Biological impact of an aerosol from a candidate modified-risk tobacco product and cigarette smoke on human organotypic nasal epithelial cultures – a view from the proteome

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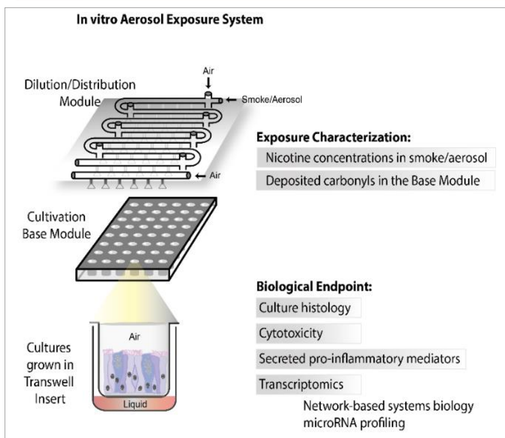
Introduction

Systems toxicology complements standard toxicological endpoints with system-level measurements and computational analysis approaches. This work reports how several proteomic techniques (isobaric tag for relative and absolute quantitation (iTRAQ), parallel reaction monitoring (PRM), and antibody-based multi-analyte profiling (MAP)) were implemented effectively to assess the impact of the exposure to a candidate modified-risk tobacco product, the Tobacco Heating System (THS) 2.2, on the human organotypic nasal model cultured at the air-liquid interface[1,2]. The term "modified risk tobacco product" indicates any tobacco product that is sold or distributed for use to reduce harm or risk of tobacco-related diseases associated with commercially marketed tobacco products"[3].
The work presented is part of a larger study that included several biological endpoints like histology, cytotoxicity, and transcriptomics (Figure 1).

Methods



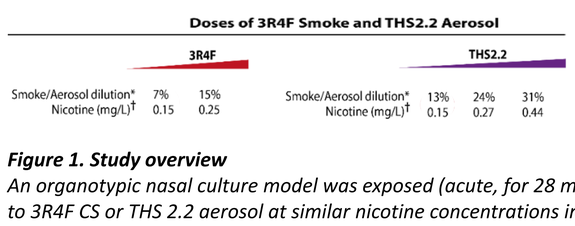


Figure 1. Study overview
An organotypic nasal culture model was exposed (acute, for 28 minutes) to 3R4F CS or THS 2.2 aerosol at similar nicotine concentrations in an Exposure System (Vitrocell 24/48®). The nasal organotypic cultures were reconstituted from the primary nasal epithelial cells of a 30 year-old male non-smoker. Figure adapted from Iskandar et al. (2017)[1].
** Dilution refers to the percent 3R4F smoke or THS 2.2 aerosol diluted with air in the Dilution/Distribution Module of the Exposure System.*
† Nicotine concentration (mg/L) measured by trapping the diluted smoke/aerosol in EXtrelut® 3NT columns.

