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Proteomics for Systems Toxicology

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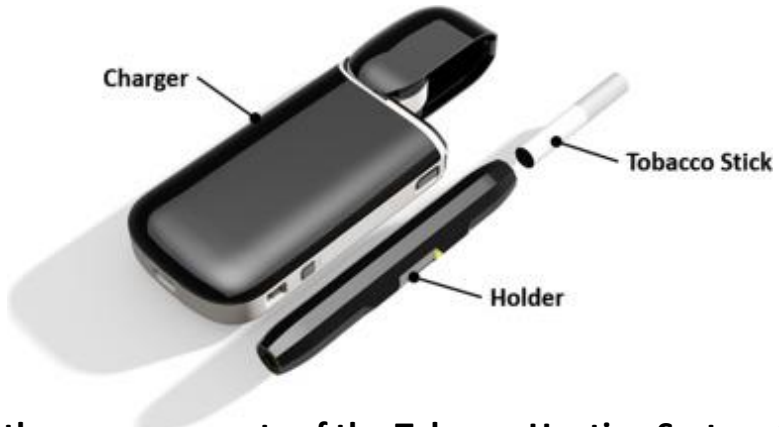
Philip Morris International is the sole source for this project.

Harm Reduction & The Tobacco Heating System (THS)

The harm reduction approach



The three components of the Tobacco Heating System (THS).

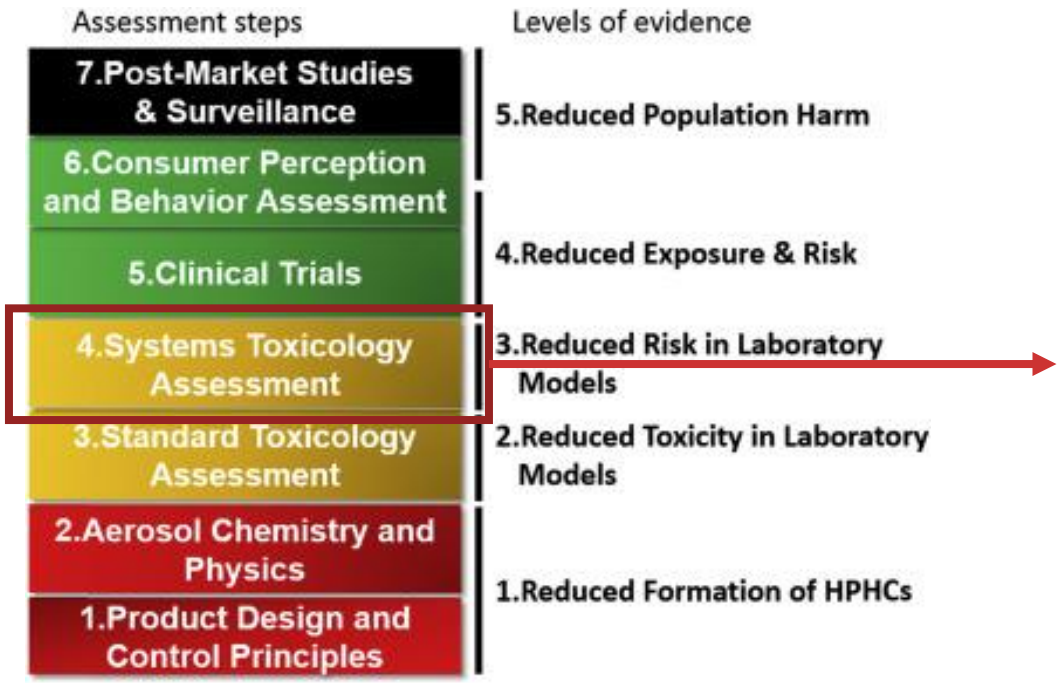
Offering adult smokers satisfying products that reduce risk

- Smoking is addictive and causes a number of serious diseases
- Worldwide it is estimated that more than one billion people will continue to smoke in the foreseeable future (The Tobacco Atlas 3rd Edition, American Cancer Society, 2009)
- Successful harm reduction requires a range of Reduced Risk Products (RRPs)* that are accepted by adult smokers
- Philip Morris International's ambition is to lead a full-scale effort to ensure that non-combustible products ultimately replace cigarettes
- The Tobacco Heating System (THS) is a potential RRP* based on the heat-not-burn principle. Tobacco is heated with an electronically controlled heating blade to temperatures that do not exceed 350°C. This absence of combustion is designed to reduce significantly the formation of Harmful and Potentially Harmful Constituents (HPHCs) by the THS product compared with cigarettes.

* Reduced-Risk Products ("RRPs") is the term we use to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment and commercialization. Because our RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.

References: Smith et al., Regulatory Toxicology and Pharmacology 81 (2016) & *The Science behind the Tobacco Heating System (2017), www.pmiscience.com*

Our Assessment Strategy



Insights into molecular mechanisms supported by OMICs approaches, including transcriptomics, proteomics, and lipidomics.

The RRP assessment program. Seven steps of assessment lead to five levels of evidence. Taken together, these levels of evidence provide the scientific evidence to demonstrate that a novel product significantly reduces harm and the risk of tobacco-related disease to individual smokers and benefits the health of the population as a whole, taking into account both smokers and nonsmokers.

