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PHILIP MORRIS INTERNATIONAL

ORIGINAL PAPER

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aerosol · heat-not-burn tobacco products · non-clinical assessment · systems toxicology

Development, validation, and characterization of methods for generating, collecting, and using aerosols from heat-not-burn tobacco products

- Heat-not-burn (HNB) tobacco products produce a nicotine-containing aerosol that is both quantitatively and qualitatively different from cigarette smoke.
- The methods and experimental setups that have been developed for studying cigarette smoke cannot be transposed directly for studying the aerosols from HNB products.
- The ability to reproducibly generate, collect, and use HNB aerosols is critical for characterization and preclinical assessment of HNB products

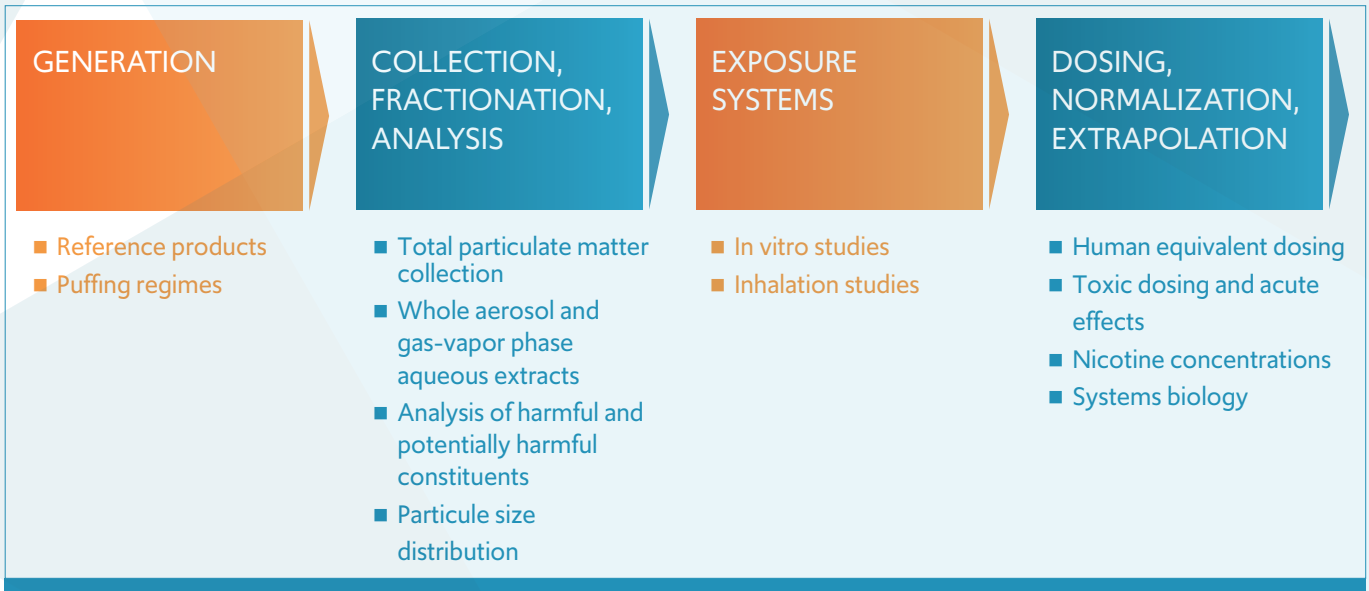

Figure 1 Methodological considerations

HNB products produce far lower quantities of harmful and potentially harmful compounds (HPHC) than cigarette smoke. The aerosol produced by one HNB product, the Tobacco Heating System 2.2, reduces HPHCs by an average of 95% relative to cigarette smoke.

Transparent sharing of protocols, tools, and data is needed to achieve international harmonization of methods, improve the objective evaluation of evidence, and develop scientifically substantiated heat-not-burn products.

While efforts to reduce the harm caused by smoking have traditionally focused on preventing smoking initiation and promoting smoking cessation, tobacco harm reduction is becoming increasingly recognized as a promising complementary method.[1] Tobacco harm reduction aims to reduce total morbidity and mortality without complete elimination of tobacco and nicotine use by providing less harmful tobacco products to adult smokers who would otherwise not quit. Modified risk tobacco products (MRTPs) are defined by the U.S. Food and Drug Administration as those that 1) significantly reduce harm and the risk of tobacco-related disease to individual tobacco users and 2) benefit the health of the population as a whole (users of tobacco products and people who do not currently use tobacco products).

