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Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the system and the scientific assessment program

Maurice R. Smith*, Bruce Clark, Frank Lüdicke, Jean-Pierre Schaller, Patrick Vanscheeuwijck, Julia Hoeng, Manuel C. Peitsch

Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland[1]

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ABSTRACT

This publication introduces a series of eight other publications describing the non-clinical assessment and initial clinical study of a candidate modified risk tobacco product (MRTP) – the Tobacco Heating System 2.2 (THS2.2). This paper presents background information on tobacco harm reduction, to complement the approaches aimed at increasing smoking cessation and reducing smoking initiation to reduce the morbidity and mortality caused by cigarette smoking. THS2.2 heats tobacco without combustion, and the resulting formation of harmful and potentially harmful constituents (HPHC) is greatly reduced compared with cigarette smoke. Assessment of the THS2.2 aerosol *in vitro* and *in vivo* reveals reduced toxicity and no new hazards. Additional mechanistic endpoints, measured as part of *in vivo* studies, confirmed reduced impact on smoking-related disease networks. The clinical study confirmed the reduced exposure to HPHCs in smokers switching to THS2.2, and the associated transcriptomic study confirmed the utility of a gene expression signature, consisting of only 11 genes tested in the blood transcriptome of subjects enrolled in the clinical study, as a complementary measure of exposure response. The potential of THS2.2 as an MRTP is demonstrated by the assessment and additional publications cited in this series.

1. Introduction

1.1. General

The U.S. Family Smoking Prevention and Tobacco Control Act defines a Modified Risk Tobacco Product (MRTP) as any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco related disease associated with commercially marketed tobacco products (Family Smoking Prevention and Tobacco Control Act). This publication is part of a series of nine publications describing the nonclinical and part of the clinical assessment of a candidate MRTP, THS2.2 regular and a mentholated version (THS2.2M). The series of publications provides part of the overall scientific program to assess the potential for THS2.2 to be a reduced risk product. This first publication in this series describes THS2.2 and the assessment program for MRTPs. This is followed by six publications that describe the nonclinical assessment of THS2.2 regular and THS2.2M (Kogel et al., 2016; Oviedo et al., 2016; Schaller et al., 2016a; Schaller et al., 2016b; Sewer et al., 2016; Wong et al., 2016). The eighth publication in the series describes a clinical study to assess whether the reduced formation of Harmful and Potentially Harmful Constituents (HPHC) for THS2.2 regular also leads to reduced exposure to HPHCs when the product is used in a clinical setting (Haziza, 2016). A final publication utilizes data gathered from the reduced exposure clinical study on THS2.2 regular to determine if a systems pharmacology approach can identify exposure response markers in peripheral blood of smokers switching to THS2.2 (Martin et al., 2016).

Abbreviations: MRTP, Modified Risk Tobacco Product; THS2.2, Tobacco Heating System version 2.2 regular; THS2.2M, Tobacco Heating System version 2.2 menthol version; HPHC, Harmful and potentially harmful constituents; PMI, Philip Morris International; FSPTCA, Family Smoking Prevention and Tobacco Control Act; FDA, Food and Drug Administration; CTP, Center for Tobacco Products; CDER, Center for Drug Evaluation and Research; CC, Combustible Cigarette; CVD, Cardiovascular disease; COPD, Chronic obstructive lung disease; NPA, Network perturbation amplitude; BIF, Biological Impact Factor; OECD, Organization for Economic Cooperation and Development; PHIM, Population health impact model; HCI, Health Canada intense smoking regime; 3R4F, University of Kentucky Reference Cigarette; miRNA, Micro-ribonucleic acid; MRC, Mentholated reference cigarettes; SA, Smoking abstinence.

* Corresponding author.
E-mail address: Maurice.Smith@pmi.com (M.R. Smith).

[1] Part of Philip Morris International group of companies.

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1.2. Tobacco harm reduction

Cigarette smoking is one of the leading causes of preventable death both in the United States and globally. For many decades, the foundational principles of reducing this harm have been focused on preventing smoking initiation and promoting smoking cessation. In recent years, a third opportunity to reduce the harm from combusted tobacco products has emerged, based on switching consumers to less harmful products that have significantly reduced levels of toxic and harmful compounds. The United States Surgeon General (US Department of Health and Human Services (2010)) concluded that '*Inhaling the complex chemical mixture of combustion compounds in tobacco smoke causes adverse health outcomes, particularly cancer and cardiovascular and pulmonary diseases, through mechanisms that include DNA damage, inflammation and oxidative stress.*' It has long been known that the best way for smokers to reduce the adverse health consequences of smoking is to quit. However, though many smokers are interested in and attempt to quit, it can be very difficult to quit smoking cigarettes and hence the rates of long-term smoking cessation remain low. For example, according to the United States Surgeon General (US Department of Health and Human Services (2010)) although about 45% of smokers quit for a day, only approximately 5% succeed in achieving long-term abstinence for one year or longer.