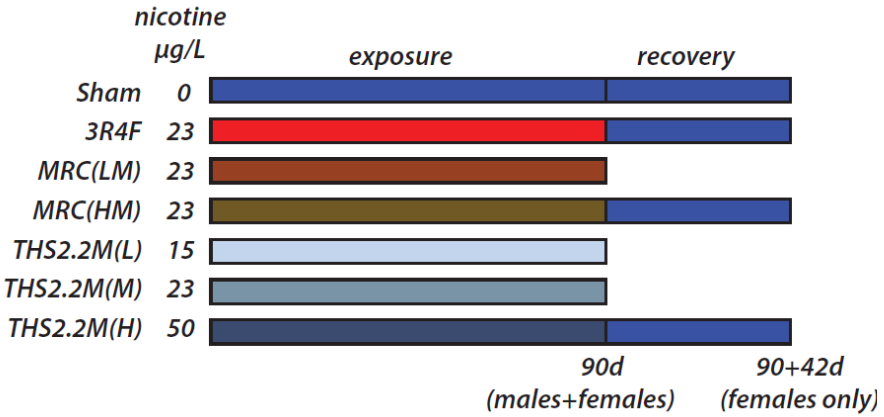
Source: Smith et al., The Science behind the Tobacco Heating System (2017), www.pmiscience.com

Case Study 1: iTRAQ analyses within an *in vivo* study

Evaluation of the Tobacco Heating System 2.2. Part 7: Systems toxicological assessment of a mentholated version revealed reduced cellular and molecular exposure effects compared with mentholated and non-mentholated cigarette smoke

Kogel et al., Regulatory Toxicology and Pharmacology 81 (2016)

Study Design of 90-day Rat Inhalation Study



MRC, mentholated reference cigarette; LM/HM, low/high menthol

OECD TG413

(molecular) endpoints

	nose	lung
Histopathology	X	X
Transcriptomics	X	X
Proteomics	X	X
Lipidomics		X

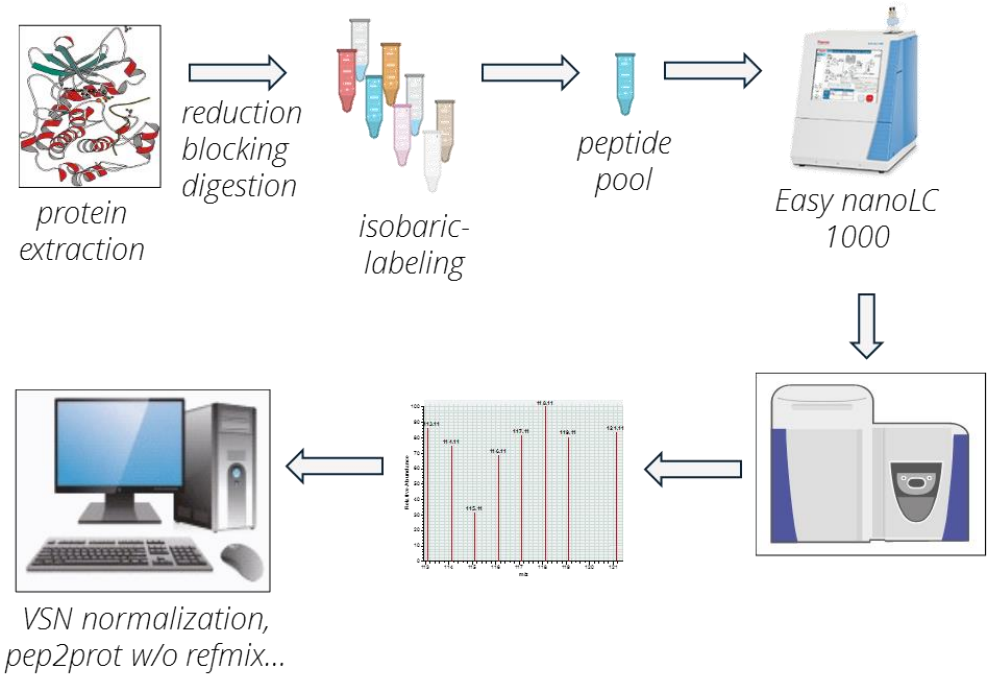
N = 6 for omics endpoints

- 90-day rat inhalation study according to Organisation for Economic Co-operation and Development (OECD) test guideline 413 to assess the effects of aerosol from mentholated version of THS2.2 (THS2.2M)
- Seven 90-day exposure groups + recovery groups: Nicotine concentration of THS2.2M(M) aerosol matched to reference smoke exposures
- Standard toxicological endpoints complemented with systems toxicology endpoints: **transcriptomics**, **proteomics**, and lipidomics

Reference: *Kogel et al., Regulatory Toxicology and Pharmacology* 81 (2016)

iTRAQ® Workflow & Label Sets

Quantitative proteomics workflow



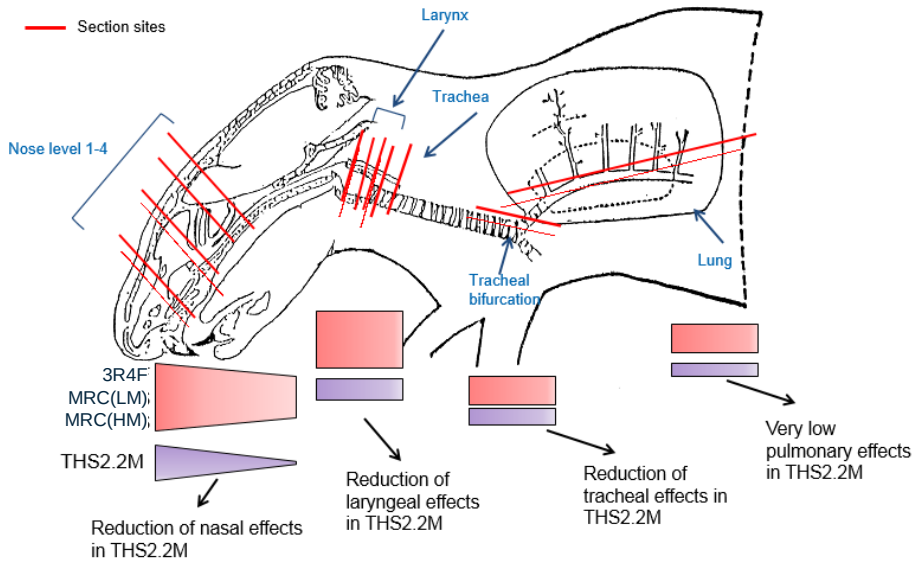
iTRAQ® Labeling Sets

Analysis	Samples	Sex	each iTRAQ set	#sets
#1	all 90d samples	Males/ Females	7 sample types + refmix	12
#2	90+42d and corresponding 90d samples	Females only	8 sample types	6

