The impact of aerosol from Carbon Heated Tobacco Product 1.2 compared with cigarette smoke on the cardiovascular system of ApoE$^{-/-}$ mice in a six-month inhalation study

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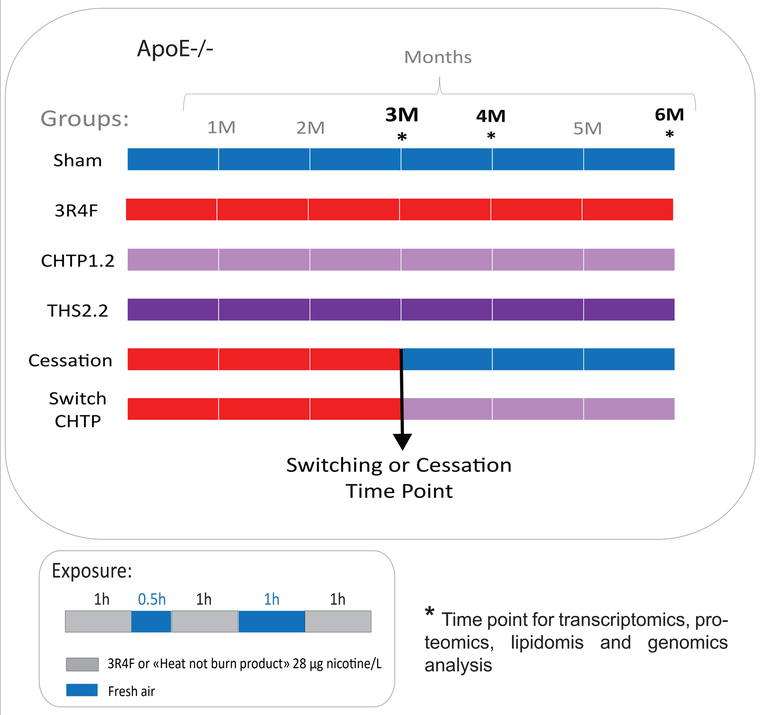
Introduction and Objectives

Cigarette smoking is the main risk factor for the development and progression of a series of diseases, including cardiovascular disease (CVD) (1-4) and chronic obstructive pulmonary disease (5). Suitable animal models play an important role in understanding smoke-induced pathogenesis. This study examined the development of hallmarks of CVD in ApoE$^{-/-}$ mice exposed to either cigarette smoke (CS) or to an aerosol from two heated tobacco products, the Tobacco Heating System (THS) 2.2, and the Carbon-Heated Tobacco Product (CHTP) 1.2, over a six-month period. In addition to chronic exposure regimes, a comparison of exposure cessation or switching to CHTP 1.2 after three months of exposure to CS was performed. Assessment of effects within this System Toxicology study leveraged a battery of assays: physiological, morphological, and molecular analysis.

Study Design and Endpoints



* Time point for transcriptomics, proteomics, lipidomis and genomics analysis

ApoE$^{-/-}$ mice were exposed to air (control group), to CHTP 1.2 or THS 2.2 aerosol, or to 3R4F CS at a concentration of 28.0 µg nicotine/L (equivalent to 600 µg total particulate matter/L) for six months.

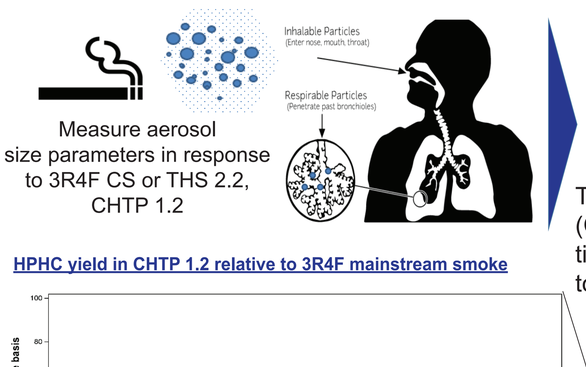
Additionally, the impact of smoking cessation or switching to CHTP 1.2 aerosol exposure after three months of 3R4F CS exposure was assessed. Blood lipid profiling, histopathological evaluation, computed tomography scans, and transcriptomic analysis of thoracic aorta and heart ventricle were performed to investigate the impact of CHTP 1.2 aerosol and CS exposure on the cardiovascular system.

