In Vitro Test Systems for Aerosols Toxicology Assessment- Characterization of Exposure, Metabolism and Kinetics

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Introduction

Direct exposure to inhaled mainstream cigarette smoke is known to cause smoking-related damage in the human lung. Aerosol exposure of human three dimensional (3D) organotypic airway epithelial tissue cultures, growing at the air-liquid interface is a well-established in vitro model enabling systems biology-based Reduced Risk Product (RRP*) assessment.

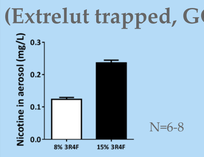
The **characterization of RRPs exposure** is an essential part of the whole product assessment strategy. Furthermore, investigations on the **migration kinetics** including adsorption and excretion occurring in 3D organotypic tissue cultures have been started to better understand the bioavailability of smoke compounds over time. To better understand the role of **xenobiotic metabolism** after cigarette smoking, human subcellular liver and lung fraction models (microsomes, S9) have been established to assess metabolite profiles and reactive metabolites relevant for toxicology. The integration of the exposure characterization results together with the systems toxicology approach to measure the biological impact of the exposure has the potential to further highlight the mode of action associated to RRPs exposure.

*Reduced Risk Products ("RRPs") is the term we use to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. PMI's RRPs are in various stages of development, and we are conducting extensive and rigorous scientific studies to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when compared to smoking cigarettes. Before making any such claims, we will rigorously evaluate the full set of data from the relevant scientific studies to determine whether they substantiate reduced exposure or risk.

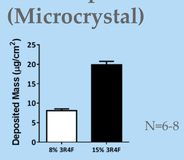
Vitrocell Characterization

Deposition of smoke of the reference cigarette 3R4F and aerosol of the THS2.2*

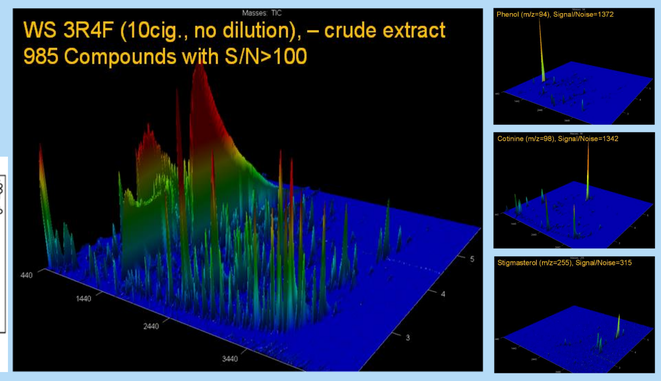
Nicotine in aerosol (Extrelut trapped, GC-FID)



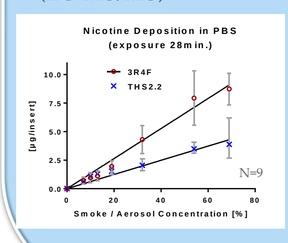
Mass deposition (Microcrystal)



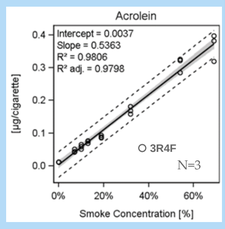
Nontargeted screening of smoke deposited at a microcrystal insert (GCxGC-TOF)



Nicotine in PBS (LC-MS/MS)



Carbonyls in PBS (LC-MS/MS)

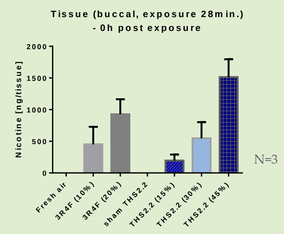


*THS2.2 = Tobacco Heating System 2.2

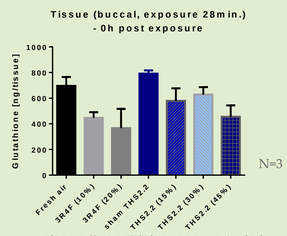
Exposure Characterization

Exposure of organotypic airway epithelial tissue cultures to smoke from the reference cigarette 3R4F or aerosol of the THS2.2

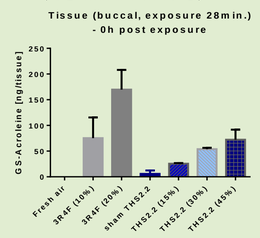
Nicotine (LC-MS/MS)