Sample and channel assignments randomized

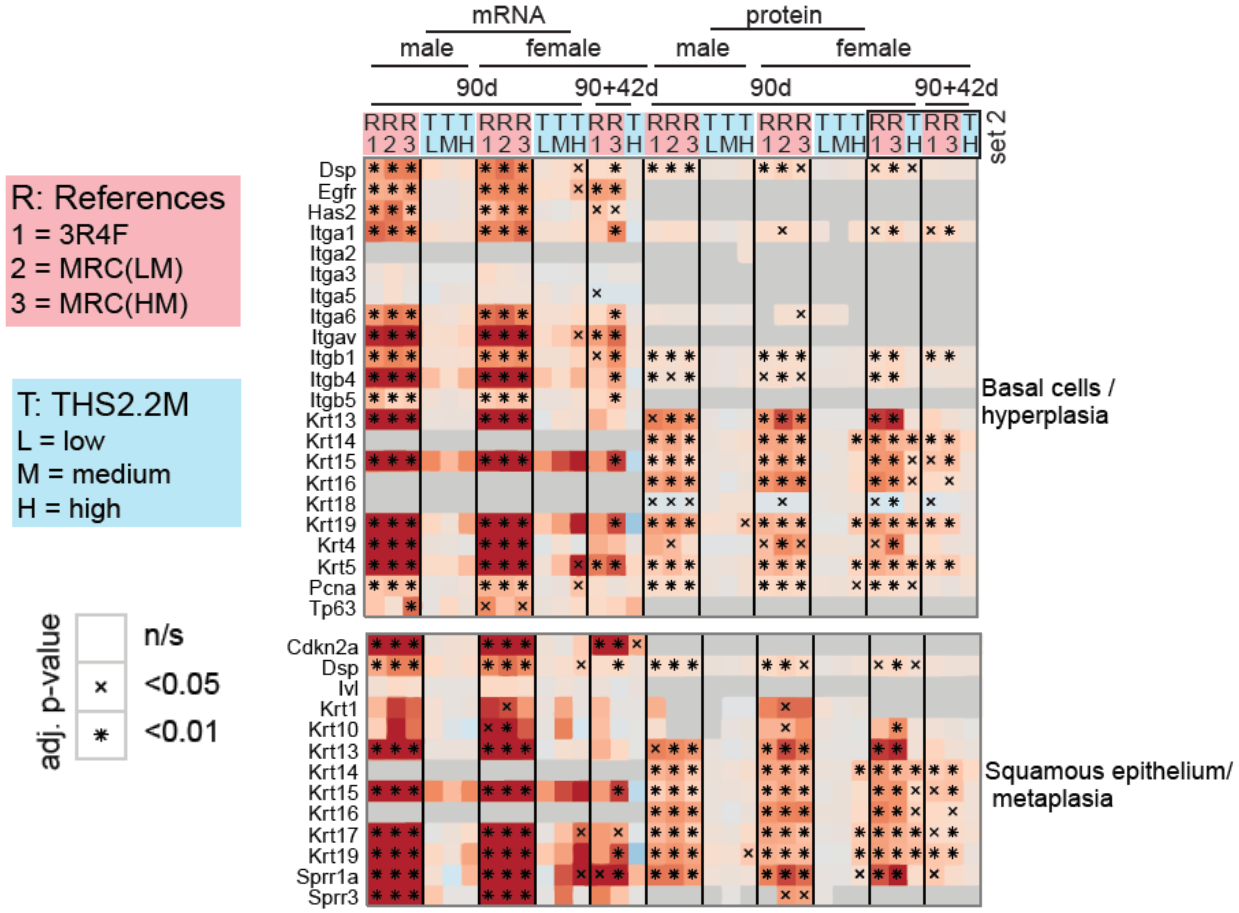
Exposure Effects on the Nasal Epithelium

Histopathology



- Adaptive response of the nasal epithelium (RNE) to cigarette smoke (CS) included basal cell hyperplasia, squamous cell metaplasia and an inflammatory response.
- Adaptive tissue changes to THS2.2M aerosol exposure were much weaker and were limited mostly to the highest THS2.2M concentration in female rats

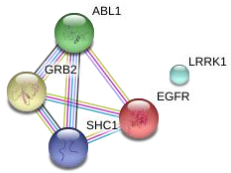
mRNA/protein tissue marker profiles



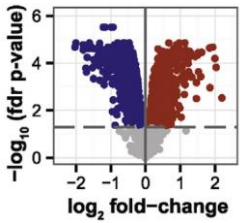
References: *Kogel et al. & Oviedo et al., Regulatory Toxicology and Pharmacology* 81 (2016)