Animals were observed on a daily basis, body weight progression was monitored twice per week, and exposure and uptake parameters (including nicotine metabolites in urine) were measured three times during the study.

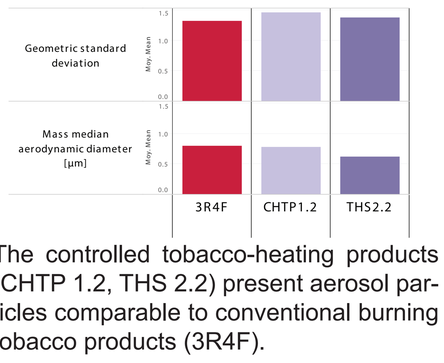
Dissections were performed after two, three, four, and six months of exposure.

Characterization of Aerosol: 3R4F, CHTP 1.2, and THS 2.2

Characterization of aerosol particles

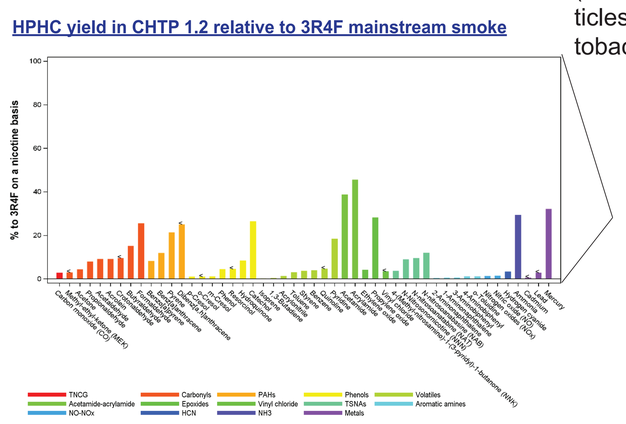


Measure aerosol size parameters in response to 3R4F CS or THS 2.2, CHTP 1.2



The controlled tobacco-heating products (CHTP 1.2, THS 2.2) present aerosol particles comparable to conventional burning tobacco products (3R4F).

HPHC yield in CHTP 1.2 relative to 3R4F mainstream smoke



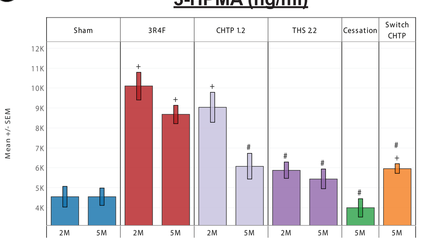
The controlled tobacco-heating approach of CHTP 1.2 reduces the delivery of harmful smoke constituents as compared with conventional burning tobacco products, as shown for 49 priority toxicants (6).

Biomarkers of Aerosol Exposure and Uptake

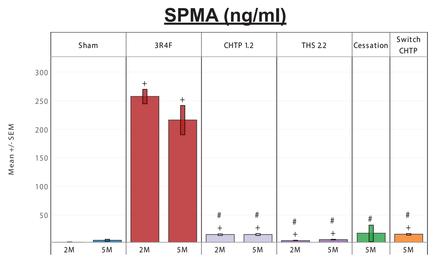
In Urine

Biomarker of CS exposure as well as nicotine exposure were measured in urine

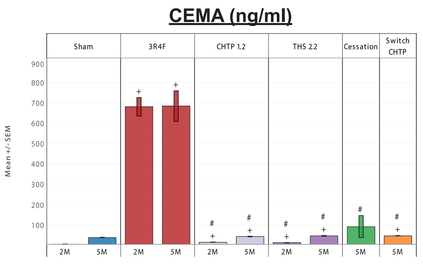
3-HPMA (ng/ml)



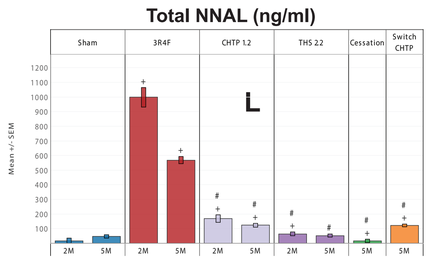
SPMA (ng/ml)



CEMA (ng/ml)

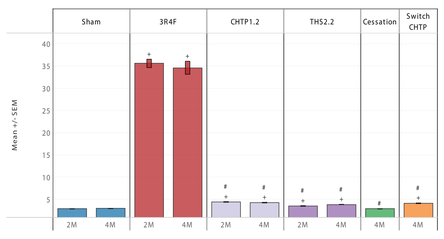


Total NNAL (ng/ml)