As outlined by the U.K. Royal College of Physicians (Royal College of Physicians (2016)), '*Smoking is completely preventable, yet, more than half a century after the health harm of smoking first became widely known, almost 1 billion people worldwide still smoke. They do so primarily because they are addicted to the nicotine in tobacco smoke and, as this addiction can be extremely difficult to overcome, many will continue to smoke until they die.*'

Referring to an earlier report (Royal College of Physicians (2007)) that promoted the principle of harm reduction in nicotine addiction, the Tobacco Advisory Group of the U.K. Royal College of Physicians (Royal College of Physicians (2016)) stated that '*as most of the harm caused by smoking arises not from nicotine but from other components of tobacco smoke, the health and life expectancy of today's smokers could be radically improved by encouraging as many as possible to switch to a smoke-free source of nicotine. While recognizing the primacy of complete cessation of all tobacco and nicotine use as the ultimate goal to prevent harm from smoking, the report argued that promoting widespread substitution of cigarettes and other tobacco combustion products would, for smokers who made the change, achieve much the same thing. Harm reduction, as a complement to conventional tobacco control policies, could therefore offer a means to prevent millions of deaths among tobacco smokers in the UK alone.*'

As noted by McNeil (McNeil, 2012) '*Since nicotine itself is not a highly hazardous drug, encouraging smokers to obtain nicotine from sources that do not involve tobacco combustion is a potential means to reduce the morbidity and mortality they sustain, without the need to overcome their addiction to nicotine.*'

The harm reduction approach can be used to complement the existing strategies of reducing smoking related harm (i.e., preventing initiation and promoting cessation of smoking), to provide smokers with novel tobacco or nicotine containing products that are substantially less toxic than cigarettes. However, the potential public health benefit of such an approach will only be achieved if these novel nicotine products are scientifically substantiated to reduce risk and are acceptable alternatives that allow smokers to switch to the reduced-risk products.

Philip Morris International (PMI) is developing a portfolio of such novel nicotine products to address a wide range of adult smoker preferences where each product type is designed to significantly reduce or eliminate the formation of HPHCs in the inhaled aerosol while preserving as much as possible the taste, sensory experience, nicotine delivery profile and ritual characteristics of cigarettes.

The novel nicotine product described in this series of papers is a '*heat-not-burn*' tobacco product, which heats tobacco at a temperature below that required to initiate combustion. Different classes of tobacco constituents decompose at different temperatures, releasing chemical compounds into the aerosol. Heating at much lower temperatures than those found at the tip of a burning cigarette generates fewer and lower levels of HPHCs. The resulting aerosol contains nicotine but has significantly reduced levels of HPHCs compared with cigarette smoke.

The development of *heat-not-burn* tobacco products is not new and earlier efforts to develop such products (notably Premier and Eclipse products from R.J. Reynolds and Accord from Philip Morris) have been reviewed (Baker, 2006). Baker concluded that consumer acceptance of these products was low primarily because of sensory and usability issues, explaining their lack of commercial success. Consumer acceptance of reduced-risk products is crucially important if they are to be used in place of cigarettes and realize the potential to reduce risk for the individual smoker and for harm reduction at the population level (Fig. 1).

The studies presented in this series of papers form part of an assessment strategy to characterize a potentially reduced-risk product that generates an inhalable aerosol by heating tobacco instead of burning it. A description of this Tobacco Heating System (THS) version 2.2 is provided below, followed by an overview of our MRTP assessment strategy.

2. Product characteristics of THS2.2

THS 2.2 is a novel tobacco product type. It has three distinct components that perform different functions (Fig. 2): (i) a *tobacco stick* - a novel patent-pending tobacco product with processed tobacco made from tobacco powder, (ii) a *holder* into which the *tobacco stick* is inserted and which heats the tobacco material by means of an electronically controlled heating blade, and (iii) a *charger* that is used to recharge the *holder* after each use.

The THS2.2 product differs from a cigarette in significant ways. First, the *tobacco stick* does not contain tobacco cut-filler (tobacco leaf cut in small pieces found in cigarettes). Instead, the tobacco is ground and reconstituted into sheets (termed cast-leaf) following the addition of water, glycerin, guar gum and cellulose fibers. Second, the *tobacco stick* (Fig. 3) contains much smaller amounts of tobacco compared with a cigarette. The weight of the tobacco plug in the *tobacco stick* is approximately 320 mg compared with the 550–700 mg cut-filler found in conventional cigarettes. The reconstituted tobacco cast-leaf is fashioned into a small plug through a proprietary process known as '*crimping*'. Third, unlike a cigarette, the *tobacco stick* contains two unique and independent filters: (i) a polymer-film filter to cool the aerosol and (ii) a low-density cellulose acetate mouthpiece filter to mimic the sensory aspects of a cigarette. Furthermore, a hollow acetate tube separates the tobacco plug and the polymer-film filter.

To operate the THS2.2 product, the user inserts a *tobacco stick*

Fig. 1. The Harm Reduction Equation. Harm reduction at the population level is the result of the availability of a scientifically substantiated reduced-risk product that is an acceptable alternative to adult smokers and is not likely to attract non-smokers.

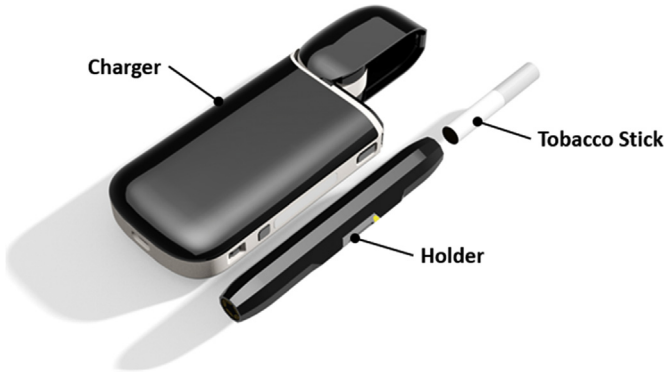

Fig. 2. The three components of the THS 2.2 product.

analysis of the inhalable aerosol delivered by the THS2.2 product in comparison with the smoke of a 3R4F reference cigarette (Schaller et al., 2016a).

