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PHILIP MORRIS INTERNATIONAL

SCIENTIFIC UPDATE FOR SMOKE-FREE PRODUCTS

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Issue 01 can be found here

This Scientific Update explains the science behind Philip Morris International's (PMI) approach to achieve a smoke-free future through a range of alternatives to cigarettes which do not burn tobacco. The following pages include our product development and assessment efforts, as well as our activities to share our methodologies and results. More detailed information can be found at www.pmiscience.com.

WHAT'S INSIDE?

A NOTE FROM OUR R&D DEPARTMENT HEADS

Even a product whose relative risk compared to cigarettes would be close to zero would not benefit public health unless smokers adopt it as a substitute for cigarettes. And providing smokers with a range of better solutions increases the likelihood that they will switch.

It is well known in the scientific community that the **main cause of the harm from smoking are the toxicants generated by the combustion of tobacco** and other materials contained in cigarettes. As the UK's National Institute for Health and Care Excellence (NICE) stated in its guidance on tobacco harm reduction: "It is primarily the toxins and carcinogens in tobacco smoke – not the nicotine – that cause illness and death." These toxicants can be significantly reduced or eliminated in products that do not burn tobacco.

For that reason, to reduce the harm from smoking, we are committed to developing alternative products that do not burn tobacco. We know, however, that this is not enough. **Even a product that would have relatively no risk compared to cigarettes would not benefit public health unless smokers adopt it as a substitute for cigarettes.** That's why non-burning alternatives have to be appealing and satisfying for smokers, replicating to the extent possible the taste, satisfaction and ritual characteristics associated with cigarettes.

"If you have a really safe product that nobody wants to use, that's no good because nobody switches."

Clive Bates, former head UK ASH, presenting his Harm Reduction Equation, below, at the E-Cigarette Summit (19 Nov 2013)

In addition, we know smokers have different preferences, and **providing them with a range of better solutions increases the likelihood that they will quit** cigarette smoking and switch to them.

Our portfolio of smoke-free products includes four products falling in two main categories: products that heat tobacco below the temperature required for burning; and others that do not use tobacco but either vaporize a nicotine-containing liquid or use other novel technologies to deliver an aerosol with nicotine and flavors.

Two of those products are already available to adult smokers in key cities in over 25 markets globally. *IQOS*, our first heated tobacco product to be commercialized, is delivering very promising results. 2 million smokers have already fully adopted *IQOS*. Of every smoker who purchased the device, **over half have completely stopped smoking and switched to *IQOS*.** By providing more smoke-free options and constantly improving them, we believe we can reach even more smokers.

This is why, besides working on new products and technologies, we are also investing in research to improve upon existing ones – for example, by improving e-cigarette technology (see page 5) and enhancing *IQOS* with a range of features, including connectivity, to encourage switching.

We are also making considerable progress in the scientific assessment of the potential of our smoke-free alternatives to present less risk of harm than continued smoking. We published online the scientific dossier for *IQOS* that we have submitted to regulatory authorities in line with the EU's Tobacco Product Directive and submitted both a Modified-Risk Tobacco Product Application (MRTPA) and a complementary pre-market tobacco product application (PMTA) to the U.S. Food and Drug Administration (FDA). We have begun or will soon be starting clinical trials across our full suite of platforms.

By the end of this year we expect to have commercial-tested our entire smoke-free product portfolio. To highlight the work that goes into developing a range of better products smokers can switch to, we are **focusing this issue on the development behind our latest commercialized smoke-free product: *MESH*.** In the following pages you will also find an overview of the latest results from our research, our most recent scientific publications, presentations made at scientific conferences and other events, and our latest regulatory milestones.

If you have any comments or questions regarding our Research & Development work, we look forward to hearing from you. Please see the last page for contact details.

