Systems toxicology assessment of potential modified risk tobacco products: effects on lung, liver, and heart in ApoE$^{-/-}$ mice using iTRAQ

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Introduction and objectives

Cigarette smoke (CS) causes adverse health effects that may occur shortly after smoking initiation and lead to the development of respiratory disease (chronic obstructive pulmonary disease), cardiovascular disease, and cancer. To reduce the risk of smokers to develop smoking-related diseases, Philip Morris International is developing smoke-free tobacco products such as Carbon-Heated Tobacco Product and Tobacco Heating System.

The Carbon-Heated Tobacco Product (CHTP) 1.2 is a potential Modified Risk Tobacco Product (MRTP) in which the tobacco plug, in a specially designed stick, is heated to less than 350°C using a carbon heat source [1]. The Tobacco Heating System (THS) 2.2, a candidate MRTP, utilizes an electronically controlled heating system to heat tobacco [2]. The operating temperature in both systems is below the combustion temperature of tobacco, resulting in generation of aerosols with significant reduction in levels of harmful and potentially harmful constituents compared with CS.

In a six-month inhalation toxicity study with ApoE$^{-/-}$ mice, aerosols from THS 2.2 and CHTP 1.2 were compared with CS at matching aerosol/CS nicotine concentrations. Fresh air exposure served as a control, and the effects of smoking cessation or switching to CHTP 1.2 after three months of CS exposure were also evaluated. Within this systems toxicology assessment study, effects on classical toxicological endpoints as well as omics endpoints were assessed. Here, we present the proteomics results on lung, liver, and heart ventricle analyzed using isobaric tag for relative and absolute quantitation (iTRAQ).

Study overview and iTRAQ analysis

In a six-month inhalation toxicity study with ApoE$^{-/-}$ mice, one candidate and one potential MRTP, the THS 2.2 and CHTP 1.2, respectively, were compared with CS from a 3R4F reference cigarette at matching aerosol/CS nicotine concentrations (28 µg nicotine/L, three hours per day). Fresh air exposure (Sham) served as a control, and the effects of smoking cessation or switching to CHTP 1.2 after three months of CS exposure were also evaluated. Eight replicates per group were analyzed at three time points (three, four, and six months).

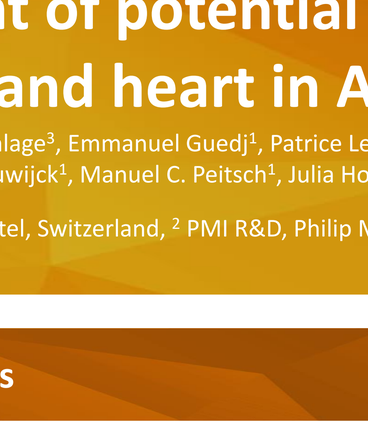

Figure 1. Overview of the study design.

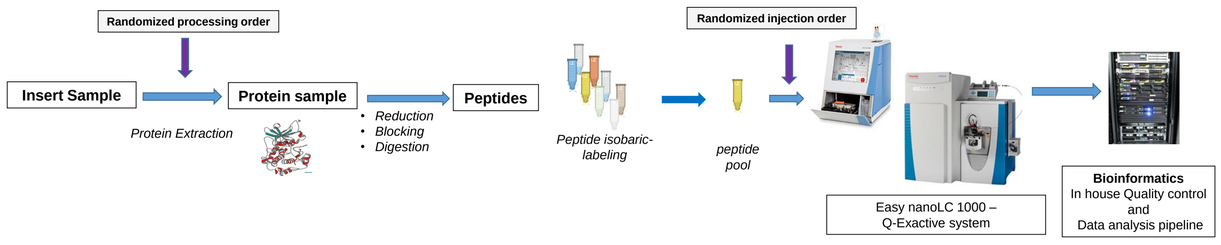

Figure 2. Schematic overview of the iTRAQ workflow.

