Toxicological evaluation of the Tobacco Heating System 2.2, a candidate modified risk tobacco product in a OECD inhalation study complemented with systems toxicology

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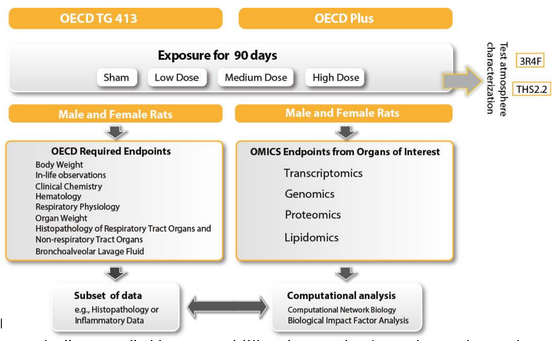
Introduction and study design

Smoking causes serious diseases, such as lung cancer, cardiovascular disease, and chronic obstructive pulmonary disease. Undoubtedly, the best way for smokers to prevent the adverse health effects of tobacco is to quit smoking. In recent years, tobacco harm reduction has also emerged as a policy that can complement traditional tobacco control interventions, such as prevention and cessation.
Here we show results of a 90-day rat inhalation study that was conducted in accordance with Organization for Economic Co-operation and Development (OECD) test guideline 413[1] to characterize potential adverse effects caused by subchronic exposure to aerosol from the Tobacco Heating System (THS) 2.2, a heat-not-burn tobacco product, and to compare with those induced by the smoke generated from the 3R4F reference cigarette.

In the frame of the OECD 413 study, additional animals were employed to further characterize the exposure effects on the transcriptome and proteome of the lung. These animals underwent the same exposure conditions at the same time as the animals in the OECD study. Organs of interest (e.g., lung) were collected from the exposed animals and used for the generation of omics data. This study arm is referred to as "OECD Plus." Here we present only the data obtained from male rats, although both genders were included in the study.

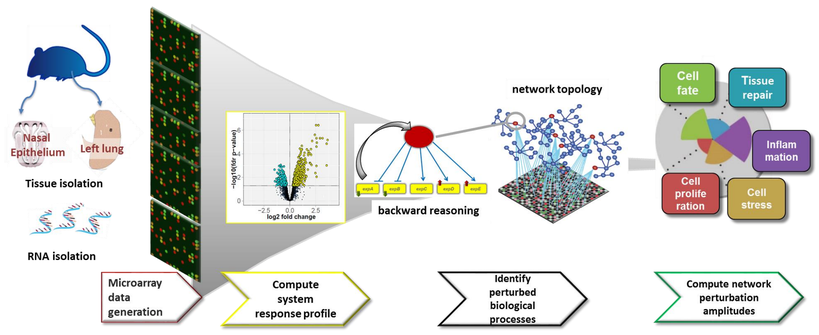


THS 2.2 consists of three distinct components: (1) an electrically heated tobacco produ
holder into which the EHTP is inserted that heats the tobacco material by means of an electronically controlled heater, and (3) a charger that is used to recharge the holder after each use. The holder heats the EHTP at a temperature not exceeding 350°C.

Methods

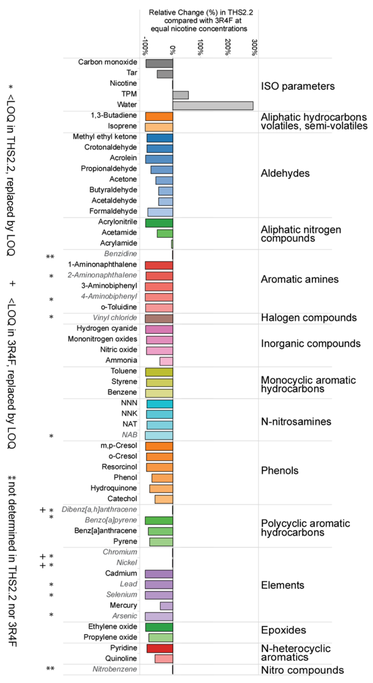
Sprague Dawley rats were exposed for a period of 13 weeks to filtered air (sham), to three concentrations of mainstream 3R4F smoke (8, 15, 23 µg/L nicotine), or to THS 2.2 aerosol (15, 23, 50 µg/L nicotine). Care and use of the rats was in accordance with the National Advisory Committee for Laboratory Animal Research Guideline 2004[2]. Exposure was confirmed by numerous biomarkers, well reflecting test atmosphere constituent concentrations. All animal experiments were approved by the Institutional Animal Care and Use Committee. Nicotine metabolites were determined by high-performance liquid chromatography after derivatization in 24-hour urine. Respiratory minute volume was determined using head-out plethysmography (EMKA Technologies, France). The histopathological evaluation was performed at defined anatomical sites of the left lung, according to a defined grading system. Free lung cells were determined in bronchoalveolar lavage fluid (BALF) by flow cytometry, and inflammatory mediators were measured by multi-analyte profiling (RodentMAP® v3.0).

