Characterization of aerosol deposition on human organotypic respiratory epithelial tissue exposed at the air-liquid interface and metabolism assessment

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Introduction and objectives

Human organotypic respiratory epithelial tissue cultures are routinely used for *in vitro* toxicological assessment of the biological impact upon exposure to smoke from a 3R4F reference cigarette and aerosol from smoke-free products at the air-liquid interface (ALI). Metabolism of deposited compounds is a critical parameter to be considered for their bioavailability and assessment of potential toxicity. Nicotine is commonly used as a marker of exposure, and its metabolism pathway is well established in the liver, responsible for the major part of nicotine biotransformation.

Cigarette smoke (CS) is a complex heterogeneous mixture of over 6,000 compounds. Various scientific bodies have acknowledged the presence in tobacco and CS of more than 100 harmful and potentially harmful constituents (HPHCs).

As CS or aerosol from smoke-free products represent a challenging matrix for *in vitro* testing and metabolite screening, a multi-compound method using dual-column liquid chromatography coupled to high-resolution accurate mass spectrometry was established. Nicotine and its major metabolites were quantified in extracts of organotypic respiratory epithelial tissue cultures following exposure to CS and aerosol from smoke-free products (THS 2.2*). Quantification was performed by stable isotope-labeled internal standards for each analyte or analyte group.

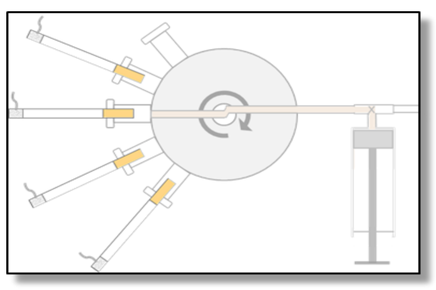
An additional exposure marker correlated with nicotine was investigated to further understand the deposition of aerosols and CS on organotypic respiratory epithelial tissue cultures.

***THS 2.2*:** *Tobacco Heating System 2.2*

Methods

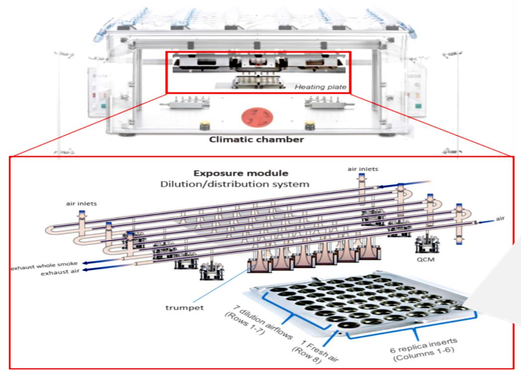
Aerosol generation and exposure of organotypic tissue cultures at the ALI

Aerosol generation



Smoking machine: SM2000, Philip Morris International Puff profile: Health Canada regimen (2 puffs/min. of 55 mL and 2 sec. aspiration and 8 sec. exhaust).

Tissue exposure system



Exposure system: VITROCELL® 24/48 system can be used to simultaneously expose 48 cultures to up to 7 different smoke concentrations, with 6 replicates per concentration.

Cell culture inserts & exposure at ALI

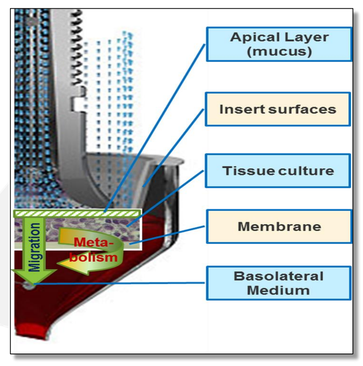
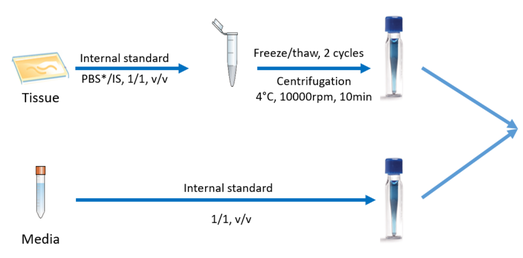


Figure 1: *Aerosol generation and exposure of tissue at the ALI.*

Analytical method

Sample preparation



Analytical system

Q Exactive™ and Vanquish™ Flex Binary UHPLC System

***PBS*:** *Phosphate-buffered saline*

Figure 2: *Workflow for samples preparation and analysis.*

Results

Metabolism and migration kinetics

Human organotypical tissues exposed to whole CS from the 3R4F reference cigarette:

- Biotransformation routes of nicotine with main metabolites in the liver *(**Figure 3**)*.
- Nicotine migrates rapidly upon exposure through the tissue into the basolateral media (***Figure 4***).
- Membrane has a significant effect on nicotine's half-life – Nicotine (M) ***(Figure 5)***. Impact of material has to be investigated more in detail.