Lung

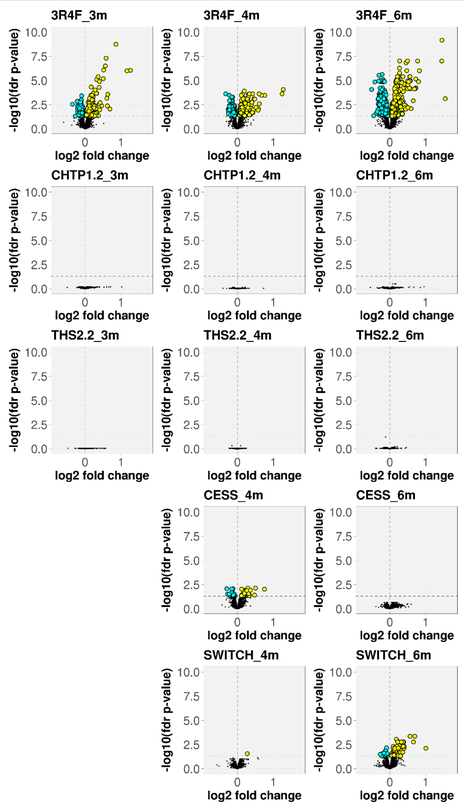

Figure 3. Volcano plots representing the lung proteome profiles. *For each protein, the change in expression in the exposed group compared with the respective Sham group, calculated as the log2 fold change, is plotted on the x-axis; statistical significance, proportional to the −log10-adjusted p-value, is plotted on the y-axis. Bold dots indicate proteins that were statistically significantly up- (yellow) or down- (cyan) regulated compared with the Sham group at each time point (FDR-adjusted p-value <0.05).*

Figure 4. Numbers of differentially expressed proteins in the lung. *Significantly differentially expressed proteins with an FDR-adjusted p-value <0.05. DEP: Differentially expressed proteins.*

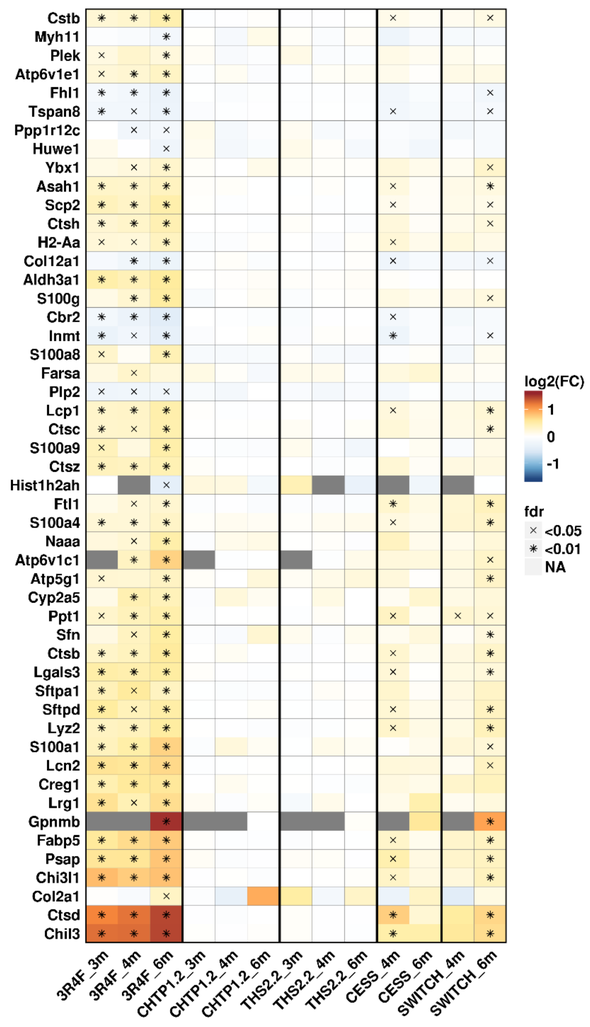

Figure 5. Expression profiles of differently expressed protein in the lung. *The protein expression fold changes compared with the respective Sham group are color-coded, and statistical significance is marked (FDR-adjusted p-value, x = <0.05, * = <0.01). Only the top 50 differentially expressed proteins by absolute fold change across all conditions are shown. Missing values are marked in grey.*