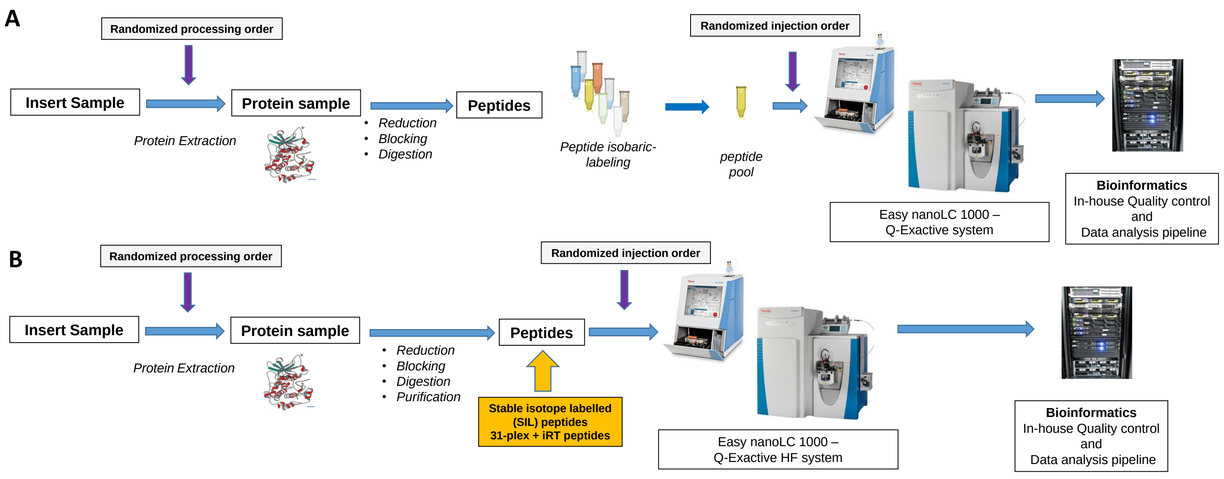


Figure 2. Schematic overview of the iTRAQ workflow (A) and the PRM workflow (B). *Absolute quantification of target proteins was achieved by spiking stable isotope labelled (SIL) peptides in known concentration as reference standard. For analysis, quality control and retention time scheduling indexed retention time kit was used[4].*

iTRAQ Analysis

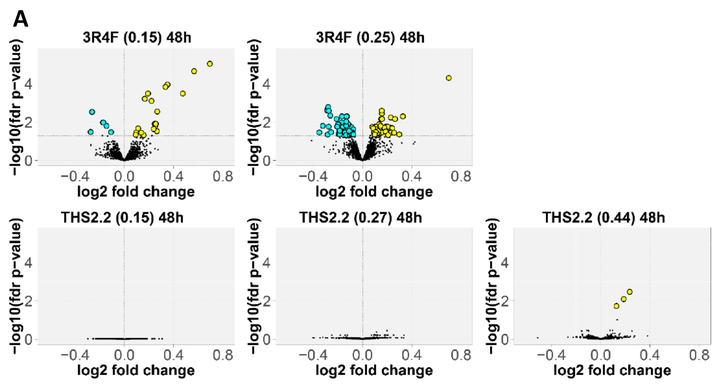


Figure 3. Volcano plots of differentially expressed proteins (DEP) in nasal epithelial cultures 48 hours post-exposure: iTRAQ analysis. *For each comparison with the sham group, a volcano plot shows the amplitude (log-(fold-change), x-axis) and statistical significance (-log$_{10}$(false discovery rate-adjusted p-value), y-axis) for the proteins quantified by iTRAQ. fdr: false discovery rate.*

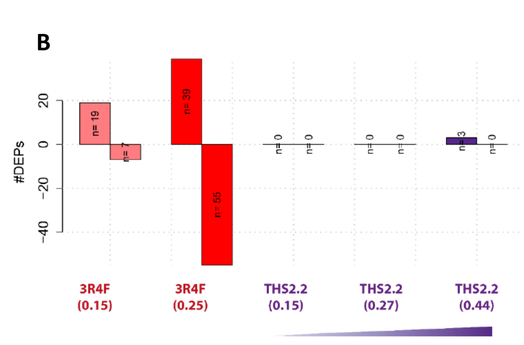


Figure 4. Number of DEPs in nasal epithelial cultures 48 hours post-exposure. *Significant differentially expressed proteins with a false discovery rate-adjusted p-value<0.05.*

