PMI SCIENCE
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ORIGINAL PAPERS

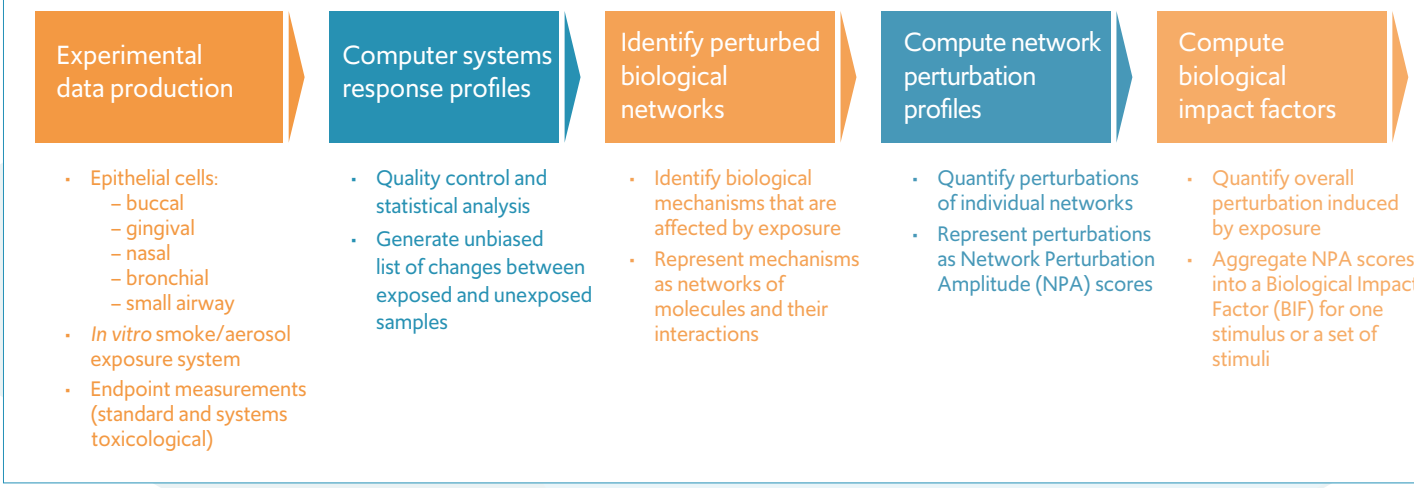
bit.ly/THS-buccal
bit.ly/THS-gingival
bit.ly/THS-nasal
bit.ly/THS-bronchial
bit.ly/THS-SA
bit.ly/THS-meta

heated tobacco · organotypic *in vitro* cultures · smoking · systems toxicology

Effects of aerosol from the Tobacco Heating System 2.2 on human organotypic cultures of the aerodigestive tract

- Exposure to aerosol from the Tobacco Heating System 2.2 had minimal biological impact on human epithelial buccal, gingival, nasal, bronchial, and small airway cells in comparison with exposure to cigarette smoke.
- Standard toxicological assays were combined with network-based systems toxicological analyses to reveal otherwise undetectable cellular-level effects.

Fig 1. Network-Based Systems Toxicology Approach



Cigarette smoke is responsible for a multitude of health risks and is one of the major modifiable risk factors for many smoking-related diseases. The aerosol produced by the Tobacco Heating System 2.2, which heats tobacco rather than burning it, has a different chemical composition to CS, with levels of harmful and potentially harmful constituents reduced by an average of 95%.

Advances in tissue engineering have enabled the development of sophisticated, three-dimensional organotypic culture systems that closely resemble human physiology.