3. The MRTP assessment strategy

In 2009, the United States Congress passed the Family Smoking Prevention and Tobacco Control Act (FSPTCA) granting the United States Food and Drug Administration (FDA) authority to regulate tobacco products, which, among other things, established the first regulatory procedure for reviewing an application and authorizing to market a reduced-risk tobacco product, (referred to in the U.S. law as a 'Modified Risk Tobacco Product,' or MRTP) (Family Smoking Prevention and Tobacco Control Act). An MRTP is defined by the FSPTCA as '*any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco related disease associated with commercially marketed tobacco products.*' The FDA stated in its MRTP Draft Guidance document (Food and Drug Administration, 2012) that the MRTP provisions of the FSPTCA '*may be valuable tools in the effort to promote public health by reducing the morbidity and mortality associated with tobacco use, particularly if companies take advantage of these provisions by making bold, innovative product changes …* '

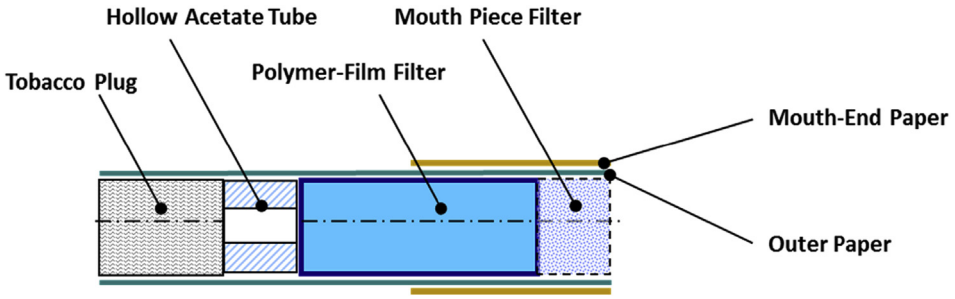

Fig. 3. Cross-sectional view of the *tobacco stick*.

into the *holder* and turns on the device by means of a switch. This initiates the heating of the tobacco via the heating blade inserted into the tobacco plug. The tobacco neither ignites nor burns. The electronically controlled heating, in combination with the uniquely processed tobacco, prevents combustion from occurring. Heat is supplied to the *tobacco stick* for a fixed period of approximately 6 min and allows up to 14 puffs to be taken during that time. The temperature of the heating blade is carefully controlled and the energy supply to the blade is cut if its operating temperature exceeds 350 °C.

When a cigarette is lit, the combination of tobacco (fuel) and oxygen in the air generates a self-sustaining combustion process that consumes the tobacco. During the period between puffs, the tobacco smolders at temperatures ranging from 600 to 800 °C in the center of the combustion zone. During a puff, the temperature increases to more than 900 °C at the periphery of the combustion zone (Baker, 1975). The combustion of tobacco results in formation of heat, smoke and ash. The smoke formed is a complex aerosol mixture estimated to contain more than 8000 compounds (Rodgman and Perfetti, 2013).

In contrast, the operating temperature of the THS 2.2 product is substantially lower than that required to cause ignition and combustion of tobacco and the temperature measured in the tobacco does not exceed 300 °C. When a puff is taken from the *tobacco stick* the tobacco temperature drops as ambient air is drawn through the *tobacco stick*. Since combustion does not occur, the structural integrity of the *tobacco stick* is retained after use. The tobacco is not consumed as in a cigarette and no ash is formed. The experimental confirmation that no combustion takes place during use of the THS 2.2 product as intended have been presented recently (Cozzani et al., 2016.) (and a separate publication is in preparation). This absence of combustion, because of controlled heating, is designed to significantly reduce formation of HPHCs by the THS2.2 product compared with cigarettes. This is confirmed by the chemical

The FSPTCA provides for the authorization of an MRTP when reduced exposure or reduced risk has been substantiated by applying a rigorous scientific assessment. Different levels of evidence are required for these respective authorizations, with ability for communicating product attributes with a reduced risk versus a modified exposure order. The FSPTCA requires applicants to demonstrate that the product, as actually used, will (i) significantly reduce harm and the risk of tobacco-related disease to individual tobacco users, and (ii) benefit the health of the population as a whole, taking into account both the users of tobacco products and persons who do not currently use tobacco products.

In contrast, the approach adopted by the European Union Tobacco Products Directive (European Parliament and Council Directive 2014/40/EU, 2014) is less detailed in its requirements than the approach adopted by FDA. The EU requires the submission of available product-specific studies and information in its notification process, but currently has no mechanism to authorize consumer information relating to reduced exposure or reduced risk.

Since the European Union Tobacco Products Directive (European Parliament and Council Directive 2014/40/EU, 2014) was implemented in May 2016, manufacturers and importers are required to submit a notification to the competent authorities of Member States for any novel tobacco product they intend to market. A novel tobacco product is defined as one that does not fall into any of the existing categories of tobacco products and is placed on the market after 19 May 2014. The notification should include:

a) Available scientific studies on toxicity, addictiveness and attractiveness of the novel tobacco product, in particular as regards its ingredients and emissions,
b) Available studies, executive summaries thereof and market research on the preferences of various consumer groups, including young people and current smokers,
c) Other available and relevant information, including a risk/benefit analysis of the product, its expected effects on cessation of tobacco consumption, its expected effects on initiation of tobacco consumption and predicted consumer perception.

The reduced exposure evaluation of an early *heat-not-burn* product (electrically heated cigarette smoking system – 'Heatbar') developed and assessed by PMI prior to the enactment of the FSPTCA, has been described previously (Schorp et al., 2012); it was recognized that '*a comprehensive assessment of reduced exposure is necessary, but is not sufficient for determining a modified tobacco product's potential to reduce risk.*'

The approaches to assess the risk of MRTPs relative to cigarette products have been described by the Institute of Medicine (Institute of Medicine (2012)) and reviewed recently (Berman et al., 2015). In this context, we have developed an assessment strategy designed to meet the more stringent requirements of the FDA's draft MRTP guidance that would also be applicable for the assessment of candidate MRTPs to be marketed in other jurisdictions.