Protein/Gene Clusters Affected in the Lung

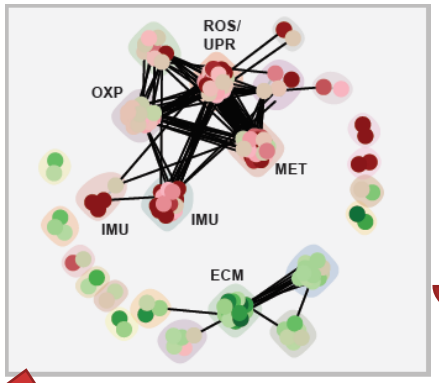
STRING database

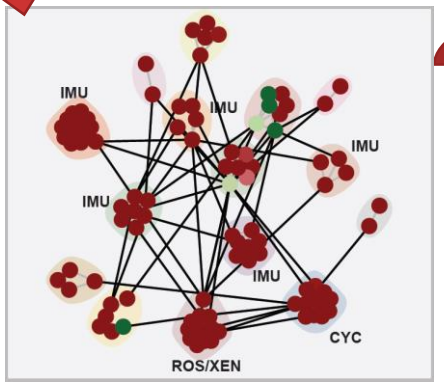


Expression profiles



Identified clusters





Functional categories

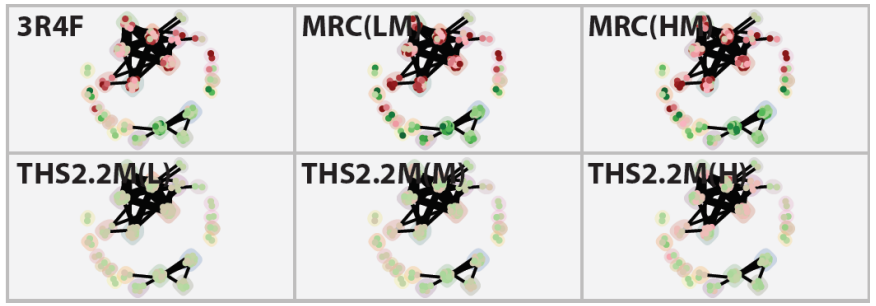
Proteomics

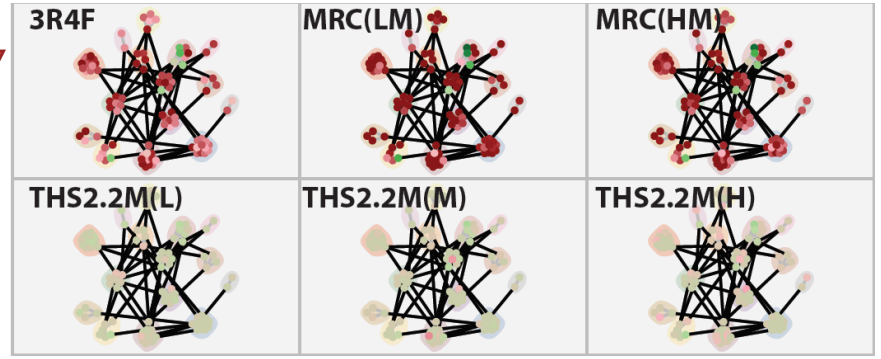
Transcriptomics

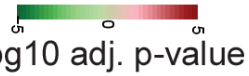
Functional categories

IMU	immune-related
ROS	oxidative-stress response
XEN	xenobiotic response
UPR	unfolded-protein response
MET	metabolism
CYC	cell cycle
ECM	extracellular matrix
OXP	oxidative phosphorylation

90-day response profiles for female rats







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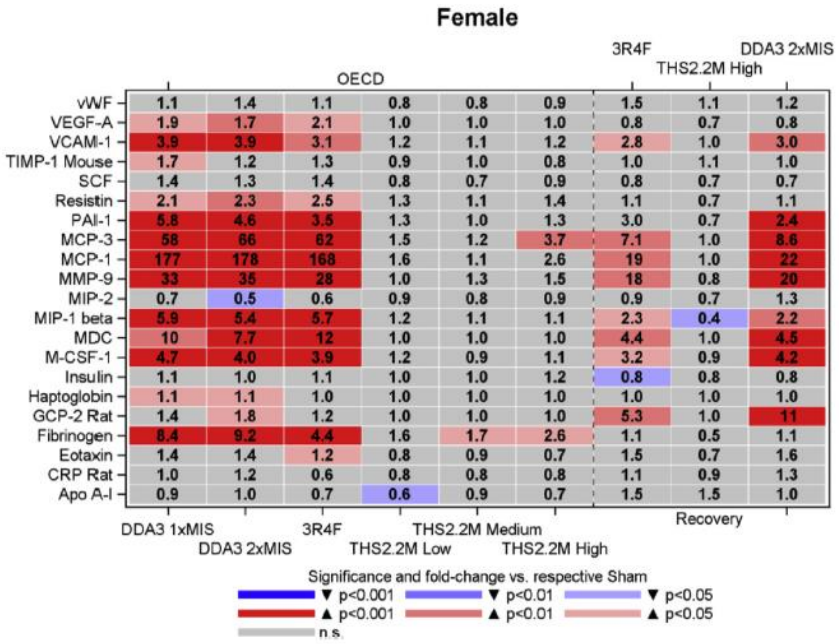
References: *Kogel et al. & Oviedo et al., Regulatory Toxicology and Pharmacology* 81 (2016) & STRING database (string-db.org)