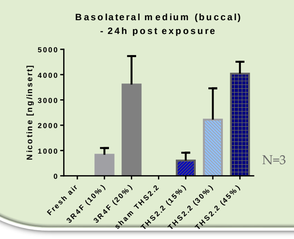
Glutathione (LC-HRAM-MS)



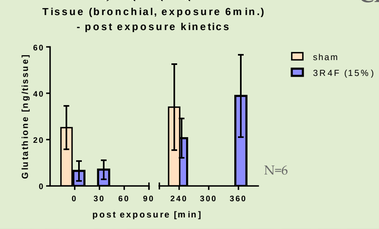
Acrolein-glutathione adduct (LC-HRAM-MS)



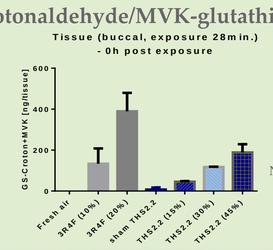
Basolateral medium (buccal) - 24h post exposure



Tissue (bronchial, exposure 6min.) - post exposure kinetics

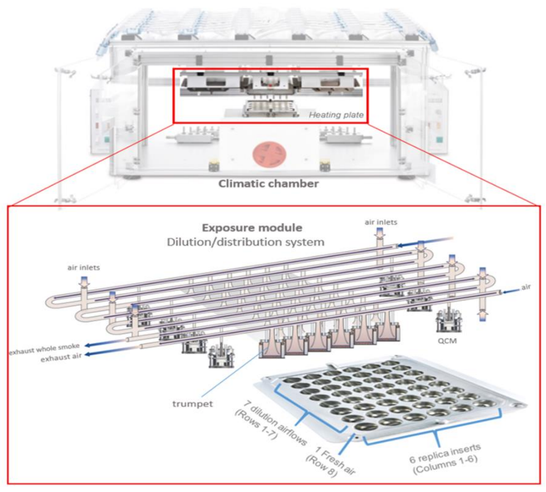


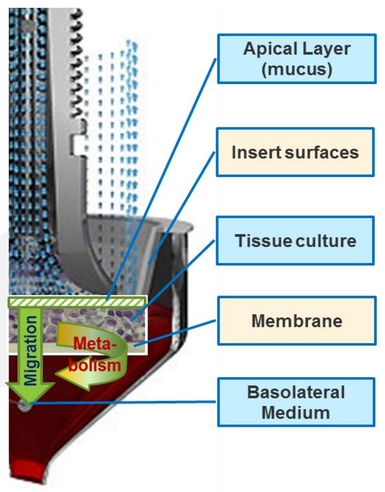
Crotonaldehyde/MVK-glutathione adduct



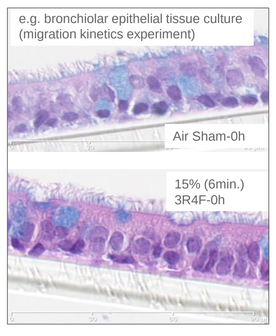
Vitrocell 24/48

- Cytotoxicity
- Xenobiotic response
- Gene expression
- Cytokine release
- Histology





Primary human bronchial, nasal, buccal and gingival epithelial cells (organotypic)



Metabolism

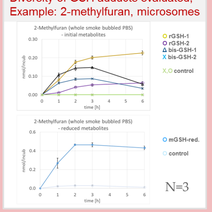
Cell-free Model 

In-vitro Model 

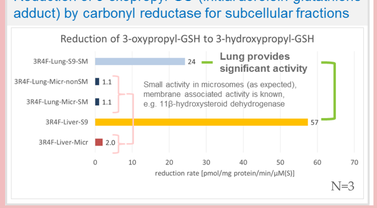
Case study metabolism of furan-type compounds using human lung and liver microsomes / S9 (cytosolic fraction)

Analytical challenge: Multiplicity of initial adducts is formed, which are also subject to subsequent metabolic reduction

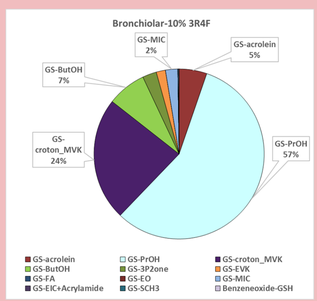
Diversity of GSH adducts evaluated, Example: 2-methylfuran, microsomes



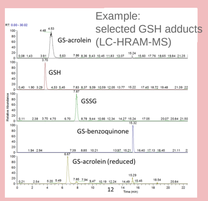
Reduction of 3-oxopropyl-GS (initial acrolein-glutathione adduct) by carbonyl reductase for subcellular fractions