The draft guidance from the FDA Center for Tobacco Products (CTP) indicates that the basis for authorizing an MRTP is somewhat different to the criteria applied by the Center for Drug Evaluation and Research (CDER) to the approval of a drug product. In the case of a drug, the general approach is to focus on a single or a very limited number of well-substantiated clinically relevant endpoints as indicators of a therapeutic effect. In the case of an MRTP, however, where product-specific epidemiological evidence is not available and clinical experience is limited, a different approach to product assessment has to be developed. It is well understood that the relative risk of smoking is not defined by a single endpoint or even endpoints reflective of a single disease or biological mechanism. Therefore, the evaluation of relative risk must take into account the complex nature of the whole organism and the many biological mechanisms that are affected by smoking. The approach to assess a candidate MRTP therefore needs to address this complexity by exploring a broad array of disease indicators to demonstrate that the use of the candidate MRTP has a reduced impact – compared with cigarettes – on mechanisms leading to tobacco-related diseases. This approach needs to be based on the best available science short of long-term epidemiological studies, which can be initiated once the product is on the market and under actual use conditions. The CTP has acknowledged this limitation in the draft guidance (Family Smoking Prevention and Tobacco Control Act; Food and Drug Administration, 2012). We understand this to mean that initial authorization of an MRTP will be based on non-clinical and clinical data which will be supplemented with post-marketing data. Evaluation of the relative level of risk of long-term use of an MRTP can begin once the product is authorized based on a weight-of-evidence approach confirming the potential for risk reduction relative to cigarette use.

3.1. The MRTP assessment framework

In this context, we have formulated a framework that utilizes what is known about combustible cigarette (CC) smoking and incorporates both epidemiological and mechanistic evidence to define our assessment approach. Epidemiological studies inform about the causal relationship between CC smoking and disease risk, as well as the benefits of smoking cessation. Ongoing exposure to cigarette smoke leads to both a time- and dose-dependent increase in the risk of developing smoking-related diseases, such as cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), and lung cancer. The accrued health risk over time can be reduced gradually by smoking cessation. The cause-and-effect relationships between smoking and these diseases are based on sound epidemiological evidence, conceptually depicted in Fig. 4 (red and green lines). The United States Institute of Medicine (Institute of Medicine (2012)) states that cessation is the '*gold standard*' for the assessment of an MRTP, providing '*an aspirational goal for risk and exposure*.' This sets the fundamental objective of an MRTP: '*switching to an MRTP must reduce the risk of developing smoking-related diseases with a risk profile approaching that of cessation*' (Fig. 4, orange lines). This MRTP assessment framework lays out the foundations for the assessment approach. In brief, if the changes observed in adult smokers who switch from cigarettes to an MRTP consistently approach the changes observed following smoking cessation, and those changes are further supported by coherent findings from non-clinical research, it is reasonable to conclude that the product will reduce risk.

Smoking-related diseases have a complex etiology. Broadly accepted mechanisms underlying many smoking-related diseases are related to impaired organ function from progression of pathological changes and comorbidity. Exposure to cigarette smoke induces molecular changes in the exposed organism and disrupts various biological processes. This in turn causes alterations at the cell and tissue level that result in physiological changes that eventually manifest themselves as diseases (Fig. 5).

Recent advances in molecular measurement and imaging technologies, mathematical modeling, and computational biology enable the integrative analysis of large data sets to quantify the biological impact of exposure to toxicants (Hoeng et al., 2012, 2014; Sturla et al., 2014). The integration of these methods with standard toxicological endpoints defines our systems toxicology-informed risk assessment approach. Using computable biological network models (Boue et al., 2015) of the key mechanisms affected by toxicants, systems toxicology enables the quantification of the biological network perturbation amplitudes (NPA) (Martin et al., 2012) caused by exposure to such toxicants and their numerical aggregation into an overall biological impact factor (BIF) (Thomson et al., 2013). This approach permits a systematic and quantitative mechanism-based comparison of the biological impact of switching to a candidate MRTP with continued smoking of CC as well as the

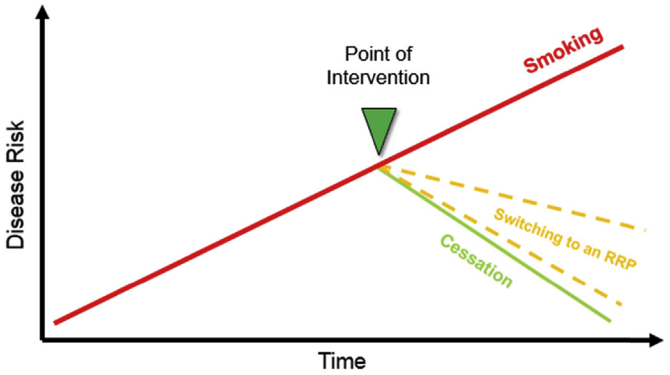

Fig. 4. Risk framework for MRTP assessment. Conceptual depiction of the cumulated risk of smoking and the effect of cessation over time. These represent the two boundaries for the assessment of an MRTP: 1) comparing switching to an MRTP with continued smoking and 2) benchmarking switching against smoking cessation (gold standard). Note that the straight lines used in this figure are for illustration purposes only as the accumulation of disease risk and the reduction upon cessation and switching to an MRTP follow different trajectories for specific diseases.

