spontaneously in Sham animals but significantly different from those in 3R4F CS-exposed mice.

References

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Acknowledgements

We would like to acknowledge the technical assistance and support of Abdelkader Benyagoub, Camille Schilt, Anaelle Dubois, Laurent Neau, and the Bioresearch, Bioanalytical, and Aerosol Teams at PMIRL Singapore and PMI Neuchatel. The pathological evaluation was managed by Laboratory of Pharmacology and Toxicology GmbH & Co. KG, expertly conducted by Ansgar Büttner (Histovia GmbH), and peer reviewed by Carole Meschter (Comparative Biosciences Inc.). We are also grateful to Sam Ansari for sample management and biobanking and Stephanie Boué for generating figures and graphs for presentation purposes.

4th German Pharm-Tox Summit **Stuttgart, Germany**
25–28 February 2019

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