Studies of MRTPs aim to show that the aerosol delivers significantly fewer and lower amounts of harmful and potentially harmful constituents (HPHCs) and is significantly less toxic than cigarette smoke (CS). Additionally, MRTP aerosols should present no new hazards. Heat-not-burn (HNB) products are MRTPs that produce an aerosol containing nicotine by heating instead of burning tobacco.

Aerosols generated by HNB products are qualitatively and quantitatively different from mainstream CS and the product category is much more heterogeneous than cigarettes. Commercially available HNB products vary in style from tobacco sticks heated by an element, to carbon-heated tobacco plugs, to hybrid products with aerosol created from a non-tobacco source that passes through a tobacco plug. A review was performed to assess the development, validation, and characterization of methods to generate, collect, and use HNB aerosols, with a particular focus on PMI's Tobacco Heating System (THS) 2.2.

STUDY REFERENCE PRODUCTS AND PUFFING REGIMES

HNB products offer an alternative to continued smoking, and, therefore, cigarette smoke should be used as reference in analyses of HNB aerosols.

Puffing regimens do not directly reflect user inhalation behavior but provide standardized conditions for comparison. The Health Canada Intense and ISO intense regimens have been widely used in research on HNB aerosols, mainly because they provide the most relevant basis for evaluating aerosol composition in comparison with CS. However, not all parameters are applicable to or possible to fulfill for HNB aerosols, which has led to development of new methods or adaptation of existing experimental setups for aerosol generation. For instance, commercially available linear smoking machines need added supports for holding the THS 2.2 devices. Rotary smoking machines, which allow continuous generation of CS, require docking stations for recharging the device batteries and acting as an interface with the smoking machine.

AEROSOL COLLECTION, FRACTIONATION, AND ANALYSIS

The aerosol collection and fractionation process (Fig. 2), while widely used, has several limitations. Methods used for trapping aerosols might substantially alter the physical and chemical composition of aerosols, and no single method can efficiently trap all constituents in all phases.