Inflammatory Effects in the Lung

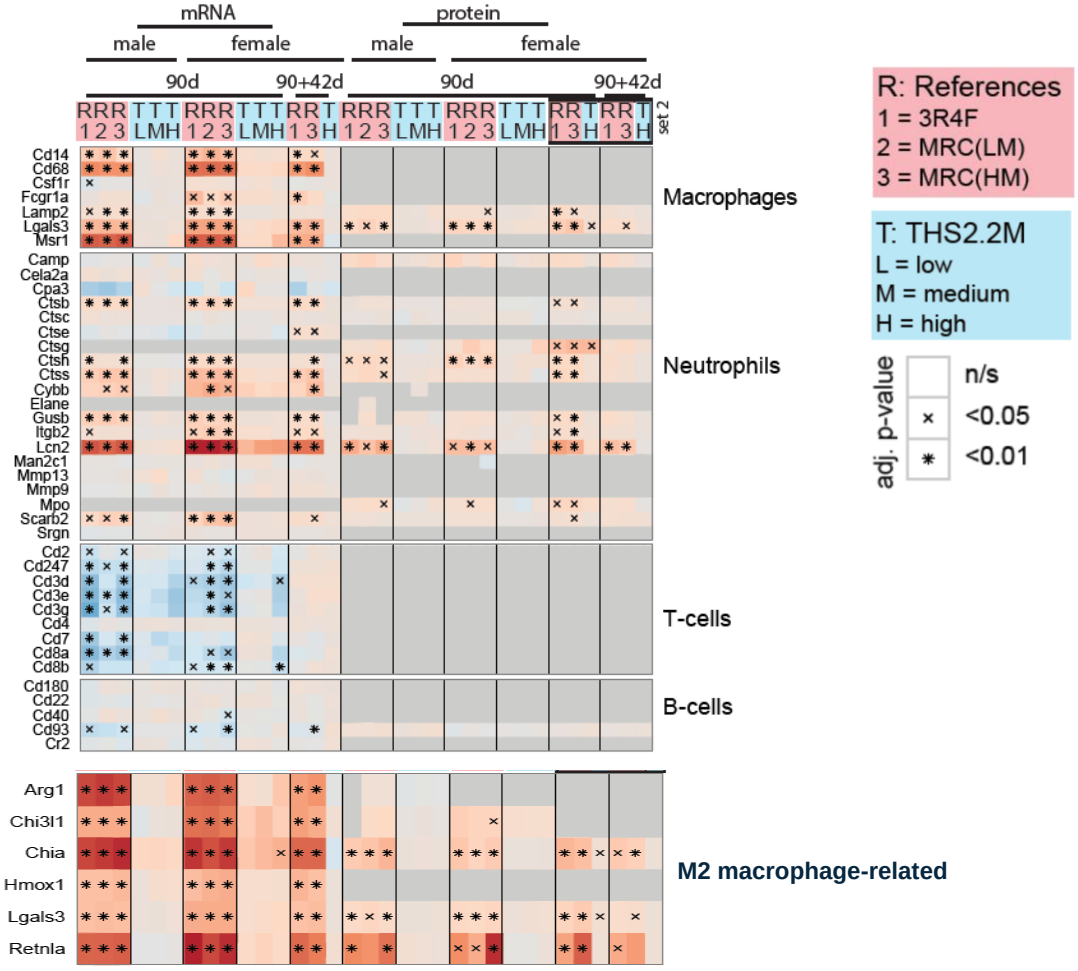
Bronchoalveolar lavage fluid marker profiles

Luminex technology



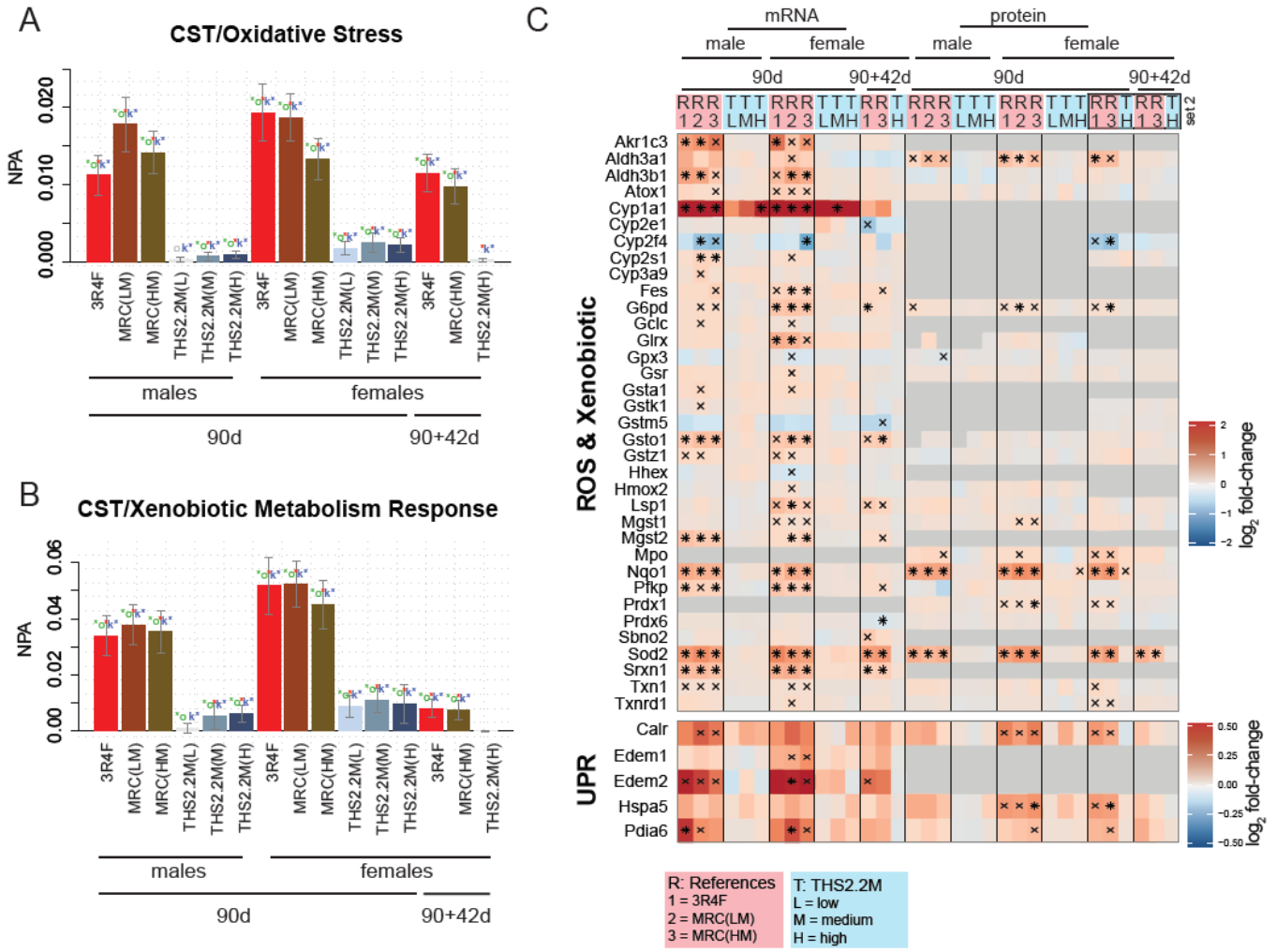
Immune-cell marker profiles

iTRAQ & Affymetrix gene chips



Reference: Kogel et al., Regulatory Toxicology and Pharmacology 81 (2016)