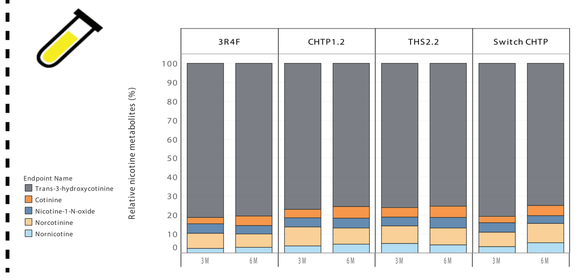
In Blood

Biomarker of CS exposure COHb (carboxyhaemoglobin concentration) measured in blood



In Urine

Nicotine metabolites in exposed groups

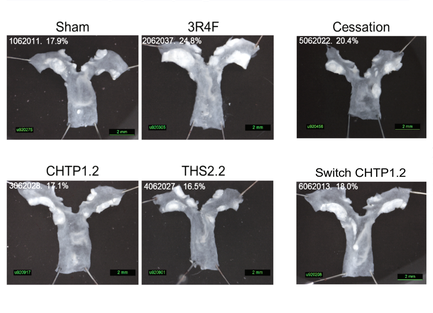


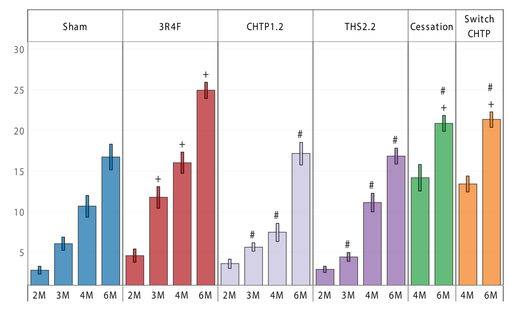
+ p<0.05 significant versus Sham # p<0.05 significant versus 3R4F

The controlled tobacco-heating approach of CHTP 1.2, as well as that of THS 2.2, reduces the delivery of harmful smoke constituents, such as SPMA, CEMA, and COHb, as compared with conventional burning tobacco products (3R4F). Levels of nicotine metabolites (Trans-3-hydrocotinine, Cotinine, Nicotine-1-N-oxide, Norcotinine, Nornicotine) are similar in CHTP 1.2, THS 2.2, and 3R4F-exposed animals.

CVD Endpoint

Aortic arch plaque progression measurements



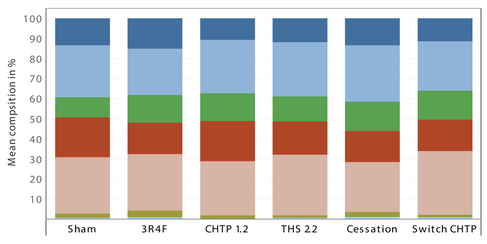


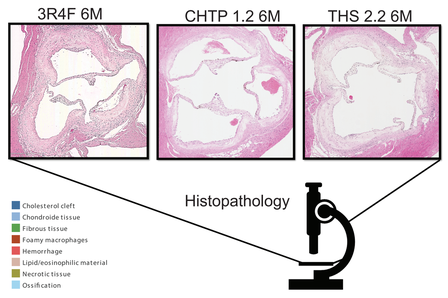
+ p<0.05 significant versus Sham
# p<0.05 significant versus 3R4F

Exposure to 3R4F CS resulted in increased plaque formation in the aortic arch of ApoE$^{-/-}$ mice compared with the sham exposure, starting from Month 3. Cessation or switching to CHTP resulted in a slowing of the plaque formation, as the plaque area in these groups was trending lower than the continuous 3R4F-exposed group in Month 4 and continuing to Month 6, where it was significantly lower.

Even three months after 3R4F exposure, the cessation and switch groups did not return to baseline (continuous fresh air) plaque area levels. There was no difference in plaque area in animals exposed to CHTP 1.2 or THS 2.2 for six months compared to the fresh air-treated animals.