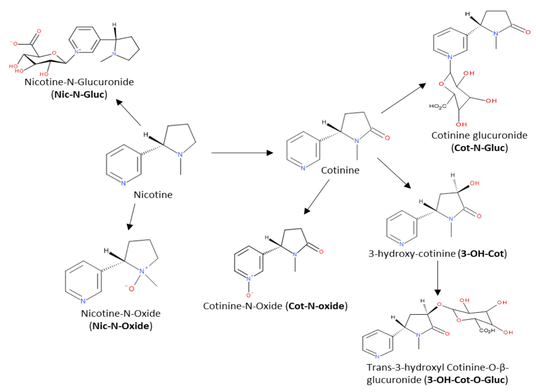


Figure 3: *Major metabolism routes of nicotine in the liver.*

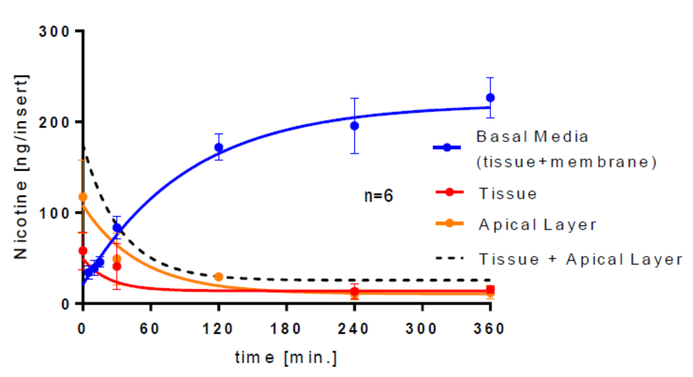


Figure 4: *Migration kinetics of nicotine in bronchial tissue, exposed to 15% 3R4F for 6 min.*

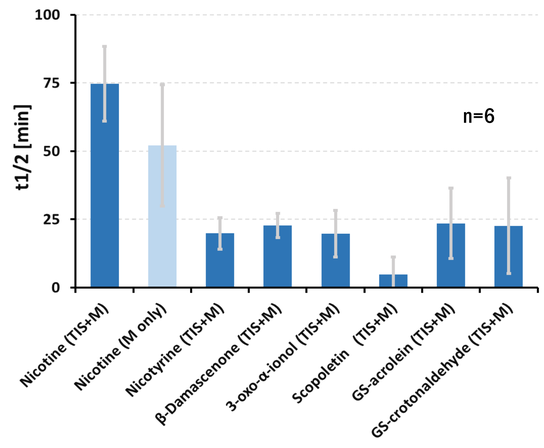


Figure 5: *Calculated half-life of nicotine and additional compounds in tissue (TIS) and membrane (M).*

Exposure characterization

Nicotine and its major metabolites were quantified after exposure of organotypic respiratory epithelial tissue cultures to 24% 3R4F CS or 24% THS 2.2 aerosol.

Cotinine as a major metabolite of nicotine represents less than 1% of total nicotine quantified. Others metabolites (nicotine-N-oxide, cotinine-N-oxide, 3-hydroxy cotinine) constitute trace amounts after exposure.

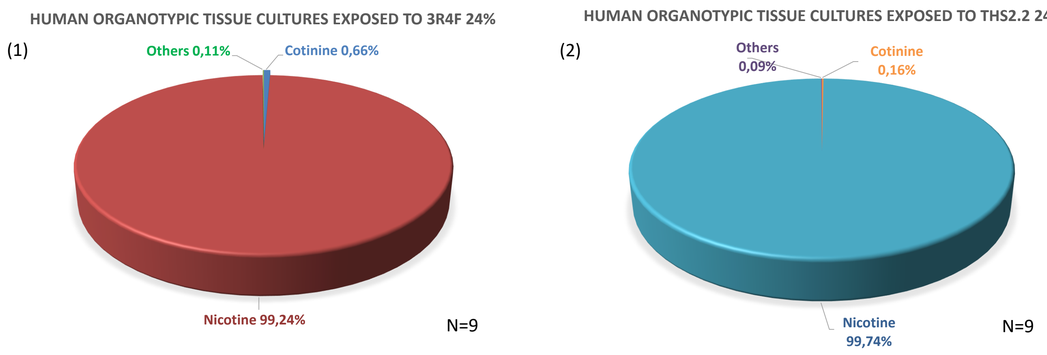


Figure 6: *Nicotine and its major metabolites after exposure on tissue from 24% 3R4F CS (1) and 24% THS 2.2 aerosol (2).*