Table 1. Experimental Repetitions, Dosing, and Endpoints

Tissue	Experimental repetitions	Nicotine concentrations in smoke and aerosols (mg/L)*							
		Cigarette Smoke				Tobacco Heating System 2.2			
Buccal	4	–	0.32	0.51	–	–	0.31	0.46	1.09
Gingival	3	–	–	49.4	84.6	–	–	54.6	100.4
Nasal	4	0.15	0.25	–	–	0.15	0.27	0.44	–
Bronchial	6	0.13	0.25	–	–	0.14	0.25	0.42	–
Small Airway	3	0.14	0.26	–	–	0.14	0.30	0.45	–

Selected Endpoints
Cytotoxicity
Culture histology
Multi-omics and network-based mRNA profiling

* **For bronchial, nasal, buccal, and small airway studies:** nicotine was determined in the aerosol (expressed in mg nicotine/L smoke or mg nicotine/L aerosol). **For gingival study:** nicotine was determined as a concentration deposited in phosphate-buffered saline (PBS) located in the exposure chamber (expressed in mg nicotine/L PBS).

Toxicological evaluations designed for mechanistic investigation (e.g., cellular and molecular assessment) are often conducted using animal models to assess the effects of a given toxin on the whole organism. However, because of species differences, results obtained from these studies are limited and cannot fully reflect the pathophysiology of human diseases.

In vitro cellular models using human cells can overcome interspecies differences, and advances in tissue engineering have enabled the development of sophisticated, three-dimensional organotypic culture systems that closely resemble human physiology. In addition, in line with 21st Century Toxicology, these models support the internationally recognized "3Rs" of animal research: **replace**, **reduce**, and **refine** the use of animals in research.

The objectives of the five studies outlined here were to assess the effects of aerosol from the Tobacco Heating System 2.2 (THS) on, respectively, human epithelial buccal, bronchial, nasal, gingival, and small airway cells using three-dimensional, organotypic culture systems.[1-6]

STUDY DESIGNS

All five studies used comparable methodologies. A further meta-analysis was conducted on the buccal, bronchial, and nasal studies, as these studies were completed earlier that the gingival and small airway studies.

Cells from human donors were grown in specially developed inserts at the air-liquid interface, representing the physiology of epithelial cells in human biology. Cells were exposed to either THS aerosol, cigarette smoke (CS), or filtered air, for 28 minutes (repeated for three consecutive days in the gingival study). Nicotine concentrations were matched as closely as possible between THS aerosol and CS (Table 1). Concentrations varied across the studies due to the morphological differences of tissue types (e.g., thickness of buccal cultures was approximately five times that of bronchial and nasal cultures). Endpoints were assessed 4- and 24-hours after exposure in the gingival study. In all other studies, endpoints were assessed 4-, 24-, 48-, and 72-hours after exposure.

RESULTS

Cytotoxicity and Histology. Effects of exposure were first assessed by measuring the release of adenylate kinase as a marker of cytotoxicity. This was followed by histological analyses of exposed tissue cultures. CS exposure resulted in similar cytotoxicity profiles in bronchial, nasal, and small airway cultures, with cytotoxicity increasing significantly in line with dose and duration of exposure. In contrast, THS aerosol exposure resulted in only minimal cytotoxicity in bronchial, nasal, and small airway cultures at all doses and post-exposure time-points.

Histological analyses identified a number of features commonly associated with cytotoxicity in buccal cultures exposed to CS, but only minor changes in buccal cultures exposed to THS aerosol.

24-hours after exposure, major cytotoxicity was observed in gingival cells exposed to CS, but not in gingival cells exposed to THS aerosol. Histological analyses identified severe damage to gingival cells after exposure to high concentrations of CS, while only minor changes were observed after exposure to THS aerosols at all concentrations.