Aortic root plaque composition

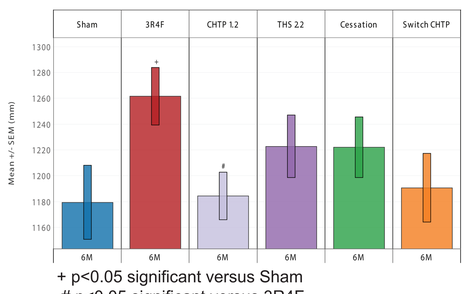




Aortic root plaque composition was evaluated in response to 3R4F CS, CHTP 1.2, or THS 2.2 exposure. Histopathologic analysis based on the evaluation of Cholesterol cleft, Chondroid tissue, Fibrous tissue, Foamy macrophages, Hemorrhage, Lipid eosinophilic material, Necrotic tissue, and Ossification highlight that aortic plaque composition is not modified in response to 3R4F, THS 2.2, or CHTP 1.2 as well as cessation or switching after 6 months of exposure.

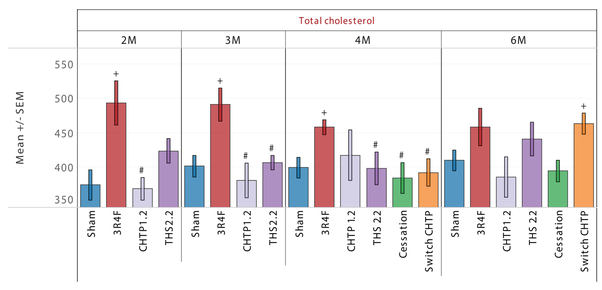
Heart left ventricle thickness

Heart left ventricle thickness was measured after six months of exposure. The 3R4F-exposed animals had higher left ventricular thickness compared with sham-exposed animals at six months of exposure. There was no difference in left ventricle thickness in animals exposed to CHTP 1.2 or THS 2.2 as well as cessation or switching.



+ p<0.05 significant versus Sham
# p<0.05 significant versus 3R4F

Serum cholesterol level

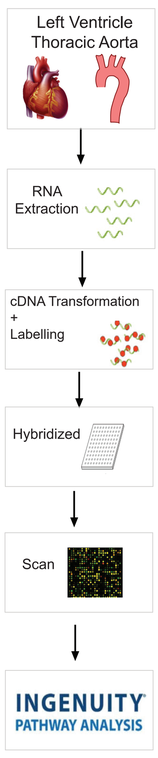


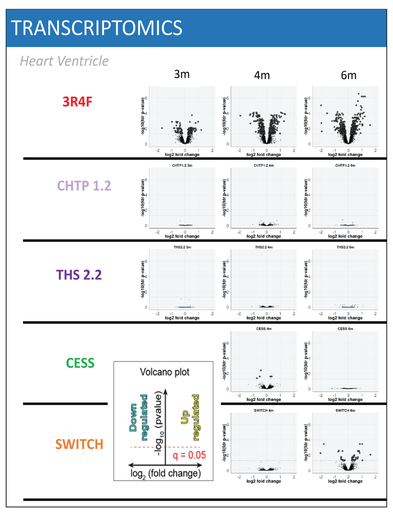
+ p<0.05 significant versus Sham
# p<0.05 significant versus 3R4F

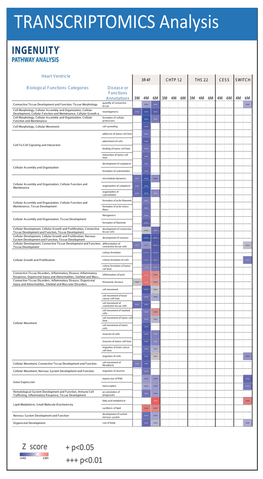
Serum samples were collected during necropsy, and total cholesterol was measured.

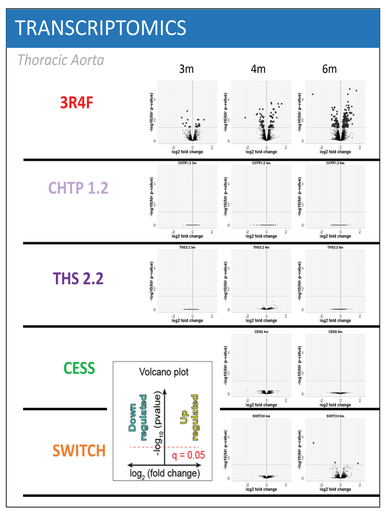
The 3R4F-exposed animals had higher total cholesterol compared with the other exposure groups up to Month 4. By Month 6, there was a higher variability in the total cholesterol levels in the groups, and treatment differences were less obvious.

