Toxicological evaluation of the Tobacco Heating System 2.2, a candidate Modified Risk Tobacco Product in a 90-Day OECD inhalation study complemented with systems toxicology.

 # 91

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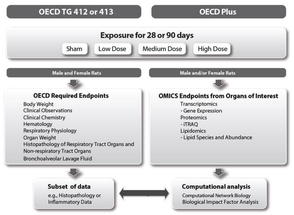
Introduction

Smoking causes serious diseases such as lung cancer, cardiovascular and chronic obstructive pulmonary diseases. Undoubtedly, the best way for smokers to prevent the adverse health effects of tobacco is to quit smoking. Over the last years, tobacco harm reduction has also emerged as a policy that can complement traditional tobacco control intervention 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 the tobacco heating system (THS) 2.2 aerosol, a heat-not-burn tobacco product, and to compare with those induced by the smoke generated from the reference cigarette 3R4F. In addition, a systems toxicology approach with additional animals was included to further characterize the exposure effects on the transcriptome and proteome of the lung, also referred as OECD(+).

THS2.2 consists of three distinct components: (i) an electrically heated tobacco product (EHTP), with unique processed tobacco made from tobacco powder, (ii) a holder into which the EHTP is inserted and which heats the tobacco material by means of an electronically controlled heater, and (iii) a charger that is used to recharge the holder after each use. The holder heats the EHTP at a temperature not exceeding 350°C.

Study Design



In the frame of the OECD 413 study additional animals for the purpose of obtaining "omics" endpoints were employed. These animals underwent the same exposure conditions at the same time as the animals in the OECD study. Organs (e.g., lung) of interest were collected from the exposed animals and after a recovery period, and used for the generation of omics data. This study arm is referred to as OECD(+). Here we present only the data obtained from female rats although both genders were included in the study.

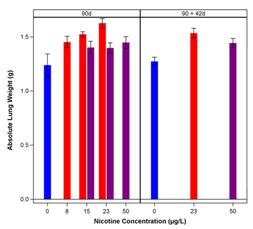
Methods

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 THS2.2 aerosol (15, 23, 50 µg/L nicotine). Exposure was confirmed by numerous biomarkers, well reflecting test atmosphere constituent concentrations (data not shown). In addition, some of the rats were allowed to recover from exposure for 42 days prior to analysis. Care and use of the rats was in accordance with the National Advisory Committee for Laboratory Animal Research Guideline 2004[2]. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC). Organ weight was determined by gravimetry. The histopathological evaluation was performed at defined anatomical sites of the nose and 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). For the quantitative proteomics analysis, peptides were labeled with isobaric tags (iTRAQ®) and analyzed by LC-MS/MS on an Q-Exactive mass-analyzer (Thermo Scientific, Bremen, Germany). RNA samples of the lung were analyzed on whole genome Affymetrix microarrays (GeneChip® Rat Genome 230 2.0). For data analysis commercially available pathway data bases were used[3,4].

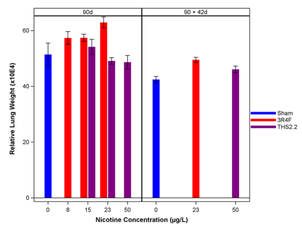
Results

Lung weight including trachea and larynx

Absolute weight



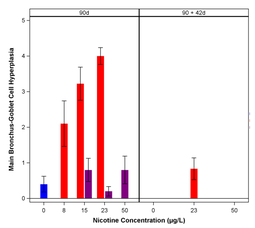
Relative weight



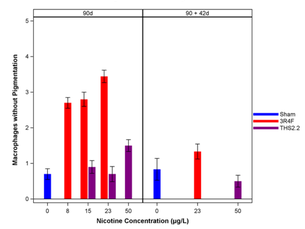
During dissection, the organ weights were measured as absolute value, and the relative weights were calculated according to body weight after exsanguination. Typical smoke exposure-related changes in absolute and/or relative weights of lungs (measured with trachea and larynx) were observed in 3R4F-smoke exposed female rats and to a lower extent in THS2.2-aerosol exposed groups. The changes observed in 3R4F-smoke exposed groups were reduced after the post- exposure recovery phase.

Histopathological findings in the lung

Goblet cell hyperplasia in the main bronchus



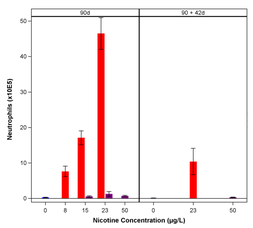
Alveolar macrophages in the parenchyma



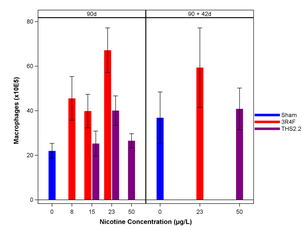
The above indicated histopathological findings were scored for their severity. The typical smoke-related as well as dose-dependent adaptive and inflammatory response after exposure to 3R4F-smoke was observed in lung, e.g., goblet cell hyperplasia in the bronchus, and increased numbers of macrophages in the lung alveolar parenchyma. Histopathology of the lungs from female rats exposed to THS2.2-aerosol showed lower response compared to animals exposed to 3R4F-smoke.