Heart ventricle

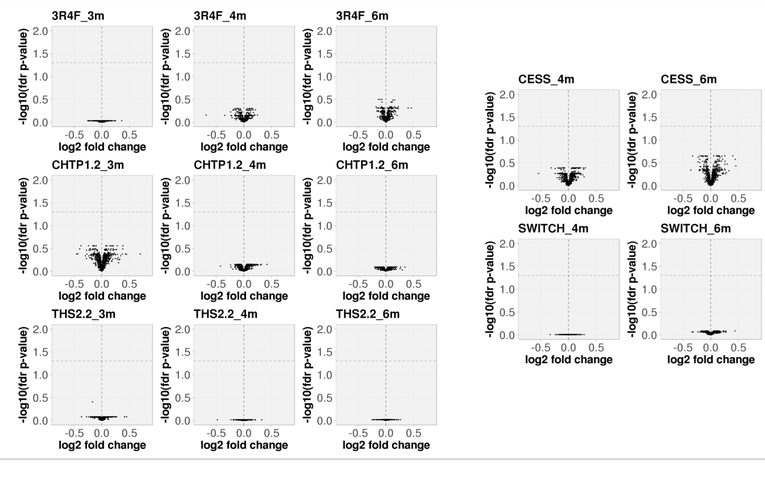

Figure 6. Volcano plots representing the heart proteome profiles. *For each protein, the change in expression in the exposed group compared with the respective Sham group, calculated as the log2 fold change, is plotted on the x-axis; statistical significance, proportional to the −log10-adjusted p-value, is plotted on the y-axis. Bold dots indicate proteins that were statistically significantly up- (yellow) or down- (cyan) regulated compared with the Sham group at each time point (FDR-adjusted p-value <0.05).*

Liver

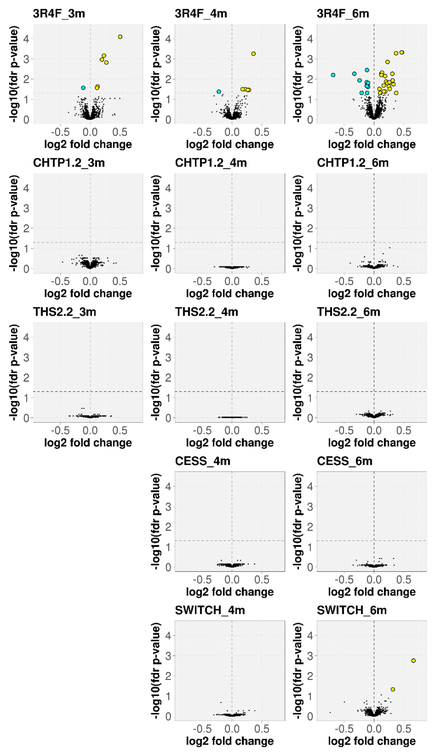

Figure 7. Volcano plots representing the liver proteome profiles. *For each protein, the change in expression in the exposed group compared with the respective Sham group, calculated as the log2 fold change, is plotted on the x-axis; statistical significance, proportional to the −log10-adjusted p-value, is plotted on the y-axis. Bold dots indicate proteins that were statistically significantly up- (yellow) or down- (cyan) regulated compared with the sham group at each time point (FDR-adjusted p-value <0.05).*

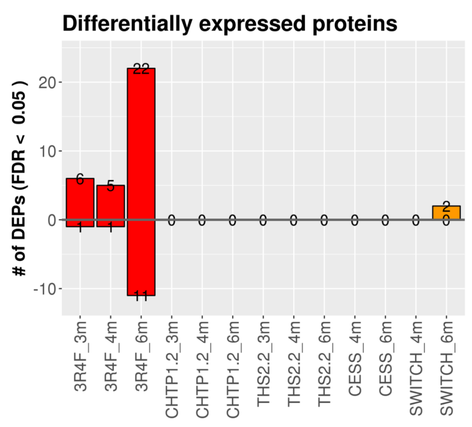

Figure 8. Numbers of differentially expressed proteins in the liver. *Significantly differentially expressed proteins with an FDR-adjusted p-value <0.05. DEP: Differentially expressed proteins.*