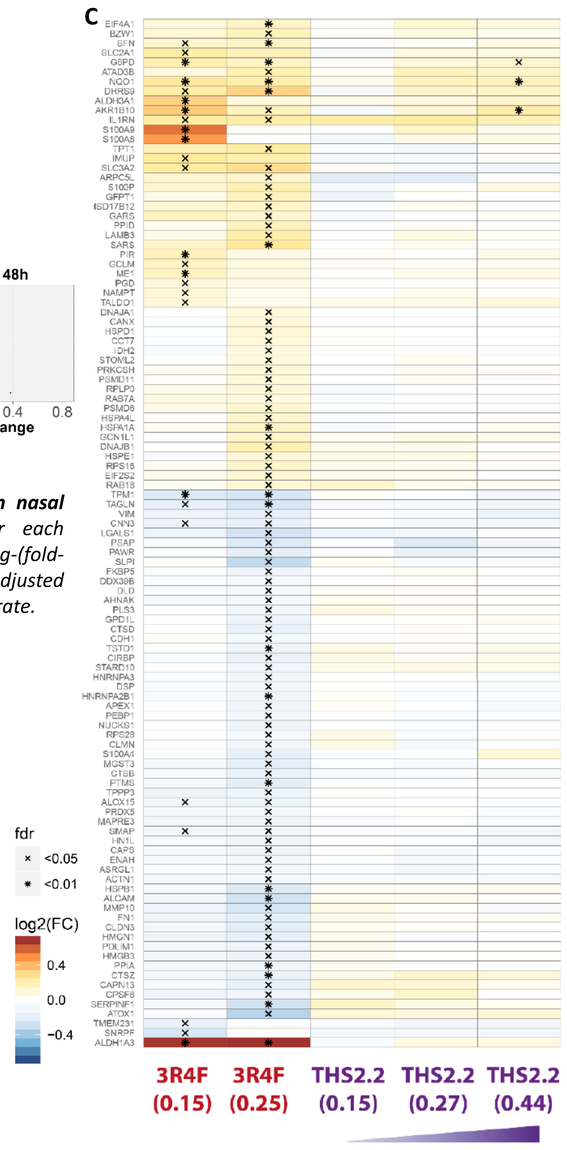


Figure 5. Differential expression heatmap of nasal epithelial cultures collected at 48 hours post-exposure. *Each row represents a DEP (with a false discovery rate-adjusted p-value<0.05). Each column is a comparison with the air-exposed control group, and the log$_2$(fold-changes) are color coded. FC: fold change.*

MAP Data

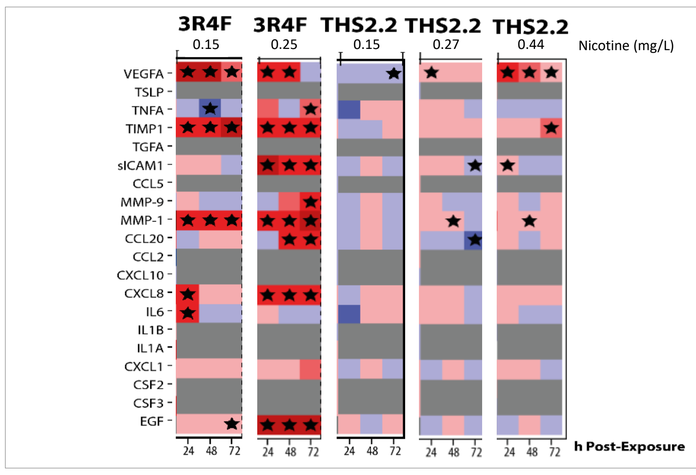


Figure 6. Exposure-induced pro-inflammatory mediators responses.
MAP data for secreted pro-inflammatory mediators measured at various post-exposure time points. Figure adapted from Iskandar et al. (2017)[1].