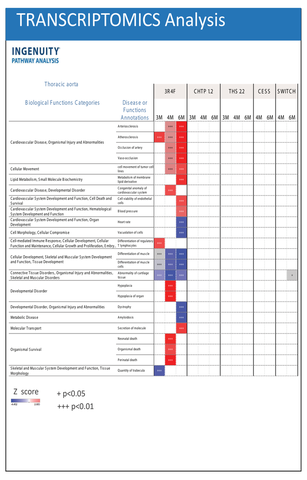
Transcriptomics, Heart Ventricle, and Thoracic Aorta











Exposure to 3R4F CS resulted in a time-dependent increase in the number of differentially expressed genes (compared to the sham-exposed mice) in the heart ventricle and the thoracic aorta. The most differentially expressed gene profile was observed in 3R4F at six months in both tissues (A-B). In mice exposed to the heat-not-burn (CHTP 1.2, THS 2.2) aerosols, no differentially expressed genes were detected compared with the sham groups at all time points evaluated in accordance with the FDR < 0.05. In cessation and switching groups, the number of dysregulated genes is strongly decreased in comparison with those observed following 3R4F exposure.
Ingenuity Pathways Analysis® (IPA) of the transcriptomics results from the heart and the thoracic aorta demonstrated that primarily, a 3R4F exposure-mediated effect was present in both tissues. Based on IPA of gene expression data, biological processes related to “Cardiovascular system development and function,” “occlusion of artery,” “atherosclerosis,” “Connective Tissue Development and Function, Tissue Morphology,” and “Cellular Assembly and Organization” were affected significantly by 3R4F CS exposure in the thoracic aorta and heart ventricle but not by exposure to aerosol from CHTP 1.2.
Cessation or switching decreased atherosclerotic plaques and restored transcriptomic profiles to profiles similar to those observed in air-exposed animals.

Conclusions

Exposure to 3R4F CS resulted in significant impact on CVD parameters: atherosclerotic plaque progression, heart ventricle thickness, lipid profile. Continuous exposure to heat-not-burn tobacco products (CHTP 1.2 and THS 2.2) resulted in a very small difference in all measured parameters related to CVD when compared with fresh air-exposed animals.The biological response to switching to CHTP 1.2 (after three months of 3R4F CS exposure) were similar to those observed in the cessation group across the spectrum of endpoints assessed and showed a generally positive effect with respect to continuous smoke exposure. Differential “omics” profiles associated with 3R4F exposure returned to nearly fresh air levels following switching to CHTP 1.2 or fresh air (cessation).These data collectively indicated a halting or regression of CVD parameters following switching from CS to CHTP 1.2 aerosol in ApoE$^{-/-}$ mice.

References

1. Aistrup GL, Balke CW, Wasserstrom JA. Arrhythmia triggers in heart failure: the smoking gun of [Ca2+]i dysregulation. Heart rhythm. 2011;8(11):1804-8.
2. Al Hariri M, Zibara K, Farhat W, Hashem Y, Soudani N, Al Ibrahim F, et al. Cigarette Smoking-Induced Cardiac Hypertrophy, Vascular Inflammation and Injury Are Attenuated by Antioxidant Supplementation in an Animal Model. Frontiers in pharmacology. 2016;7:397.
3. Bakhru A, Erlinger TP. Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. PLoS medicine. 2005;2(6):e160.
4. Bleyer AJ, Shemanski LR, Burke GL, Hansen KJ, Appel RG. Tobacco, hypertension, and vascular disease: risk factors for renal functional decline in an older population. Kidney international. 2000;57(5):2072-9.
5. Boue S, De Leon H, Schlage WK, Peck MJ, Weiler H, Berges A, et al. Cigarette smoke induces molecular responses in respiratory tissues of ApoE-/- mice that are progressively deactivated upon cessation Toxicology. 2013;314:112 - 24.
6. Phillips BW, Schlage WK, Titz B, Kogel U, Sciuscio D, Martin F, et al. A 90-day OECD TG 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of the aerosol from the carbon heated tobacco product version 1.2 (CHTP1.2) compared with cigarette smoke. I. Inhalation exposure, clinical pathology and histopathology. Food and chemical toxicology. 2018.

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