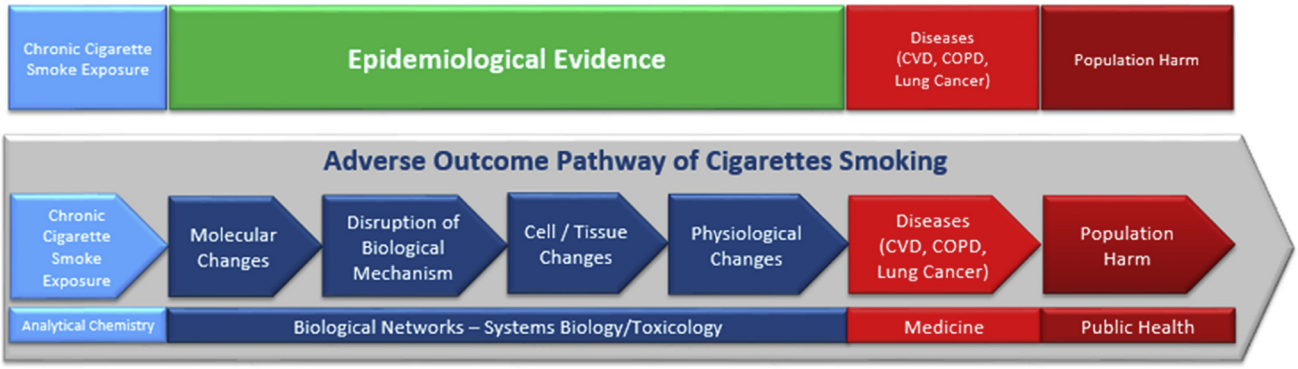

Fig. 5. Chronic exposure to cigarette smoke affects a number of biological networks associated with smoking-related diseases in a causal chain of events known as Adverse Outcome Pathways (Sturla et al., 2014 and references therein).

benchmarking of the impact of switching against that of smoking cessation in animal models of disease. We can therefore emulate disease progression and reversibility in a short timeframe.

3.2. The MRTP assessment program

We have developed a generally applicable assessment program which integrates seven assessment steps (Fig. 6) necessary to demonstrate that a candidate MRTP i) significantly reduces harm and the risk of tobacco-related disease to individual tobacco users, and ii) benefits the health of the population as a whole, taking into account both users of tobacco products and persons who do not currently use tobacco products. These assessment steps are designed to provide five levels of evidence as the assessment program is completed (Fig. 6):

1. The first step of the assessment is designed to ensure that the product is manufactured to appropriate quality standards and is sufficiently characterized to document product performance parameters. This product characterization enables the establishment of a product specification. All products entering the subsequent assessment steps must meet the established specification, as must the products that are introduced to the market. To ensure this consistency, a well-controlled Change Management process has been established. This process ensures that the established specifications are met for any proposed change to the product before it is implemented by determining any potential impact on product performance and aerosol composition. For instance, this process was utilized following changes to the tobacco blends used in the *tobacco stick* based on feedback from taste panels and to ensure sustainability of tobacco sources used to formulate the blend. The aerosol generated from *tobacco sticks* reformulated with different tobacco blends (with blend code D2 for the regular version and D1 for the menthol version) was compared with the aerosol from *tobacco sticks* using the previous tobacco blends (blend code FR1). There was no change in HPHC yields or *in vitro* toxicology findings for the different blends – further details provided in: (Schaller et al., 2016b).
2. The second assessment step is designed to analyze the chemical composition of the aerosol generated by the candidate MRTP and quantify the reduction in HPHCs formation in comparison with a cigarette. This analysis is also needed to evaluate whether new potentially harmful constituents are generated by the MRTP. During this step, it is also necessary to assess the influence of usage patterns and *puffing regimens* on aerosol composition, to ensure that the candidate MRTP delivers a stable reduction in HPHCs, as designed. Furthermore, the analysis of the aerosol chemistry data, collected under various conditions, including in the absence of oxygen, confirms the absence of the involvement of combustion in aerosol generation. The aerosol particles are also analyzed to verify i) that the aerosol contains a similar particle size distributions as the one found in CC smoke, to ensure a similar delivery of nicotine in the aerosol and ii) the significant reduction or absence of the insoluble solid particles found in cigarette smoke. Finally, during this step we also analyze the effects of MRTP use on indoor air chemistry to evaluate its impact on air quality in comparison with CC use (Mitova et al., 2016) and benchmark against national and international standards for exposure to environmental toxicants e.g.(European Agency for Safety and Health at Work, 2006). These environmental studies are conducted under well-controlled and realistic conditions based on accepted building standards (European Committee for Standardization, 2006).
3. The third step of the assessment determines whether the *reduced formation of HPHCs* leads to *reduced toxicity in laboratory models.* This step also contributes to the evaluation of any new hazards. This second level of evidence is based on toxicological studies conducted both *in vitro* and *in vivo.* First, we selected a battery of *in vitro* assays designed to assess the cytotoxicity and the genotoxicity of candidate MRTP aerosols in comparison with

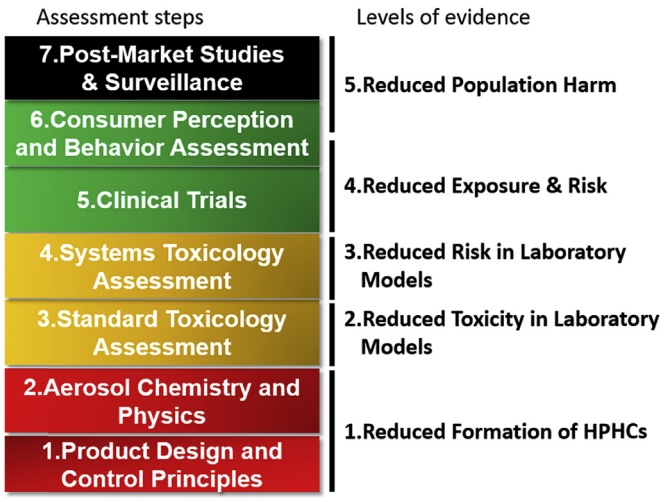

Fig. 6. The MRTP assessment program. Seven steps of assessment lead to five levels of evidence. Taken together, these levels of evidence provide the scientific evidence to demonstrate that a candidate MRTP is indeed a reduced risk product.