Targeted Proteomics – Cellular Stress and Pro-Inflammatory Responses

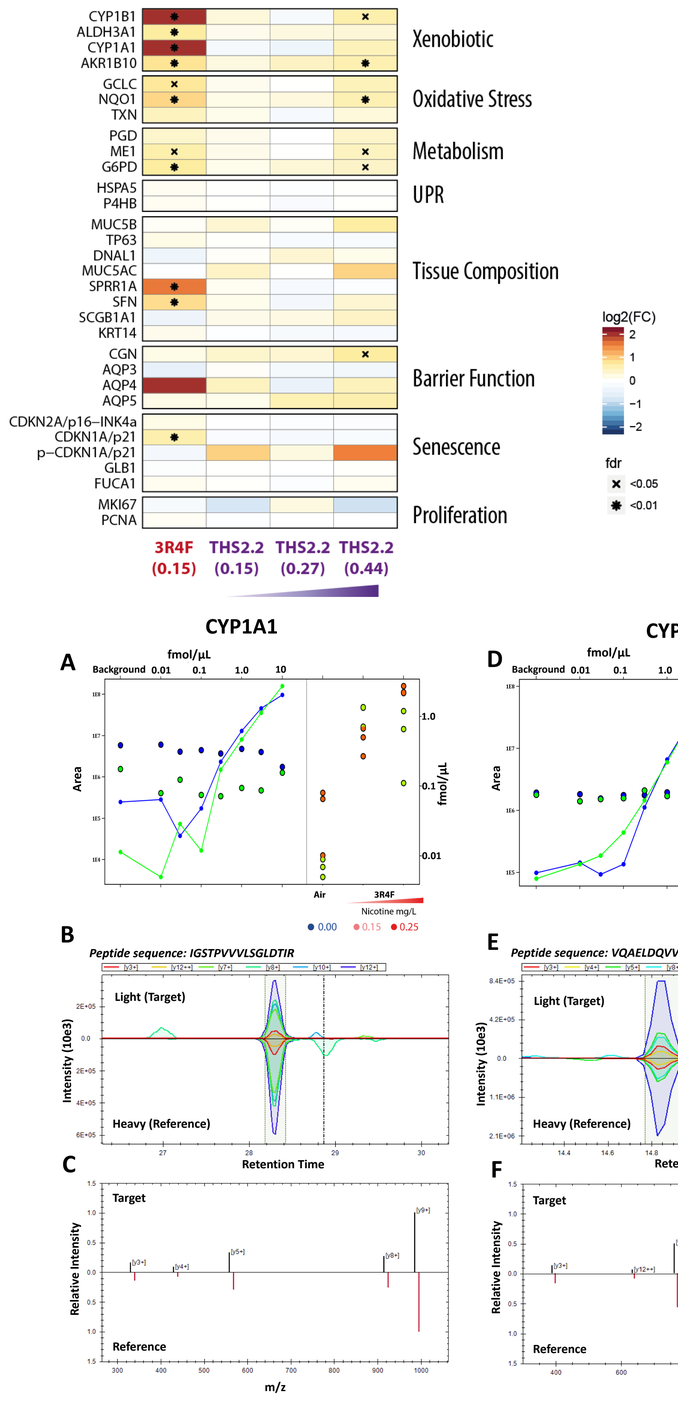


Figure 7. Alterations of selected protein targets in the nasal organotypic cultures following 3R4F and THS 2.2 exposure measured by PRM.
The log$_2$(fold-changes) compared with the air control are color-coded, and the FDR-adjusted p-values are indicated. UPR: unfolded protein response. Figure from Iskandar et al. (2017)[1].

This 31-plex PRM panel was established to capture the alterations of major biological functions in response to cigarette smoke (CS) exposure, including xenobiotic response, oxidative stress, and tissue composition.

Figure 8. Representative graphs for protein expression level analysis of the two cytochrome P450 proteins CYP1A1 and CYP1B1.
(A) CYP1A1 SIL peptide dilution curve experiments in nasal culture background and determined target protein concentration in response to 3R4F CS treatment. (B) CYP1A1 Transition ion-traces of peptide IGSTPVVVLSGLDTIR used for protein quantification. (C) Intensity correlation chart of fragment ions from SIL peptide (Reference) and target peptide for CYP1A1 specific peptide shown in B. (D) CYP1B1 SIL peptide dilution curve experiments in nasal culture background and determined target protein concentration in response to 3R4F CS treatment. (E) CYP1B1 Transition ion-traces of peptide VQAELDQVVGR used for protein quantification. (F) Intensity correlation chart of fragment ions from SIL peptide (Reference) and target peptide for CYP1B1 specific peptide shown in E.

Conclusions

- Using iTRAQ, exposure to 3R4F CS identified biological processes that included xenobiotic metabolism, oxidative stress response, metabolism, and pro-inflammatory responses, covered by 94 differentially expressed proteins. On the contrary, the highest dose of THS 2.2 aerosol exposure led to only three significantly up-regulated proteins (AKR1B10, NQO1, and G6PD).
- The 31-plex protein PRM panel confirmed that 3R4F CS exposure elicited a xenobiotic metabolism, oxidative stress, and tissue adaptation response compared with exposure to fresh air.
- Antibody-based MAP demonstrated that 3R4F CS exposure let to an increase on the release of pro-inflammatory mediators (IL-8, IL-6, TIMP1, MMP-1) into the medium, whereas the THS 2.2 aerosol exposure showed no significant increase.
- In summary, all three proteomics methods supported the reduced effects in the proteome expression changes of cultures exposed to THS 2.2 aerosol when compared with the response of 3R4F CS at comparable nicotine concentrations.
- Overall, this study demonstrated the applicability of proteomics within the Systems Toxicology approach to quantify and compare the effects of 3R4F CS and THS 2.2 aerosol on biological pathways, such as cellular stress and pro-inflammatory responses.

References
[1] Iskandar et al. (2017), Systems Toxicology Meta-Analysis of In Vitro Assessment Studies: Biological Impact of a Candidate Modified-Risk Tobacco Product Aerosol Compared with Cigarette Smoke on Human Organotypic Cultures of the Aerodigestive Tract, *Toxicol. Res.*, 2017,**6**, 631-653
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Competing Financial Interest
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