RNA samples of the lung were analyzed on whole genome Affymetrix microarrays (GeneChip® Rat Genome 230 2.0). Systems response profiles were measured as differential gene expression by pairwise comparisons. Using causal biological network models[3-7], differential gene expressions were transformed into differential values for each node of the network. The differential node values are in turn summarized into a quantitative measure of network-level perturbation amplitude[8].



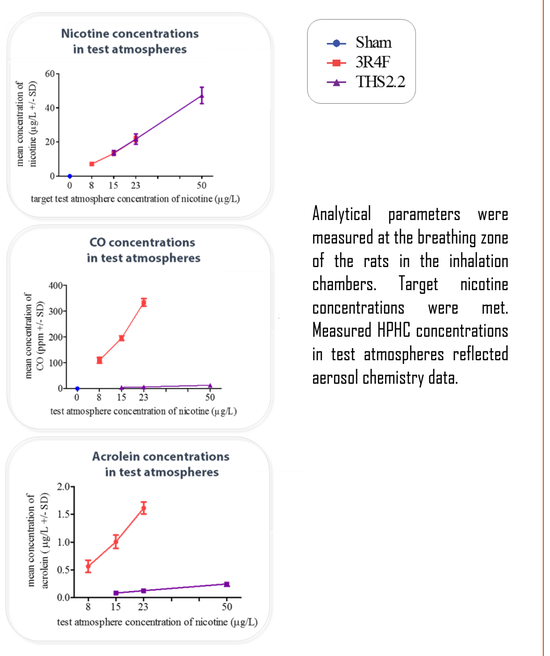
Results

Aerosol chemistry of THS 2.2 versus 3R4F



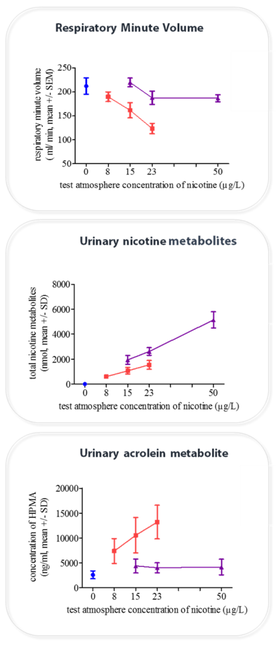
Mainstream aerosol constituents of THS compared against 3R4F. Data were normalized on mg nicotine. Data from 3R4F were set to 0%.
Aerosol chemistry of 56 smoke constituents showed a strong reduction of harmful and potentially harmful constituents (HPHC) in THS 2.2 relative to 3R4F.

Characterization of the test atmospheres



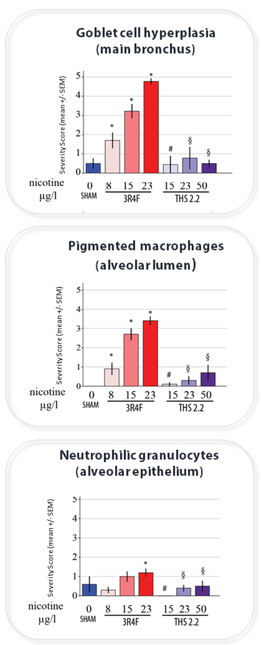
Analytical parameters were measured at the breathing zone of the rats in the inhalation chambers. Target nicotine concentrations were met. Measured HPHC concentrations in test atmospheres reflected aerosol chemistry data.

Aerosol uptake and metabolism



THS 2.2 caused less upper respiratory tract irritation, resulting in higher uptake of aerosol.
Aerosol uptake by the rats was monitored by measuring urinary meta-bolites of representative aerosol constituents. Urinary nicotine metabolites correlated with nicotine levels in test atmosphere. Urinary acrolein metabolite correlated with chemical composition of aerosols and remained low in all THS 2.2 groups.

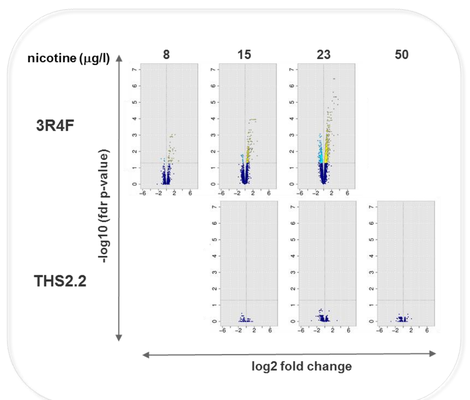
Histopathology of lung tissue



Histopathological findings were scored for their severity. The typical smoke-related as well as dose-dependent adaptive and inflammatory response after exposure to 3R4F smoke was observed in the lung (i.e., goblet cell hyperplasia in the bronchus and increased numbers of macrophages in the lung alveolar parenchyma). Histopathology of the lungs from rats exposed to THS 2.2 aerosol showed lower response compared with animals exposed to 3R4F smoke.