Prof. Manuel C. Peitsch
Chief Scientific Officer

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Vice President, Technology & Operations RRP

UNDERLYING PRINCIPLES OF DEVELOPING SMOKE-FREE PRODUCTS

PMI's R&D program is designed to address the twin conditions of harm reduction. First, our aim is to develop products that are less harmful. Second, they must be sufficiently satisfying so that adult smokers switch to them. Our R&D program is designed to develop and assess products that will meet these conditions.

Achievement of the first objective depends on developing products that do not burn. It also means providing smokers a range of products that mimic many of the aspects they look for in a cigarette and provide a range of convenience benefits they do not have with cigarettes. Combined, these elements provide a better option for the many millions of adult smokers across the world who would otherwise continue to smoke.

REDUCING TOXICANTS THROUGH THE ABSENCE OF BURNING

The high temperature associated with the lit end of a cigarette, up to 900°C, leads to the breakdown of organic matter in tobacco into the thousands of chemicals found in smoke. Many of these chemicals are toxicants, the vast majority of which are the byproducts of combustion. The scientific community has recognized these combustion-related toxicants as the main cause of smoking-related diseases, such as lung cancer and heart disease.[1]

Importantly for our product development efforts, these toxicants can be significantly reduced or even eliminated if no combustion occurs. We are exploring different technologies to achieve that.

DESIGNING PRODUCTS THAT FACILITATE COMPLETE SWITCHING

The absence of combustion is not enough; smokers need to find familiar aspects in these alternatives. For example, research from leading cessation expert Professor Jed Rose of Duke University found that a satisfactory cigarette alternative should comprise both cigarette-like nicotine delivery and the sensorial and ritual aspects of smoking.[2] In fact, there are a variety of characteristics that smokers look for besides the nicotine experience, including taste, ritual, and the sensation of smoking.[3] It is unlikely that any manufacturer could successfully replicate or mimic all of these attributes in a single product. For this reason we are developing a range of products to allow smokers to choose the one that best suits their preferences.

ELIMINATING MOST HARMFUL CHEMICALS BY HEATING TOBACCO BELOW 400°C

The chemical reactions that occur at high temperatures in the lit end of a cigarette are the overwhelming cause of the harmful chemicals in tobacco smoke. In fact, such chemical reactions occur generally at an increasing rate as the temperature rises, with the vast majority of the harmful chemicals in tobacco smoke being produced during combustion. There may still be some chemical reactions occurring to a limited extent at lower temperatures. And below 300°C small amounts of other harmful chemicals naturally present in tobacco may be present as a result of distillation (the evaporation of a substance). However, at the operating temperatures of our heated tobacco products, the harmful chemicals found in tobacco smoke are reduced on average by 90-95%. Furthermore, two specific markers of combustion, namely carbon monoxide and nitrogen oxides, are reduced by approximately 98%.

REFERENCES

1 See, e.g., U.S. Surgeon General, The Health Consequences Of Smoking – 50 Years Of Progress, 627 (2014), https://www.surgeongeneral.gov/library/reports/50-years-of-progress/ ("Inhaling the complex chemical mixture of combustion compounds in tobacco smoke causes adverse health outcomes, particularly cancer and cardiovascular and pulmonary diseases...").

2 Rose et al, Pharmacologic and sensorimotor components of satiation in cigarette smoking, Pharmacol Biochem Behavior 76: 243-50 (2003), http://www.sciencedirect.com/science/article/pii/S0091305703002491. See also Royal College of Physicians, HARM REDUCTION IN NICOTINE ADDICTION: HELPING PEOPLE WHO CAN'T QUIT (2007), p223, http://www.rcplondon.ac.uk/publications/harm-reduction-nicotine-addiction; Shahab et al, Novel Delivery Systems for Nicotine Replacement Therapy as an Aid to Smoking Cessation and for Harm Reduction: Rationale, and Evidence for Advantages over Existing Systems, CNS DRUGS, 27:1007-19 (2013), http://rd.springer.com/article/10.1007%2Fs40263-013-0116-4.