Cellular Stress in the Lung



- Network enrichment analysis of transcriptomics data (Panels A/B) and of mRNA/protein marker panel (Panel C) show that cigarette smoke induces cellular stress response in lung tissue
- THS2.2M aerosol, at the three tested concentrations, induced only a limited cellular stress response

Reference: *Kogel et al., Regulatory Toxicology and Pharmacology* 81 (2016)

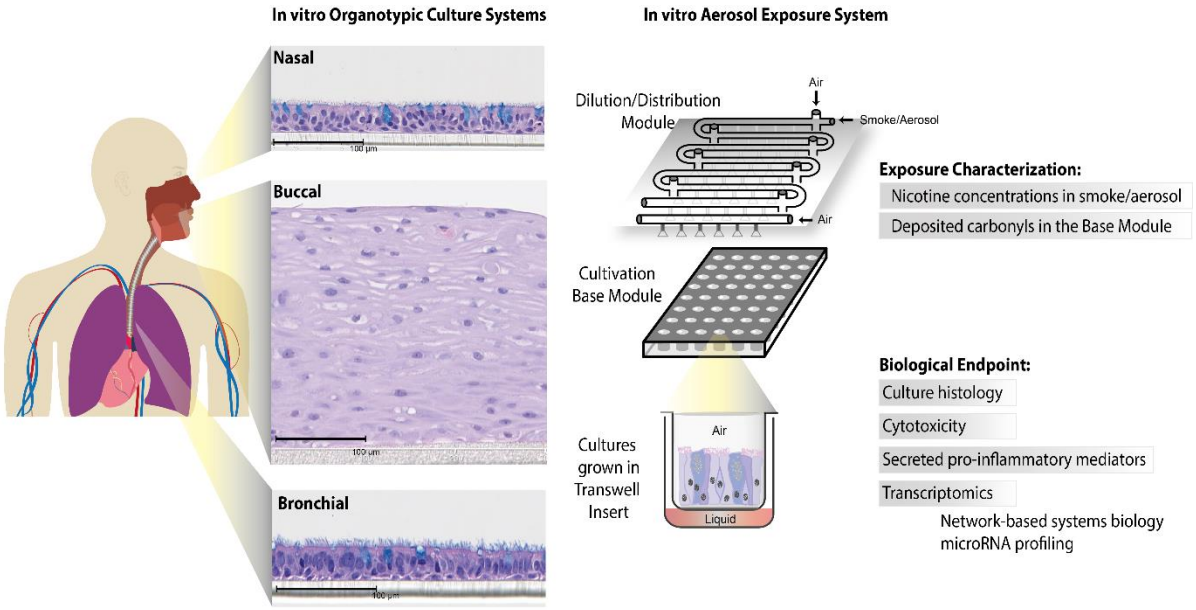
Case Study 2:
PRM panel for *in vitro* toxicity assessment using organotypic cultures

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

Iskandar et al., Toxicology Research, accepted

Meta-analysis of three THS2.2 Assessment Studies with Different Organotypic Cultures of the Aerodigestive Tract

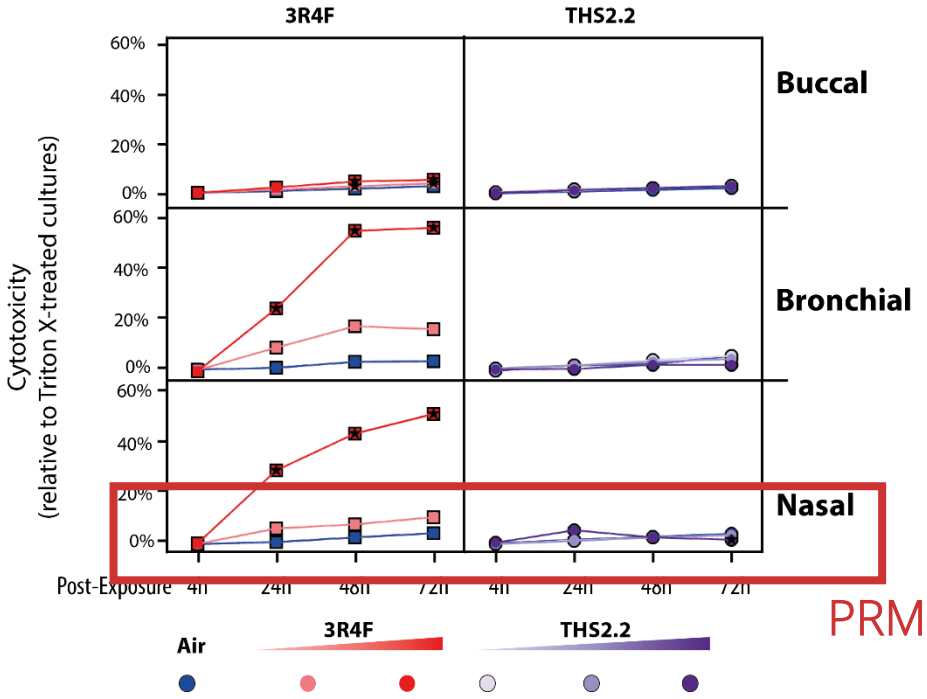
Study Design



Tissue	Experimental Repetitions	Doses of 3R4F Smoke and THS2.2 Aerosol						
	2014–2016	3R4F			THS2.2			
Buccal§	Smoke/Aerosol dilution*	NA	15%	24%	NA	25%	32%	69%
	Nicotine (mg/L)†	NA	0.32	0.51	NA	0.31	0.46	1.09
Bronchial§	Smoke/Aerosol dilution*	7%	13%	NA	13%	24%	31%	NA
	Nicotine (mg/L)†	0.13	0.25	NA	0.14	0.25	0.42	NA
Nasal§	Smoke/Aerosol dilution*	7%	15%	NA	13%	24%	31%	NA
	Nicotine (mg/L)†	0.15	0.25	NA	0.15	0.27	0.44	NA