Accurate measurement of nicotine deposition on tissue

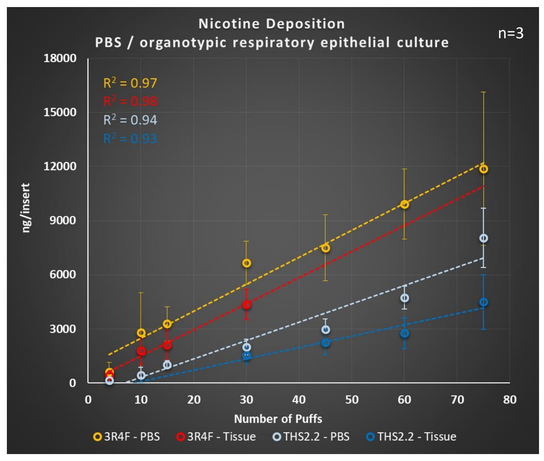


Figure 7: *Linear correlation of deposition to puff regime of nicotine on tissue and PBS after 3R4F CS and THS 2.2 aerosol exposure.*

- Human organotypic respiratory epithelial tissue cultures were exposed to undiluted 3R4F CS and undiluted THS 2.2 aerosol in various puff-based conditions.
- Tissue cultures were located in the exposure chamber without touching the media to prevent migration of nicotine into basolateral media.

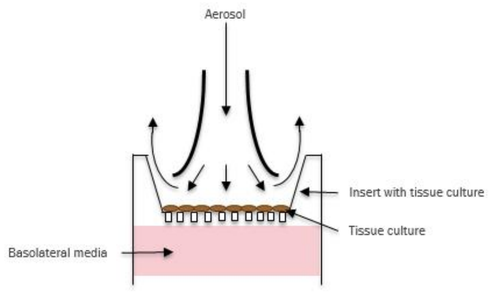


Figure 8: *Exposure chamber with tissue cultures.*

Scopoletin – additional exposure marker

A feasibility study demonstrated huge potential for additional aerosol constituents that can be used to describe in more detail the exposure of PBS and tissues in the VITROCELL® system.

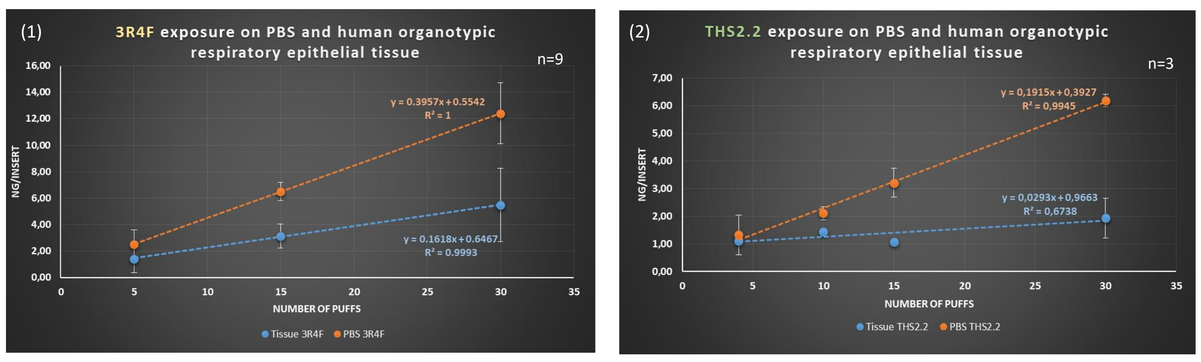


Figure 9: *Linear correlation of deposition to puff regime on tissue and PBS after 3R4F CS (1) and THS 2.2 aerosol exposure (2).*

Conclusions

- ❑ The multi-compound method is suitable for investigating metabolism pathway experiment as well as kinetic assessment in the complex matrix.
- ❑ Avoiding direct contact of exposed media and tissue during exposure was facilitated by lowering the volume of the media, which prevents migration of the nicotine and its metabolites during exposure in order to accurately measure the deposited nicotine in the tissue.
- ❑ The assessment of migration kinetics for the exposed organotypic tissue cultures is important for accurate characterization of the exposure.
- ❑ Scopoletin, used as an additional exposure marker, provides information on linear correlation of deposition to number of puffs on exposed tissue and PBS to 3R4F CS and THS 2.2 aerosol.

References

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Metabolomics, The Hague 23-27 June 2019

Competing Financial Interest – The research described in this poster was sponsored by Philip Morris International