3 See, e.g., Center for Substance Use Research, The Pleasure Of Smoking: The Views Of Confirmed Smokers, p.7 (Dec 2016), http://static1.1.sqspcdn.com/static/f/782462/27390673/1482788582747/CSUR_Pleasure_of_Smoking.pdf?token=E6AIOgD6VR2QXEGDLbUy7lEgBLo%3D Iskandar, A. R., I. Gonzalez-Suarez, S. Majeed, D. Marescotti, A. Sewer, Y. Xiang, P. Leroy, E. Guedj, C. Mathis, J. P. Schaller, P. Vanscheeuwijck, S. Frentzel, F. Martin, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2016). A framework for *in vitro* systems toxicology assessment of e-liquids. Toxicol Mech Methods 26(6): 389-413. (@PMI Science) (PMID: 27117495) - doi:10.3109/15376516.2016.1170251

OUR PRODUCT PORTFOLIO

HEATED TOBACCO PRODUCTS

One approach to significantly reduce the levels of toxicants is to heat tobacco to a temperature below that at which combustion occurs: less than 400°C. From a smoker acceptance standpoint, these products have the advantage of more closely approximating the taste, sensory satisfaction and ritual they are used to with cigarettes.

PRODUCTS WITHOUT TOBACCO

Another approach is to produce an aerosol without the use of tobacco. The ability to precisely design the composition of the originating substance leads to better control of the resulting aerosol. These platforms may be best suited for smokers who are not necessarily looking for the taste and sensory experience of tobacco.

PLATFORM 1

ELECTRICALLY HEATED TOBACCO PRODUCT *(EHTP)*

DESCRIPTION

An electronically controlled heating blade precisely heats a specially designed tobacco stick to temperatures below 350°C. The experience lasts six minutes or 14 puffs, similar to that of a cigarette.

ASSESSMENT PROGRESS

Our studies are very advanced and point in the direction of risk reduction. We have already completed numerous laboratory studies and eight clinical studies. An exposure response study designed to measure clinical risk markers when adult smokers switch to *EHTP* over a 12-month period is currently underway. Studies to verify understanding of risk communication and product use in real life conditions, as well as post market studies, are also well advanced, with some having been completed.

PLATFORM 2

CARBON-HEATED TOBACCO PRODUCT *(CHTP)*

DESCRIPTION

A carbon tip heat source precisely heats tobacco to a similar temperature to *EHTP*. The heat source is fully separated from the tobacco by a proprietary design.

ASSESSMENT PROGRESS

Our non-clinical and early clinical studies are progressing well and show results comparable to *EHTP*, including a five-day reduced exposure study. The clinical phase of a three-month reduced exposure study has been completed, with results expected by year-end.

PLATFORM 3

E-VAPOR PRODUCT

DESCRIPTION

Comprises products in which nicotine (a weak base) reacts with a weak organic acid to generate a respirable nicotine salt. We are exploring two routes for this platform, one with electronics and one without.

ASSESSMENT PROGRESS

Our non-clinical studies are progressing well, and we have completed a clinical study showing a comparable nicotine profile to cigarettes. A six-month clinical study will begin by the end of Q2.

PLATFORM 4

E-VAPOR PRODUCTS (COMMERCIALIZED UNDER VARIOUS TRADEMARKS)

DESCRIPTION

Battery-powered devices that vaporize a liquid nicotine solution (also known as e-cigarettes). Includes our new technology, *MESH*, designed to improve aspects such as product quality and consistency.

ASSESSMENT PROGRESS

The non-clinical assessment on our e-liquids is well advanced. For our *MESH* device, we are commencing clinical studies. An indoor air quality study on our e-vapor products demonstrates no negative impact on air quality.

OTHER DEVELOPMENTS

We continue to search for new technologies in the smoke-free product space. PMI's venture fund invests in entrepreneurs and growth companies with new solutions for products that have the potential to present less risk of harm than continued smoking.
Our Idea Submission Portal offers innovators an opportunity to provide technical solutions that can enhance our product portfolio.

The products depicted are subject to ongoing development and therefore the visuals are illustrative and do not necessarily represent the latest stages of product development.