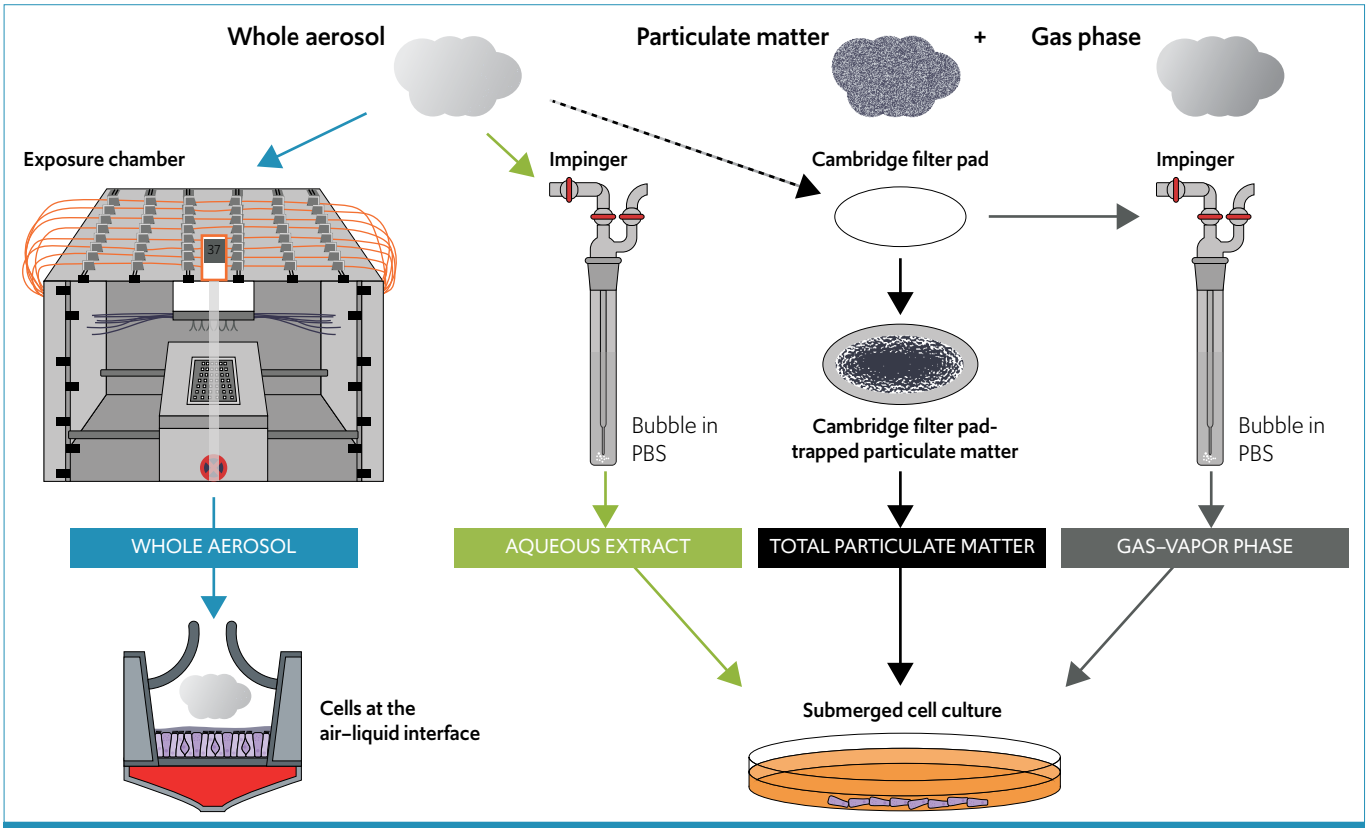

Figure 2 Experimental design for collection of and in vitro exposure to the whole aerosol (WA), aqueous extract (AE), total particulate matter (TPM), and gas–vapor phase (GVP) fractions

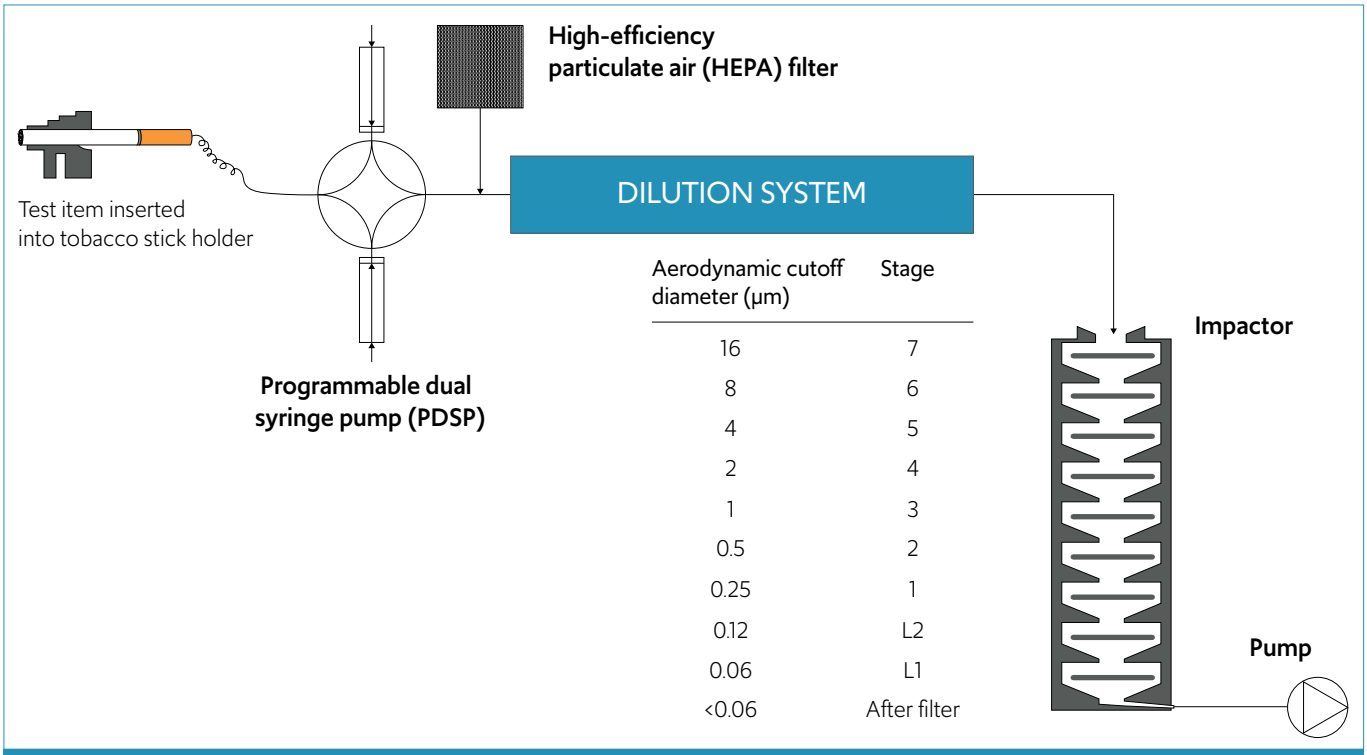

Figure 3 Experimental setup for cascade impactor measurement of particle size distribution

Total particulate matter collection The Cambridge filter pad (CFP) extraction process of total particulate matter (TPM) needs to be adjusted to take into account the high water content of HNB aerosols. Opening the CFP holder and handling the filter after exposure leads to water loss and overestimation of nicotine-free dry particular matter. Development of methods for maximizing retention of water content will yield more accurate estimates. The cytotoxicity of TPM collected for in vitro applications might be altered in the presence of metabolic activation systems. Insolubility should be assessed as precipitation in the final mixture under the actual test conditions.