Doses taken for the comparative analysis of transcriptomic and miRNA data

Cytotoxicity



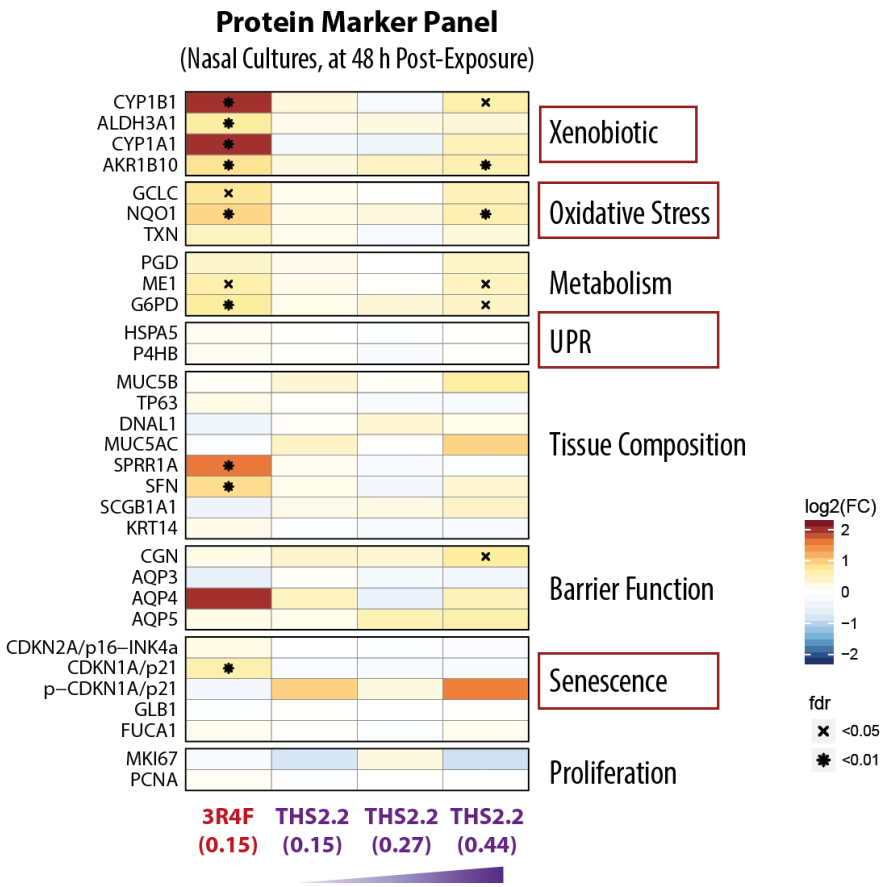
PRM panel

Reference: *Iskandar et al., Toxicology Research, 2017, accepted*

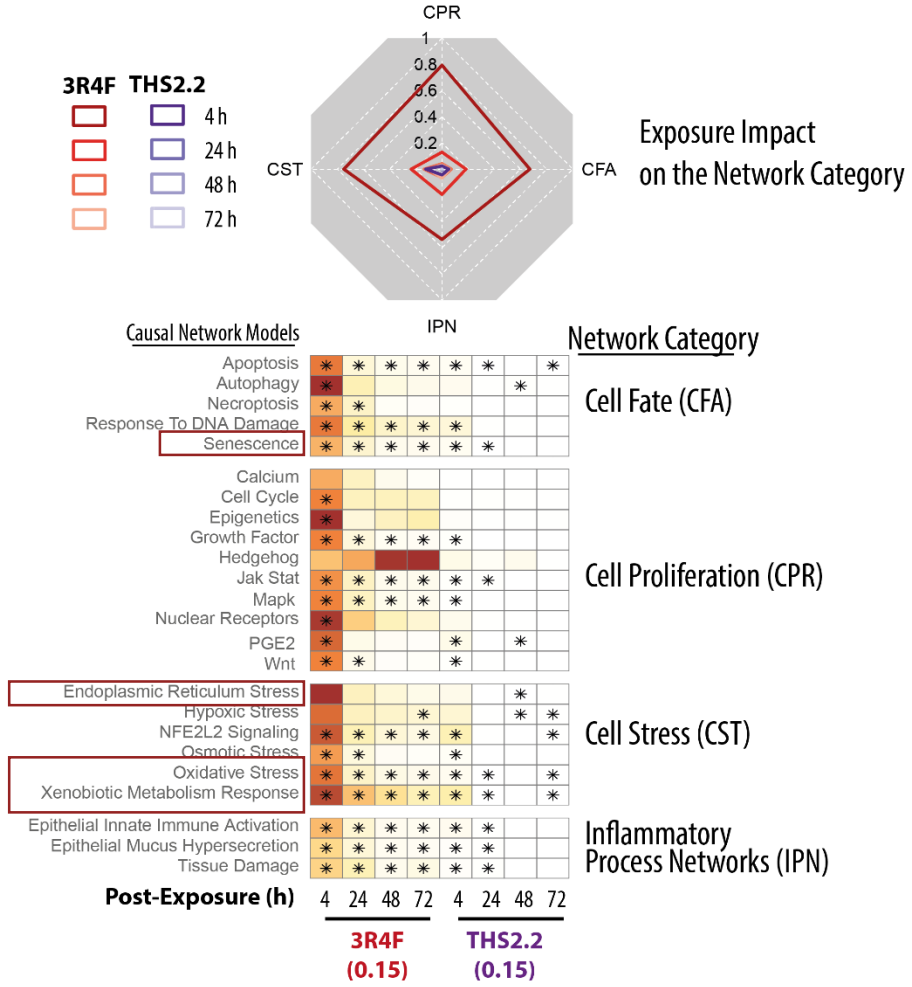
PRM Panel Complemented Other Endpoints

Parallel-reaction monitoring (PRM) panel defined to cover the main response categories in organotypic assessment studies