RECENT MILESTONES IN PMI'S RESEARCH

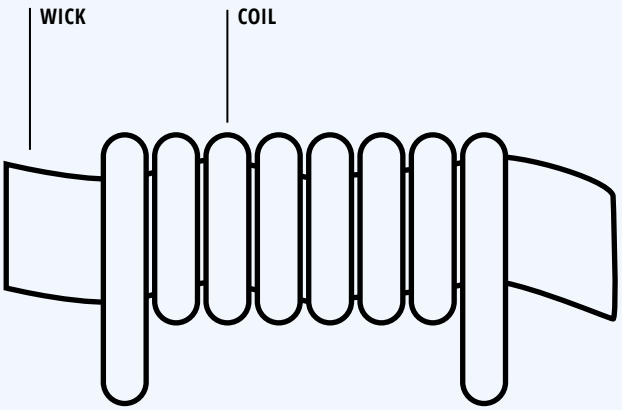
Focus on: *MESH*™ – Addressing Today's E-Vapor Challenges

PMI has been participating in the e-vapor (or e-cigarette) space since 2013. In that year we undertook a strategic initiative to commercialize U.S. based technology in the rest of the world. In 2014, we expanded our presence with the acquisition of *Nicocigs*, a leading e-cigarette company in the UK and its brand *Nicolites*. In parallel, we have been developing our R&D facility technologies to improve existing e-cigarette products, such as automating manufacturing to ensure higher product quality. This work resulted in *MESH*, a product we launched in a city test in Birmingham, UK, at the end of last year. Below we describe the efforts that went into developing this novel approach to e-vapor products, how we are ensuring high quality through manufacturing, and where we stand in terms of assessment of our products in the category.

AUTOMATION TO IMPROVE QUALITY AND CONSISTENCY

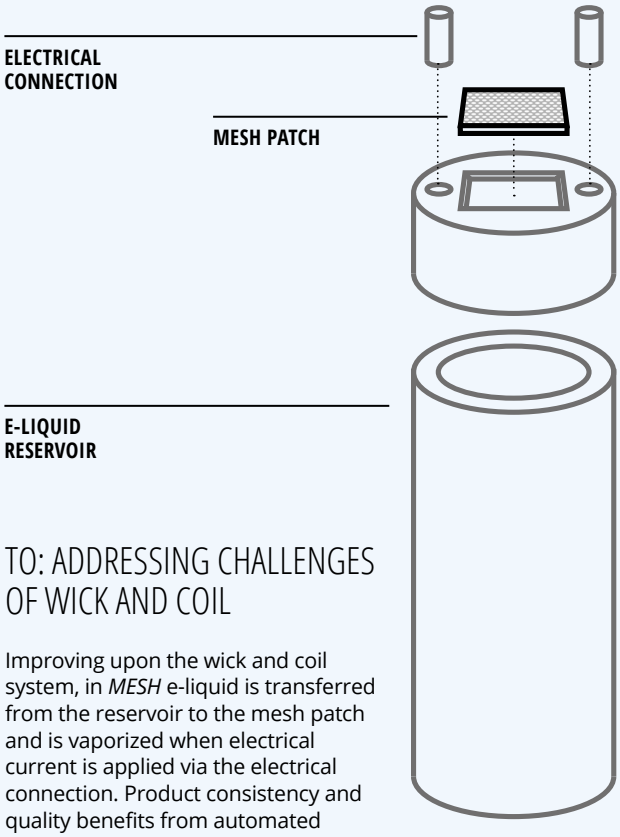
E-cigarette technology is based on a simple principle: vaporizing a liquid through exposure to heat. Virtually all of today's products accomplish this by exposing a moistened "wick" to a heated coil. The operation of these products is similar to the way a wet towel dries on a radiator. It is an effective yet simple means of delivering an aerosol. This technology at present, however, requires the wick to be threaded through a wound coil by hand. Though we currently commercialize this technology and believe it has strong potential to be less harmful than smoking, our other products with the potential to reduce risk compared to cigarette smoking are produced in automated manufacturing facilities where we can tightly control their production. One of our principle aims in developing the next generation of e-vapor technology was how to apply those same controls to e-vapor products.