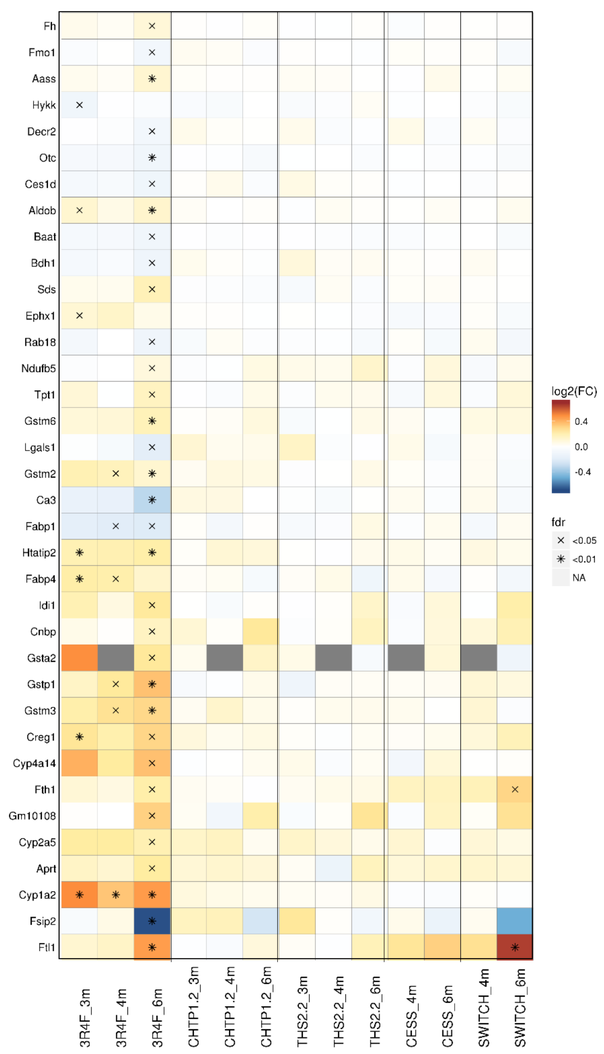

Figure 9. Expression profiles of differentially expressed protein in the liver. *The protein expression fold changes compared with the respective Sham group are color-coded, and statistical significance is marked (FDR-adjusted p-value, x = <0.05, * = <0.01). Only the top 50 differentially expressed proteins by absolute fold change across all conditions are shown. Missing values are marked in grey.*

Summary and conclusions

- A six-month inhalation exposure study was conducted to assess the effects of exposure to CHTP 1.2 and THS 2.2 aerosol compared with those of 3R4F CS on the lung, heart, and liver of ApoE$^{-/-}$ mice. In addition, the effects of cessation and switching from 3R4F CS to CHTP 1.2 aerosol were evaluated.
- 2,508, 2,008, and 1,173 proteins were quantified for lung, liver, and heart ventricle, respectively.
- CS elicited an extensive exposure response in the lung, including an immune and oxidative stress response (up to 500 differentially expressed proteins).
- THS 2.2 and CHTP 1.2 aerosol exposure were associated with lesser molecular effects than CS on these processes in the lung.
- No significantly differentially expressed proteins were detected in the heart proteome among the test groups.
- CS exposure induced significant protein alterations in the liver, including xenobiotic metabolism, oxidative stress, and iron metabolism-related proteins.
- Upon THS 2.2 and CHTP 1.2 aerosol exposure, no differential protein expression was observed in the liver.
- Overall, this work supports reduced biological effects of THS 2.2 and CHTP 1.2 aerosols, compared with CS, in the ApoE$^{-/-}$ mouse model.

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Competing Financial Interest

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