PRM Panel



Transcriptomics (Network Enrichment) Results



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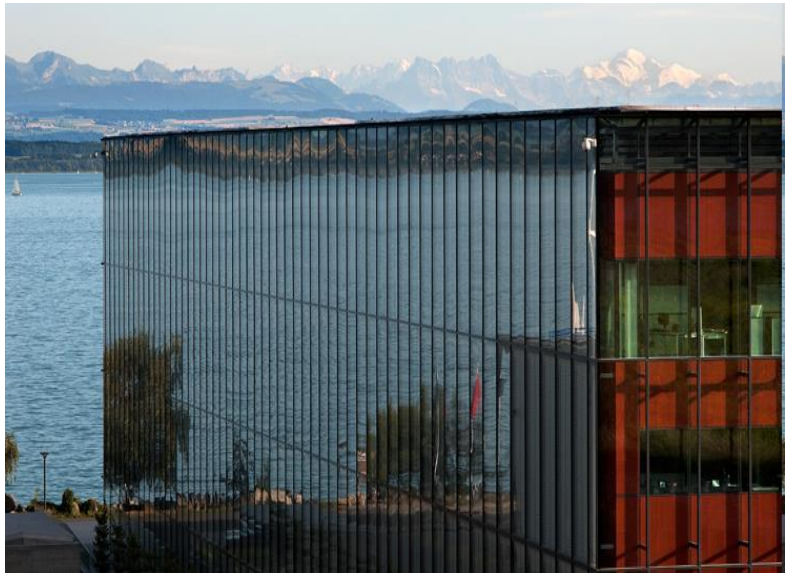
Reference: *Iskandar et al., Toxicology Research, 2017, accepted*

Summary & Conclusions

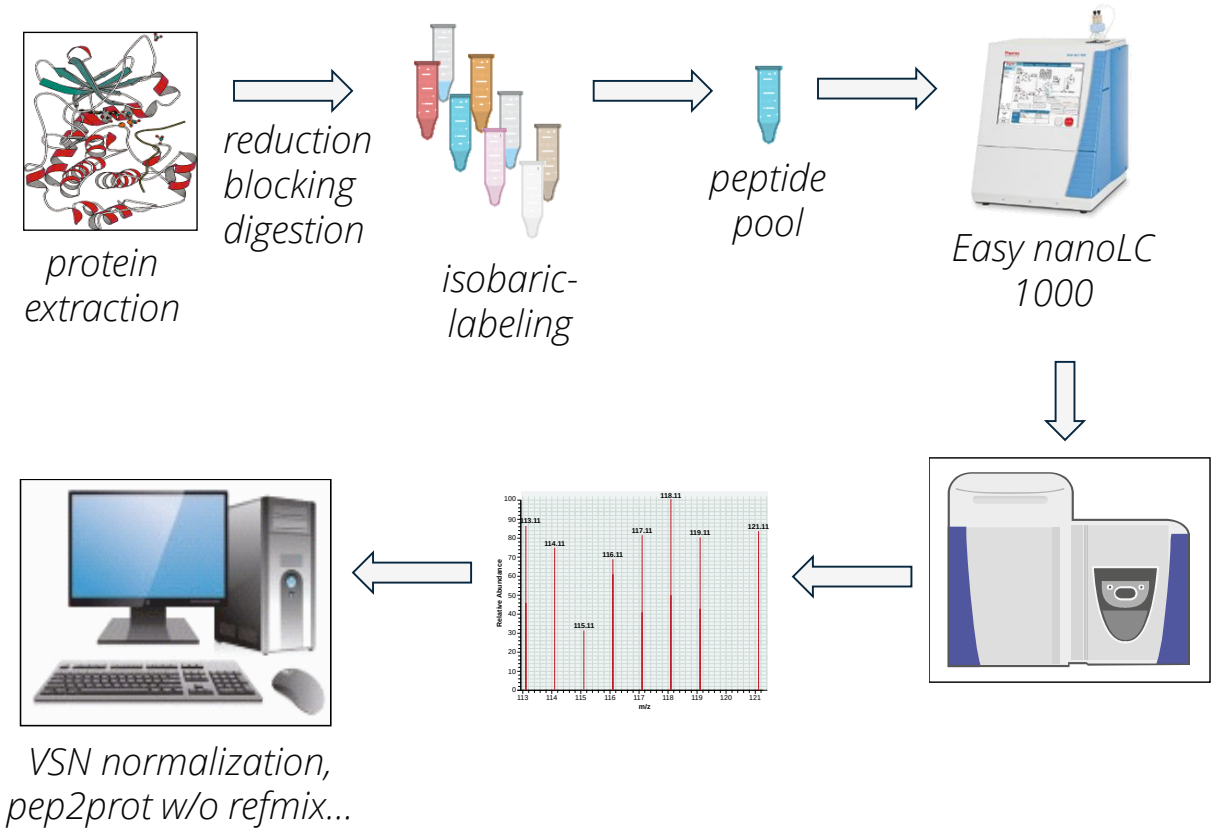
- Both untargeted (iTRAQ®) and targeted (PRM) proteomics are effectively used within comprehensive assessment framework for potential RRPs*
- Two case studies presented: 90-day rat inhalation study for THS2.2M and nasal organotypic study for THS2.2
- Proteomics complemented and further supported insights from other endpoints, e.g. on lung immune and cellular stress response in rat study and stress responses in organotypic study
- Overall, proteomics further supported the reduced biological impact of THS2.2 aerosols compared to cigarette smoke in these studies

Thanks!



Thanks to the following teams in Neuchatel & Singapore:

- Proteomics
- Transcriptomics
- Aerosol generation
- Workshop and exposure set-up
- Treatment team and animal handling
- Barrier support
- Dissection and lavage
- Bioanalytics
- Computational Biology and Statistics
- Histological processing and pathology


protein extraction
reduction blocking digestion
isobaric-labeling
peptide pool
Easy nanoLC 1000
VSN normalization, pep2prot w/o refmix...