Differential cell count of free lung cells in the BALF

Neutrophil count

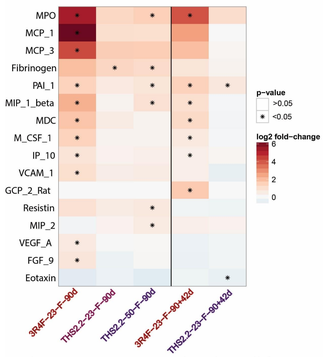


Macrophage count



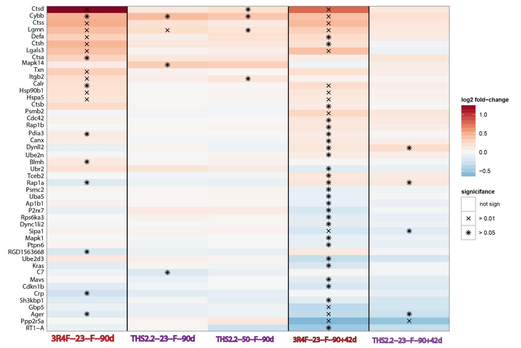
The number of macrophages and neutrophils were determined in BALF of female rats by flow cytometry. The neutrophil cell counts showed an increase in all 3R4F-smoke exposed groups compared with sham. Even at the highest THS2.2-aerosol concentration neutrophil counts were lower than in the 3R4F groups. Macrophage counts were higher in the 3R4F groups as compared with sham. The macrophage counts in THS2.2 showed no increase relative to sham with exception of a likely incidental marginal increase in the 23 µg/L nicotine THS2.2 group. The increase was lower than found in the 3R4F groups.

Multi-analyte profiling of inflammatory mediators in BALF



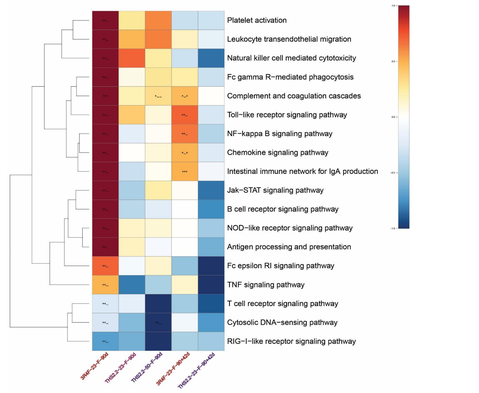
Inflammatory mediators were measured in the cell free supernatant of the BALF of female rats by a multiplex assay. 30 out of 60 analytes had at least half of their values below the lower limit of quantification for all the groups of rats. 8 out of 60 analytes did not show aerosol-dependent changes. Analysis of the remaining analytes revealed lower inflammation and oxidative stress markers in the THS2.2-aerosol exposed as compared with 3R4F-smoke exposed rats after 90 days of exposure. These markers were partially reverted to sham level after 42 days of recovery.

Proteomics profile of inflammation-related proteins



All proteins that were in the Reactome data base[3] within the category immune system were selected. This category comprises of signaling pathways from the innate and the adaptive immune system, the cytokine signaling and ROS production. Only proteins that showed a significant change in response to exposure compared with the sham group are shown. Majority of the proteins that were regulated in response to 3R4F-smoke exposure showed a lower absolute fold change or were not regulated in response to THS2.2-aerosol.

Transcriptomics profile of inflammation-related processes



Signaling pathways from the KEGG data base related to immune system and inflammatory signaling were selected. These pathways were used for gene enrichment analysis of the transcriptomics data generated from the lungs of female rats. Each row represents a gene set that was significantly enriched in at least one exposure group. Gene sets of these signaling pathways were highly enriched by 3R4F exposure but not by THS2.2-aersol exposure.

Summary & Conclusion

Increase in weights of lungs (with trachea and larynx) is typical for smoke exposed rats [5-7]. Histopathological findings in 3R4F-smoke exposed groups showed increased amount of macrophages in the parenchyma and goblet cell hyperplasia in the bronchi. These findings were also consistent with previous inhalation studies[5, 7-9]. Lower lung weights and lower severity scores in the histopathology evaluation were observed in the THS2.2-aerosol exposed animals compared with 3R4F-smoke exposed animals. Consistently, proteins related to inflammatory processes showed a lower differential expression in the lung tissue of THS2.2-aerosol exposed animals compared with 3R4F-smoked exposed. Gene set enrichment analysis revealed also that inflammation-related processes were less impacted after THS2.2-aerosol exposure than after 3R4F-smoke exposure. Overall, systems toxicology data indicated that aerosol from the THS 2.2 caused a reduced respiratory tract toxicity in comparison with cigarette smoke.

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