CC smoke (Schaller et al., 2016a). Second, the direct inhalation toxicity of candidate MRTP aerosols is analyzed in animal inhalation studies according to the guidelines from the Organization for Economic Cooperation and Development (Organization for Economic Co-operation and Development (OECD), 2009; Oviedo et al., 2016; Wong et al., 2016). In these studies we rigorously monitor the test atmosphere composition and measure biomarkers of exposure in the urine and blood of the exposed animals. This methodology allows us to assess the degree of *reduced exposure in laboratory models*, which provides an indication of what can be achieved independent of human behavior and use patterns. This evidence level also provides support to the substantiation of *reduced exposure and risk.*

4. The fourth assessment step is used to determine whether *reduced formation of HPHCs* and *reduced toxicity in laboratory models* lead to *reduced risk in laboratory models.* This evidence is based on systems toxicology studies (Sturla et al., 2014) conducted both *in vitro* and *in vivo*. The approach adopted for these *in vitro* assays is to use primary human cells and organotypic tissue cultures of human origin, as they are deemed to be more relevant than immortalized cell lines (Iskandar et al., 2016). The initial step in this assessment compares the effects of MRTP aerosol and CC smoke extracts on primary normal human bronchial epithelial cells using high-content screening (Marescotti et al., 2016) as well as a detailed mechanistic analysis of the changes occurring at the transcriptome level (Gonzalez-Suarez et al., 2014; Kogel et al., 2015). This type of study provides an initial assessment of an MRTP's impact on key pathways of toxicity. Second, we assess the impact of the whole MRTP aerosol in comparison with whole cigarette smoke on disease mechanisms using *in vitro* assay systems designed to recapitulate the structure of the human epithelial tissues exposed to aerosol/smoke (Kuehn et al., 2015; Mathis et al., 2013; Schlage et al., 2014; Talikka et al., 2014) and/or key events in disease pathways (Poussin et al., 2014, 2015; van der Toorn et al., 2015a). Third, we have complemented *in vivo* studies conducted under OECD guidelines (Kogel et al., 2014; Phillips et al., 2015a) with systems toxicology methods to quantify the impact of candidate MRTP aerosols on biological mechanisms perturbed by CC smoke, in conjunction with the physiological and toxicological endpoints measured under the OECD guidelines. Fourth, we employ animal models of disease with a study design that mimics the MRTP Assessment Framework. Briefly, these studies allow us to compare the effects of initial exposure to CC smoke followed by switching to a candidate MRTP aerosol with those caused by continuous exposure to CC smoke and benchmark them against those of cessation (Ansari et al., 2016; Phillips et al., 2015b). To enable a comprehensive analysis of these effects, these studies leverage the principles of systems toxicology through a very broad array of measurements, ranging from comprehensive molecular quantifications through transcriptomics, proteomics, and lipidomics, to full histopathological evaluation and disease risk markers, such as measurement of lung emphysema and atherosclerotic plaque formation. Furthermore, the use of an animal model such as the Apoe$^{-/-}$ mouse enables the concomitant analysis and quantification of both pulmonary and vascular effects of smoking, cessation and switching (Boue et al., 2013; Lietz et al., 2013; Lo Sasso et al., 2016a). Similarly, life-time exposure studies in the A/J mouse enable the concomitant analysis and quantification of both emphysema and cancer-related endpoints (Stinn et al., 2013) while leveraging the systems toxicology approach. Studies conducted in this assessment step provide the evidence that a candidate MRTP presents a *reduced risk in laboratory models.* It is essential to establish the performance of the product in laboratory models prior to performing clinical studies.
5. The fifth step of the assessment utilizes clinical studies to assess whether *reduced formation of HPHCs* leads to *reduced exposure and risk* in humans who use the candidate MRTP. These studies are conducted with adult smokers who are randomized into three groups reflecting the MRTP Assessment Framework: i) continued smoking, ii) cessation, or iii) switching to the candidate MRTP. Studies conducted in clinical confinement for a week can be used to quantify the maximum possible reduction in HPHC exposure compared with ongoing smoking and cessation, examples of these studies on a previous product are provided: (Tricker et al., 2012a, 2012b, 2012c, 2012d). This type of study is then complemented with studies conducted in ambulatory mode e.g. (Martin et al., 2012), to assess whether the reductions in HPHC exposure observed in short-term confinement can be sustained for a longer period in a near to real-world setting. Furthermore, with study periods ranging from three months to one year, it is possible to assess whether *reduced exposure* leads to a favorable change in smoking-related clinical risk endpoints, and hence provide assurance that *reduced exposure* leads to *reduced risk.* The endpoints to assess *reduced exposure* must be selected to reflect the range of molecular entities contained in CC smoke for which appropriate biomarkers of exposure can be reliably measured. Similarly, the endpoints to assess *reduced risk* must be selected to reflect the effects of CC smoke on different organ systems and biological mechanisms. These disease risk markers, or biomarkers of effect, must also be responsive to smoking cessation within the duration of the study and measurable using validated methods.