Human organotypic tissue cultures exposed to whole smoke from the reference cigarette 3R4F



LC-HRAM-MS: Thermo QExactive, ESI (+) ionization. Screening: full scan, compound extraction by accurate mass



Results Case Study Furans (summary):

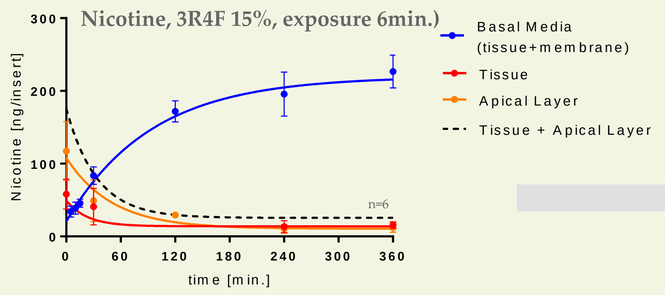
I. **Human lung microsomes provide CYP2E1 activity**
II. **Human lung microsomes are able to detoxify furan(s)** (CYP2E1, generating reactive intermediate metabolites)
III. **Human lung S9 generate reactive sulfates** of hydroxymethyl-derivates of furans (e.g. 5-HMF)

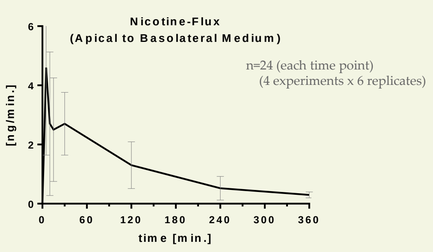
3 smoke compounds, acrolein, crotonaldehyde and MVK[1] are responsible for majority (approx. 92-93%) of the GSH-adducts identified

[1] MVK: methylvinylketone

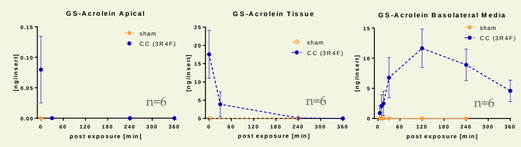
Migration Kinetics

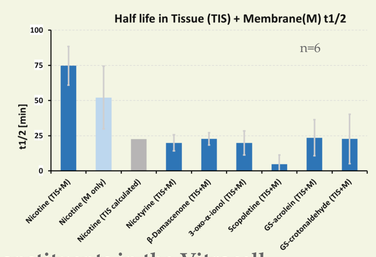
Human organotypic bronchiolar tissue cultures exposed to whole cigarette smoke





Acrolein Glutathione Adduct





- Outlook: detailed evaluation of the dynamics of smoke constituents in the Vitrocell
- Impact of materials will be evaluated

Conclusions

- **Subcellular fractions (microsomes, S9) are useful tools to study in vitro lung metabolism:**
  - Metabolite profiles, reactive metabolites relevant for toxicology & kinetics
  - Results from subcellular fractions metabolism models can be utilized in more complex models, like organotypic tissue culture
- **Analytical methodologies are established to characterize the exposure of organotypic tissue cultures:**
  - Nicotine and reactive carbonyls glutathione adducts can be determined to describe the dose in exposed tissues
  - Glutathione serves as additional marker for tissue functionality
- **Metabolism studies will further substantiate the characterization of the organotypic culture models as tools for in vitro respiratory toxicology testing**
- **Dynamics of the exposed organotypic tissue cultures help to understand the biological system:**
  - Migration kinetics (including adsorption, metabolism, excretion kinetics): => bioavailability of smoke (compounds) by area under the curve (AUC)

References

1) *Peterson L. A. Reactive metabolites in the biotransformation of molecules containing a furan ring. Chem. Res. Toxicol. 26:6 (2013).*
2) *Takakusa H. et al. Markers of Electrophilic Stress Caused by Chemically Reactive Metabolites in Human Hepatocytes . Drug Metabolism and Disposition. Vol. 36, No. 5 (2008).*
3) *Metabolism and bioactivation of toxicants in the lung. The in vitro cellular approach, Jose´ V. Castell et al., Experimental and Toxicologic Pathology 57 (2005) 189–204*
4) *University of Kentucky (www.3r4f.com), (http://www2.ca.uky.edu/refcig/3R4F%20Preliminary%20Analysis.pdf)*

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