Whole aerosol and gas–vapor phase aqueous extracts Optimization of trapping methods is critical for adequate interpretation and comparison of in vitro exposure assays. Aerosol condensate from all phases may be caught in a cold trap. Alternative methods include impaction and electrostatic trapping. Gas–vapor phase aqueous extracts can be trapped by bubbling the aerosol through a specific volume of liquid medium in an impinger (Fig. 2).

Analysis of HPHCs It is important to consider selecting the most relevant HPHCs for assessing HNB aerosols, given their diversity. Fifty-eight constituents have been selected for THS 2.2. Different trapping systems are needed for different aerosol fractions.

Particle size distribution Cascade impactors are widely used for measuring aerosol particle size distribution (Fig. 3) and are the reference method for inhaled pharmaceutical products. Of note, while dilution of the aerosol might be necessary for minimizing the effects of coagulation, it can substantially and rapidly change particle size distribution. PMI has assessed and validated the commercially available Aerodynamic Particle Sizer 3321 spectrometer (TSI, Shoreview, MN, USA), which provides high-resolution, real-time aerodynamic measurements of particles from 0.5 to 20 microns in size.

AEROSOL EXPOSURE SYSTEMS

In vitro studies Two-dimensional and three-dimensional submerged cell cultures (Fig. 2) are suitable for testing the effects of aerosol fractions, and systems toxicology approaches can enhance mechanistic understanding. However, although rapid and inexpensive, the simulation of interactions between aerosol and tissues in vivo is very limited. Exposure of complex, organotypic, three-dimensional cultures at the air–liquid interface better reflects the exposure in vivo. However, it requires the use of specialized systems such as the Vitrocell 24/48 exposure system (VITROCELL Systems GmbH, Waldkirch, Germany), which samples, dilutes, and delivers aerosol (Fig. 4).

Inhalation studies In line with 21st Century Toxicology, PMI's inhalation studies support the internationally recognized "3Rs" of in vivo research: replace, reduce, and refine the use of animals. Since animal studies cannot be fully eliminated, they should be designed to maximize sensitivity and efficacy while meeting key regulatory requirements. Static exposure systems are affected by depletion of oxygen and test agents over time. Dynamic systems are widely used, but have the drawbacks of consuming large quantities of test material and requiring complex experimental setups.

DOSING, NORMALIZATION, AND EXTRAPOLATION

While doses should span realistic human equivalent range, the need to test toxic doses and acute effects and the variability in assay sensitivity mean that in vitro data must be interpreted with care. The concentrations of HNB aerosol might need to be orders of magnitude higher than those for CS to show similar biological activity. Thus, before starting comparative studies, dose-range-finding studies should be undertaken to identify the highest concentrations that can be assessed without confounding the effects due to cytotoxicity.

The frequently applied normalization parameters in conventional CS toxicity comparisons are not meaningful for normalization of HNB aerosols because of the substantial differences in the chemical properties of aerosol. The recommended method is the use of nicotine concentration as a normalization parameter.2 A range of nicotine concentrations should be assessed, including one that matches for both products being tested.

Tobacco Harm Reduction

Cigarette smoke (CS) is the leading modifiable risk factor for many human diseases. Complete smoking cessation is the best approach for reducing 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 (RRP)* that have the potential to reduce the risk of developing smoking-related diseases as compared to continued smoking.