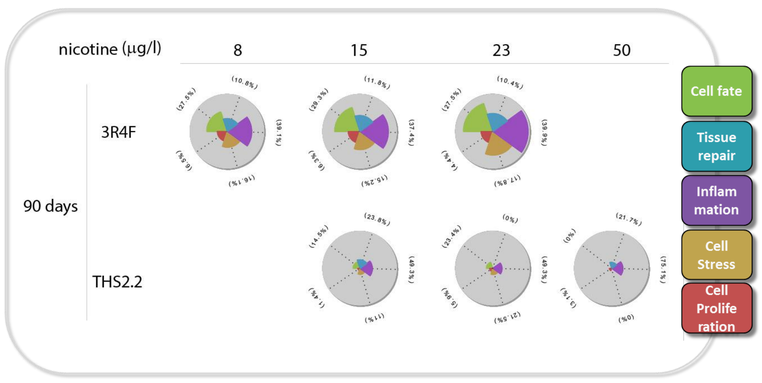
*p < 0.05 compared with sham ;
# p < 0.05 compared with 3R4F 15 µg/l
§ p < 0.05 compared with 3R4F tine 23 µg/l

Systems response profile analysis



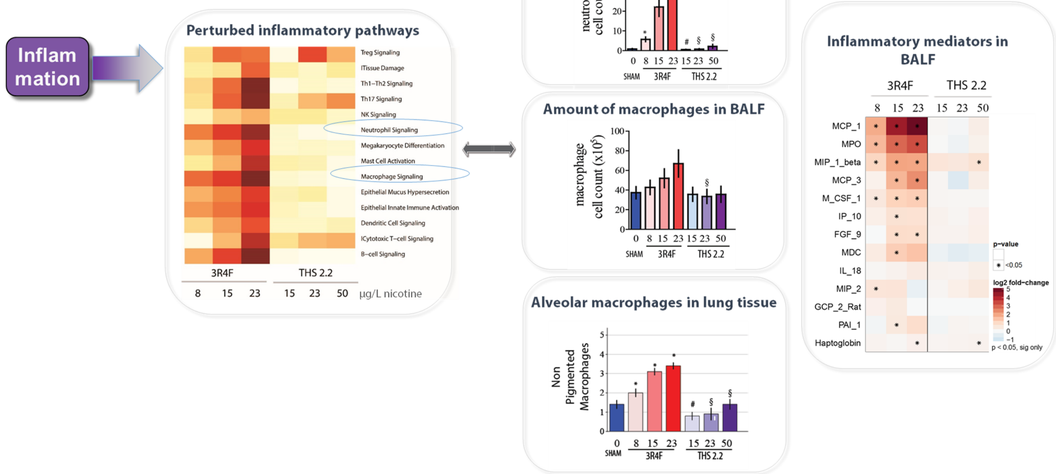
For each gene, the gene expression change is calculated as the log2 fold change, (*x*-axis). The statistical significance, proportional to the negative log10 false discovery rate (fdr)-adjusted *p*-value, is plotted on the *y*-axis. Yellow and cyan dots highlight genes that are statistically significantly upregulated and downregulated, respectively (fdr < 0.05). Compared with the 3R4F reference cigarette smoke, THS 2.2 aerosol induced a minor response in lung tissue (although applying a higher dose of nicotine).

Network perturbation analysis of lung



The relative network-level biological impact for each exposure group was compared with the sham groups after a 90-day exposure. The surface area of each segment is proportional to the contribution of each network perturbation (shown as percentage in the labels) within each exposure group. The impacted biological processes in the lung were related to cell fate, cell stress, and inflammation after exposure to 3R4F reference cigarette smoke. Groups exposed to THS 2.2 aerosol showed a much smaller network perturbation compared with 3R4F-exposed groups.

Lung inflammation



Heatmap summarizing the inflammation subnetworks that were significantly perturbed in at least one treatment after nose-only exposure. Among those subnetworks, neutrophil and macrophage signaling were majorly impacted after exposure to the 3R4F reference cigarette but much less impacted after THS 2.2 exposure. This was also reflected in the differential cell count of BALF neutrophils and macrophages as well as in the amount of inflammatory mediators measured in the BALF.

Conclusions

Histopathological evaluation showed usual dose-dependent adaptive and inflammatory responses for exposure to 3R4F reference cigarette smoke in rat lungs (i.e., goblet cell hyperplasia in the bronchi and increased amount of macrophages in the parenchyma). These findings were also consistent with previous inhalation studies[9-12]. Lower severity scores were observed in the THS 2.2 aerosol-exposed animals, even at double the aerosol concentration on nicotine basis, compared with 3R4F smoke-exposed animals.
Systems toxicology evaluation exemplified how classical toxicology endpoints (e.g., histopathology) can be complemented effectively by molecular measurements and computational analyses within a systems toxicology framework. Exposure of rats to aerosol from THS 2.2 resulted in a lower transcriptional systems response and lower biological network perturbations of lung tissue compared with exposure to 3R4F cigarette smoke. Computational network analysis also revealed that inflammation-related processes were less impacted after THS 2.2 aerosol exposure than after 3R4F cigarette smoke exposure. Consistently, cells and proteins related to inflammatory processes showed a lower differential expression in the lung tissue of THS 2.2 aerosol-exposed animals compared with 3R4F smoke-exposed animals.
Overall, toxicology data indicated that THS 2.2 aerosol caused reduced lung toxicity in comparison with 3R4F cigarette smoke.

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