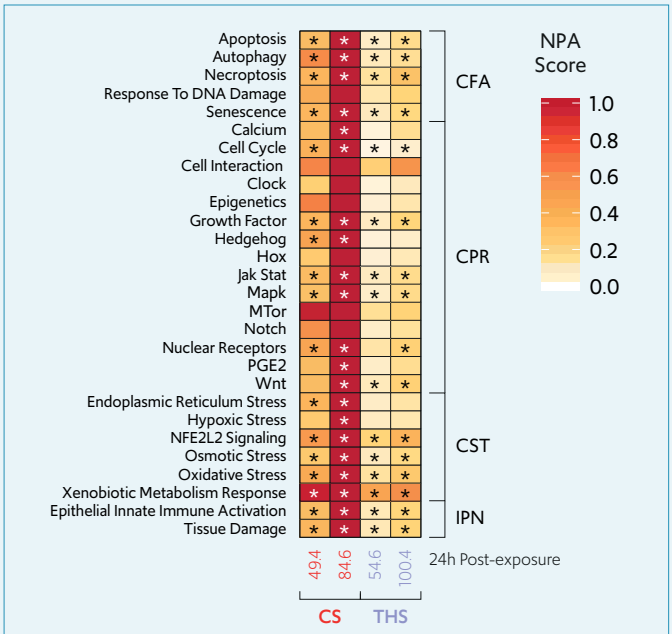


Fig 2. Gingival

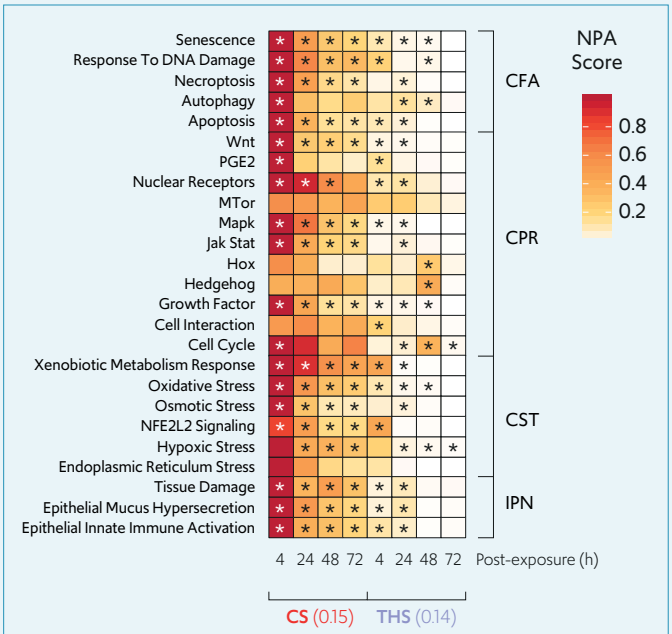


Fig 3. Small Airway

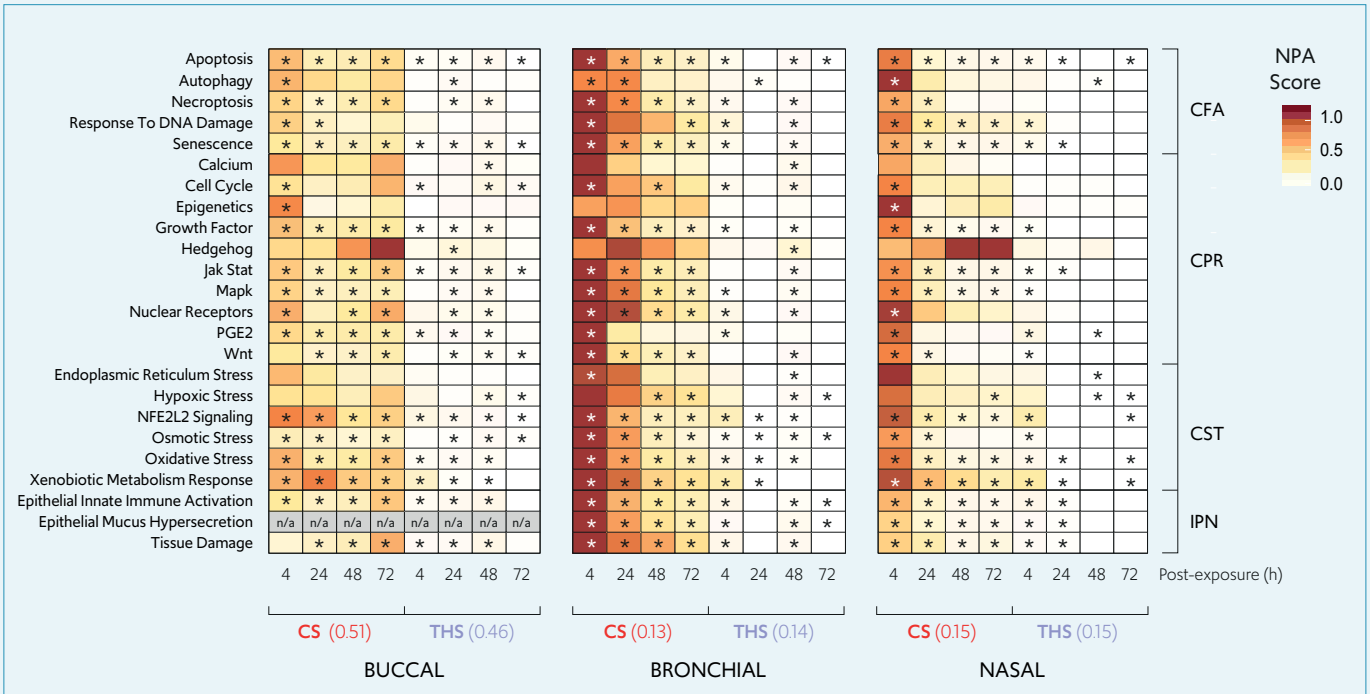


Fig 4. Buccal, Bronchial, Nasal

NETWORK PERTURBATION AMPLITUDE (NPA) SCORES. **CFA:** Cell Fate; **CPR:** Cell Proliferation; **CST:** Cell Stress; **IPN:** Inflammatory Process Networks; **CS:** cigarette smoke; **THS:** Tobacco Heating System 2.2; *: statistically significant

Multi-Omics and Network-Based Analyses. In complement to the standard toxicological measurements carried out in these studies, causal network enrichment analyses of transcriptomic, metabolomic, and mRNAomic data were used to assess otherwise undetectable cellular-level changes on the five tissue types studied. In contrast to CS, THS aerosol was found to have a significantly reduced impact on key biological networks associated with smoking-related disease (Figs 2, 3 and 4).

CONCLUSION

The five studies briefly outlined here, in addition to the meta-analysis of the buccal, bronchial, and nasal studies, all indicate a minimal biological impact on human epithelial buccal, gingival, nasal, bronchial, and small airway cells exposed to THS aerosol in comparison with cells exposed to CS. The studies further demonstrate the applicability and robustness of a systems toxicology approach for *in vitro* inhalation toxicity

Tobacco Harm Reduction

Cigarette smoke (CS) is the leading modifiable risk factor for many human diseases. Complete smoking cessation is the best approach to reduce the risks of smoking-related diseases. However, while the prevalence of cigarette smoking has been steadily declining over the years, millions of individuals across the globe continue to smoke. Smoking cessation has proven difficult for many smokers, who might benefit from using alternative products that have the potential to reduce the harm caused by CS.

For smokers who would otherwise continue smoking cigarettes, PMI's goal is to offer reduced-risk products (RRPs),* that have the potential to reduce the risk of developing smoking-related diseases as compared to continued smoking.