   As outlined in the introduction (Fig. 1), for effective harm reduction at the population level, an alternative tobacco product must not only be scientifically substantiated to reduce risk, but also be acceptable to cigarette consumers. It is unlikely that new products that do not satisfy smokers will enable them to switch. The nicotine delivery profile and the rewarding subjective effects of tobacco products are critical components of product satisfaction and their actual use. Lack of adoption of alternative nicotine delivery systems may therefore be related to ineffective nicotine delivery and/or a low level of satisfaction. To assess whether a candidate MRTP delivers satisfying levels of nicotine, with a delivery profile similar to cigarettes, we also conduct pharmacokinetic studies in the fifth step of our assessment program. These studies are conducted along the lines of a previous report (Picavet et al., 2016).
6. It is important that accurate, non-misleading, scientifically substantiated product information and benefits are communicated to adult smokers to provide them with an incentive to switch from cigarettes to an MRTP. The sixth step of the assessment involves studies that measure, prior to market introduction, the likely effect of introducing a new tobacco product with its associated communication materials on tobacco use behavior among adult smokers and non-smokers. This step assesses the likelihood that adult smokers will switch from cigarettes to an MRTP, and that former smokers, smokers who are motivated to quit, and non-smokers are not likely to use the product. Integral to the above is the assessment of consumer understanding and risk perceptions that any product communication would generate. The objective is to ensure that the intended product communication enables the public to correctly comprehend the modified exposure/risk claims and form the correct perception of the health risks of using an MRTP in comparison with cigarettes, nicotine replacement therapies, and cessation.

7. Once the product is on the market, it will be necessary to conduct post-market studies and surveillance to understand how the product is used in real-world settings. Passive surveillance measures are used to gather spontaneous reports of any adverse events related to product use. Longer-term assessment of exposure and health outcomes will be carried out, together with an ongoing assessment of consumer perception and tobacco use behavior.

To be effective, an MRTP must benefit the health of the population as a whole, accounting for current, former, and never-smokers (Family Smoking Prevention and Tobacco Control Act; Food and Drug Administration, 2012). However, population-level data regarding the risks and uptake of an MRTP prior to its market introduction are clearly lacking. To gain an understanding of the potential impact of an MRTP market introduction on population-level mortality, one can employ a mathematical model (Vugrin et al., 2015). Towards this end, PMI has developed a population health impact model (PHIM) for MRTPs (Weitkunat et al., 2015) that leverages publicly available epidemiology data. PMI's PHIM is designed to estimate the impact of the introduction of an MRTP on mortality based on assumptions about the fractional residual risk of an MRTP relative to that of cigarettes, and possible scenarios for the uptake of the MRTP in the population. The model estimates the impact on mortality in a population that survives until a specific time after the introduction of the MRTP on the market. Such a model can be used to predict the potential impact from the introduction of an MRTP based on the exploration of a wide range of scenarios describing realistically the prevalence of cigarette and MRTP use, individually and in combination.

The publications in this series are outlined below and report the results obtained having completed part of the overall assessment approach with the candidate MRTP THS2.2 in the context of the second to the fifth step of the MRTP assessment program.

4. Outline of the publications that follow in this series

4.1. Publications of studies conducted in the 2nd step of the MRTP assessment program

In Part 2 of this series of papers (Schaller et al., 2016a), the mainstream aerosol composition of both regular and mentholated THS2.2 is compared with that of the mainstream smoke from a reference cigarette (3R4F). The criteria for selection and the results for 58 HPHCs and analytes determined are provided and demonstrate that the majority of HPHCs measured in THS2.2 aerosol are reduced by more than 90% when compared with reference cigarette smoke. *In vitro* toxicological assessment of THS2.2 aerosol fractions is also described, revealing a >90% reduction in cytotoxicity, as determined by the neutral red uptake (NRU) assay, and a similar reduction in mutagenic potency in the mouse lymphoma assay (MLA). The THS2.2 aerosol fraction was not mutagenic in the Ames mutagenicity assay.

The performance of THS2.2 operated under simulated extreme climatic conditions (desert and tropical conditions) was assessed by monitoring aerosol composition to show no significant modification with climatic condition. The aerosol composition was also measured when using puffing regimens that were more intense than the standard Health Canada Intense (HCI) conditions, to confirm that HPHC levels remained lower than the levels formed in reference cigarette smoke generated with the HCI regimen.

In part 3 of this series of papers (Schaller et al., 2016b), the influence of 43 different tobacco blends (from a large range of tobacco types) on the formation of HPHCs in THS2.2 aerosol was determined. The aerosols produced by these blends in the THS2.2 contained significantly lower concentrations of HPHCs than did 3R4F mainstream smoke. For most HPHCs, the blend composition had a minimal impact on the yields in the resulting aerosols. However, some HPHCs presented significant variability across the different blends, likely resulting from the distillation of endogenous preformed compounds present in certain tobacco types. This approach provided the information required to intelligently blend tobaccos to meet consumer needs while maintaining low HPHC delivery.

4.2. Publications of studies conducted in the 3rd and 4th step of the MRTP assessment program

In part 4 of this series of papers (Wong et al., 2016), a 90-day nose-only inhalation study in rats was performed according to OECD Test Guideline 413 (Organization for Economic Co-operation and Development (OECD), 2009). The approach was modified to combine classical and systems toxicology approaches, transcriptomic analysis and miRNA expression (the latter results included in part 5 below). The effects of exposure of respiratory tract organs in THS2.2-exposed animals were much lower than those in rats exposed to 3R4F cigarette smoke. The results also confirmed that for the THS2.2 aerosol, there was no apparent new toxicity effects, compared with 3R4F cigarette smoke.

Part 5 of this series of papers (Sewer et al., 2016) demonstrated that 3R4F cigarette smoke, but not THS2.2 aerosol, caused global miRNA downregulation. Certain miRNA species, notably those associated with the inflammatory response, were upregulated in 3R4F cigarette smoke-exposed lung, but reduced following THS2.2 aerosol exposure. This work contributed to an increase in mechanistic understanding of the complex exposure responses.