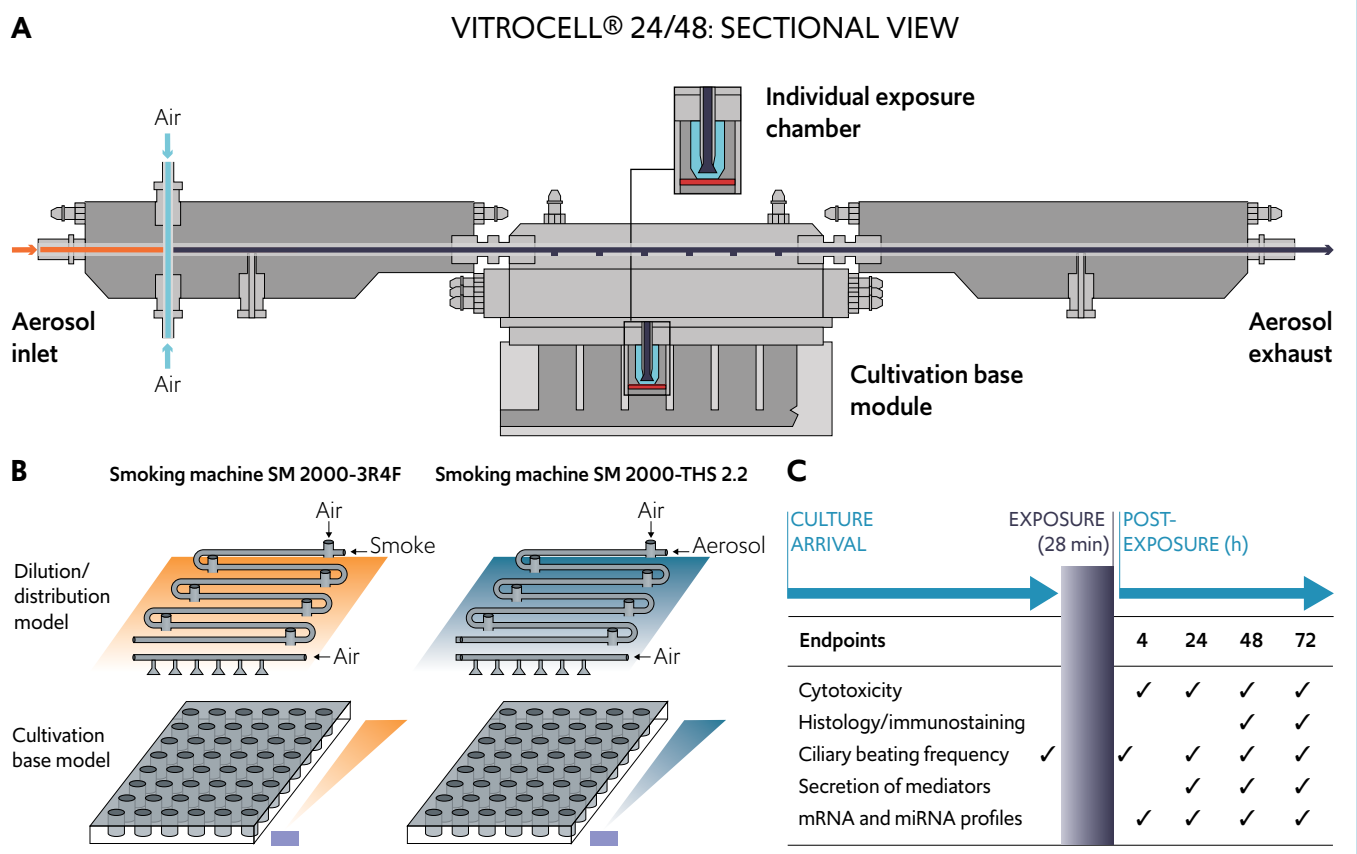

Figure 4 Schematic representations of the VITROCELL® 24/48 exposure system

(A) Schematic cross-sectional view of one row showing the dilution/distribution module, the cultivation base module, and individual exposure chambers; (B) Experimental setup for a comparative study of THS 2.2 aerosol and cigarettes smoke; (C) Experimental design of an exposure study of organotypic cell cultures.

Depending on the endpoint of interest, the conversion factor for extrapolating from animal studies to humans may be based on body surface area (for systemic toxicity) or lung surface area (for local toxicity). Because similar biomarkers can be measured in vitro, in vivo, and in human clinical studies, more direct comparisons of potential adverse events at similar plasma levels can be made between species and tissues. The use of systems biology approaches can amplify the insights.

CONCLUSIONS

Experimental methods were reviewed to support further studies, objective evaluation, and the verification of existing evidence as well as the development of scientifically substantiated products. The rapid growth of HNB products has been accompanied by the development of new testing methods and adaptation of existing methods. International harmonization of methods is needed to improve alignment and comparison of data. This will be achieved by transparent sharing of protocols, tools, and data.

REFERENCES

1. Boué S, et al. State-of-the-art methods and devices for the generation, exposure, and collection of aerosols from heat-not-burn tobacco products. *Toxicol Res Appl* 2020; published online Jan 21. https://doi.org/10.1177/2397847319897869.
2. WHO. The scientific basis of tobacco product regulation. Second report of a WHO study group. Geneva: World Health Organization, 2008, p. 289.

Data, software, and scientific protocols relating to HNB products and other RRPs are available on the open, online platform INTERVALS

www.intervals.science