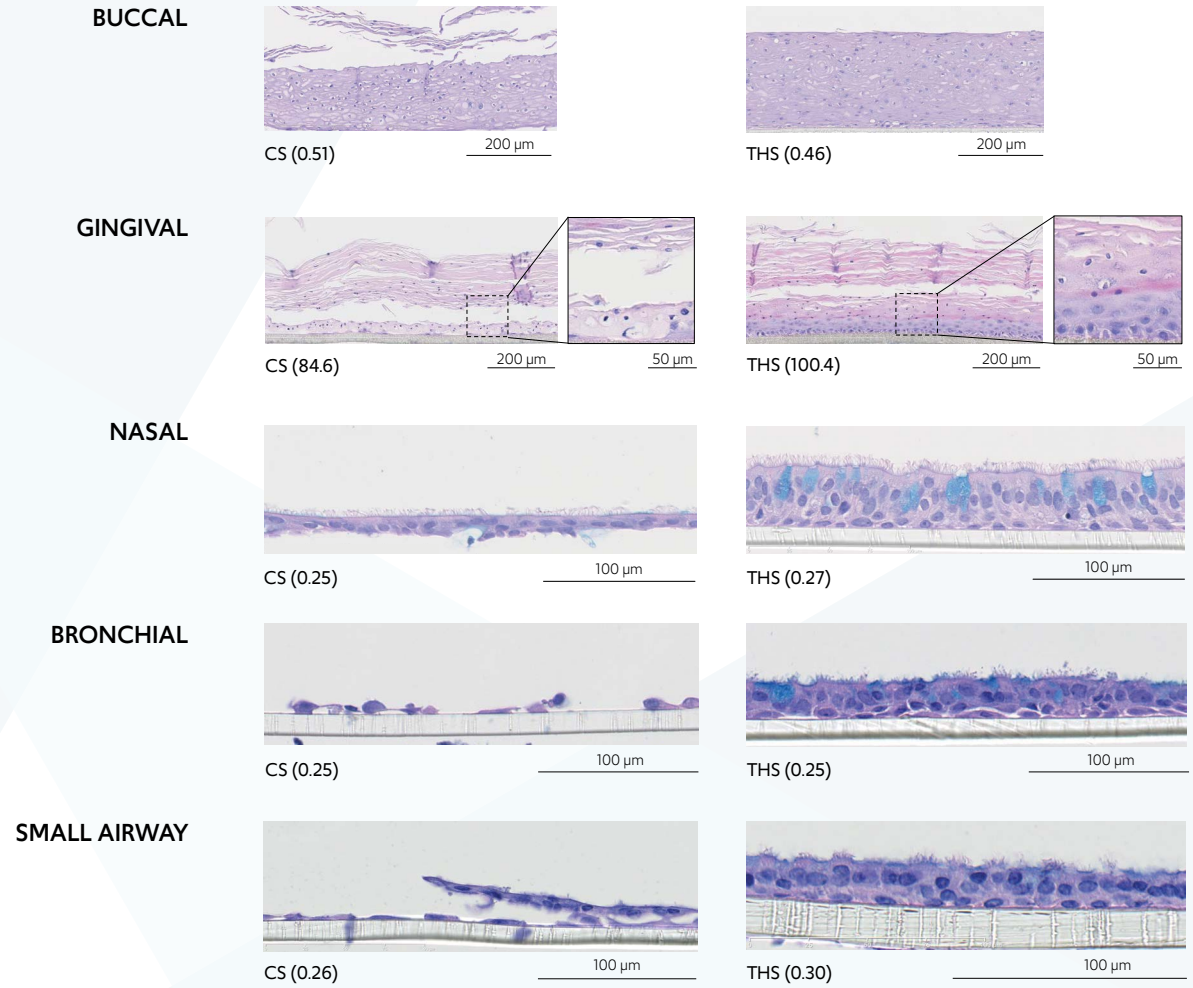


Fig 5. Selected Histology

Representative images of hematoxylin-eosin and alcian blue-stained sections observed 72 hours post-exposure for buccal, bronchial, nasal, and small airway cultures, and 24 hours post-exposure for gingival cultures. **CS:** cigarette smoke; **THS:** Tobacco Heating System 2.2.

REFERENCES

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FULL STUDY DATA AVAILABLE
https://doi.org/10.26126/

intervals.lkumkc.1
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intervals.jdnrqn.2
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