In part 6 of this series of papers (Oviedo et al., 2016), a 90-day nose-only inhalation study in rats was performed according to OECD Test Guidelines 413 (OECD, 2009) on a mentholated variant of THS2.2 (THS2.2M) assessing both classical endpoints (described in the OECD guidelines such as histopathology, etc.), and complemented with transcriptomics and quantitative proteomics analyses of respiratory nasal epithelium and lung tissue, together with lipidomic analysis of lung tissue. Rats were exposed to either filtered air (sham), THS2.2M, two mentholated reference cigarettes (MRC, designed to meet 3R4F specifications with menthol added at different levels), or the 3R4F reference cigarette. The study results show that systemic toxicity and alterations in the respiratory tract were significantly lower in THS2.2M-exposed rats than in MRC and 3R4F.

In part 7 of this series of papers (Kogel et al., 2016), the systems toxicological assessment results from the study described in part 6 are discussed. The results demonstrated adaptive responses in the respiratory nasal epithelium to 3R4F cigarette smoke; these adaptations included squamous cell metaplasia and inflammatory response, with a close correspondence of the molecular and histopathological findings. In contrast, the adaptive tissue and molecular changes to THS2.2M aerosol exposure were much weaker, and limited mostly to the highest THS2.2M concentration in female rats. 3R4F smoke exposure induced an inflammatory response, triggered cellular stress responses, and affected sphingolipid metabolism. These responses were not observed or were much lower after THS2.2M aerosol exposure.

4.3. Publications of studies conducted in the 5th step of the MRTP assessment program

Part 8 of this series of papers (Haziza et al., 2016) describes a 5-day, controlled, parallel group, open-label clinical study where 160 smoking, healthy adult subjects were randomized to three groups

and asked to: (1) switch from CC to THS2.2 (THS group, 80 participants), (2) continue to use their own non-menthol CC brand (CC group, 41 participants), or (3) to refrain from smoking (Smoking abstinence [SA] group, 39 participants). All biomarkers of HPHC exposure, except those associated with nicotine exposure, were significantly reduced in the THS group compared with the CC group, and approached the levels observed in the SA group. Greater product consumption and total puff volume were reported in the THS group, but exposure to nicotine was similar to CC at the end of the confinement period in the clinic. Reduction in the urge to smoke was comparable between the THS and CC groups, and the THS was well tolerated with few adverse events.

Part 9 of this series of papers (Martin et al., 2016) reports the results from gene expression profiling of whole blood collected during the clinical study referred to in part 8 (Haziza et al., 2016). A whole-blood-derived gene signature that can distinguish smokers from either non-smokers or former smokers with a high degree of specificity and sensitivity has been described previously (Martin et al., 2015). The small signature, consisting of only 11 genes, was tested on the blood transcriptome of subjects enrolled in the clinical study as a complementary measure of exposure response. The signature performed remarkably well in predicting significant reduction in exposure response within just 5 days after subjects switched to THS2.2 or abstained from smoking. The blood transcriptomics profiling can therefore serve as a complementary measure of exposure response.

5. Conclusions

The data presented in this series of papers demonstrates that, compared with the 3R4F reference cigarette, both regular and mentholated versions of THS2.2 yield significantly reduced levels of HPHCs. This reduced formation of HPHCs by both versions of THS2.2 leads to a reduced toxicity, assessed both *in vitro* using assays for cytotoxicity and mutagenicity and *in vivo* in two distinct 90-day inhalation studies in rats. An important finding is that the reduced formation of HPHCs (measured under standardized machine smoking conditions) also leads to the reduced exposure when used *ad libitum* in a short-term clinical study conducted in adult smokers in a controlled environment.

We have previously reported on a systems toxicology study (step four of the MRTP assessment program) conducted in an animal model assessing the impact of THS2.2 on disease mechanisms (Lo Sasso et al., 2016b; Phillips et al., 2016; Titz et al., 2016). This study has shown that exposure to THS2.2, in comparison with 3R4F exposure, leads to a reduced exposure to HPHCs, which in turn leads to a reduced perturbation amplitude of disease-associated mechanisms as well as a reduced severity of disease endpoints *in vivo*. In addition, the effects of switching from 3R4F to THS2.2 were approaching those of cessation. Furthermore, we have previously reported on five *in vitro* systems toxicology studies conducted with THS2.2 in human primary cells. These studies were designed to compare the effects of 3R4F smoke with those of THS2.2 aerosol on key cellular toxicity endpoints (Gonzalez-Suarez et al., 2016), organotypic airway epithelium (Iskander et al., 2016b; Zanetti et al., 2016), as well as on mechanisms involved in vascular inflammation (Poussin et al., 2016)and endothelial dysfunction (van der Toorn et al., 2015b). The results of these studies showed that THS2.2 aerosol is less toxic than 3R4F smoke. Taken together, all these results show that THS2.2 has the potential to be an MRTP.

6. Outlook

The data presented in this series of papers and the previously published studies (Poussin et al., 2016; van der Toorn et al., 2015b; Gonzalez-Suarez et al., 2016; Phillips et al., 2016; Titz et al., 2016; Lo Sasso et al., 2016b; Iskandar et al., 2016b; Zanetti et al., 2016) are an essential component of our MRTP assessment program applied to THS2.2. The converging lines of evidence emerging from these study results show that THS2.2 has the potential to be a reduced-risk product. However, to confirm that THS2.2 is indeed a reduced-risk product we are conducting longer-term clinical studies, designed to quantify disease risk markers in addition to biomarkers of exposure. Future publications describing in more details the absence of combustion during THS2.2 use (i.e. that the aerosol produced is not smoke), further clinical studies, perception and behavior studies, and population impact modeling will be published elsewhere.

Conflict of interest statement

The work reported in all nine parts of this supplement involved a candidate Modified Risk Tobacco Product developed by Philip Morris International (PMI) and was solely funded by PMI. All authors are (or were) employees of PMI R&D or worked for PMI R&D under contractual agreements.

Transparency document

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