The trick was to design a flat alternative to the original coil and wick technology that provided a comparable user experience to traditional e-cigarette products. By designing a flat coil, a machine could place a flat wick on top of it, inject e-liquid into an adjacent cavity, and neatly seal it all in a self-contained cartridge. Adapting techniques originally designed for fabrics, we were able to create a fine mesh patch through overlapping near-microscopic wires. This allowed the mesh patch to maximize the exposure to the wick with sufficient porosity for aerosol to pass through. We also introduced software to prevent the e-cigarette from functioning when the wick is dry. This is important, as puffing on a dry wick causes the formation of higher levels of some toxicants such as formaldehyde. While the level of such toxicants would still be much lower than in cigarette smoke, technology allows us to avoid this occurrence, often defined as "dry-puff". Our software maintains a consistent delivery until the liquid is finished, at which point the device switches off.



FROM: E-CIGARETTE "COIL AND WICK" DESIGN MUST BE ASSEMBLED BY HAND

Depiction of wick and coil design employed by present-day e-cigarettes. This design must be manually assembled.



TO: ADDRESSING CHALLENGES OF WICK AND COIL

Improving upon the wick and coil system, in *MESH* e-liquid is transferred from the reservoir to the mesh patch and is vaporized when electrical current is applied via the electrical connection. Product consistency and quality benefits from automated assembly.

ENSURING QUALITY THROUGH STRICT QUALITY CONTROLS & TESTING

Beyond the automated manufacturing controls developed for *MESH*, PMI wanted to ensure that all its e-vapor products – both liquids and devices – meet strict quality standards in practice. All our e-liquids are produced in one of our facilities in the European Union and are subject to stringent quality standards before being released into a market. This includes ensuring that all our ingredients are of high quality and Generally Recognized as Safe (GRAS) according to the U.S. FDA's list of food additives.

The nicotine in our e-liquids is pharmaceutical grade. Beyond ingredients, we also analyze emissions to ensure that the levels and numbers of harmful constituents, as well as the toxicity of the aerosol, are consistently and significantly below those found in cigarette smoke. With these controls in place, we are confident of the quality and consistency of every one of our e-vapor products.

ASSESSMENT TO DATE

To go beyond our quality controls and assess the health impact of these products, PMI is undertaking a thorough assessment of *MESH*, which incorporates both the e-liquid and the device itself. This comprises not only standard toxicology and clinical studies, but also PMI's newly developed systems toxicology approach for e-liquids and their aerosol.

As a starting point, we investigated the toxicity of the propylene glycol and vegetable glycerin that constitute the main components of e-liquid volume. Conducted with and without nicotine, a 90-day *in vivo* inhalation study showed no aerosol-related respiratory toxicity and only very mild nicotine-related effects that were expected given the very high doses that were administered. We complemented this study with another 90-day study conducted with a flavor mixture representative of all flavor variants. Results of this second study are currently being reviewed.

To explore in more detail the biological effects of e-liquids, we apply the systems toxicology approach we developed.[4] This approach uses large-scale automated measurements at physiological and molecular levels to investigate how these substances affect the mechanisms involved in smoking-related diseases. The first results of these studies, conducted with propylene glycol, vegetable glycerin and nicotine, show that the unflavored e-liquid is not more toxic than nicotine alone.[5]

Our non-clinical assessment of *MESH* is progressing, and our clinical assessment is expected to start with a pharmacokinetic study in 2017 and a reduced exposure study in 2018.

HIGH QUALITY ALTERNATIVES FOR SMOKERS

Through striving to apply the same rigor that we apply to our other smoke-free alternatives, PMI has been able to innovate and manufacture e-vapor products of very high and consistent quality. We are optimistic about the results expected from the range of assessments we are applying to our e-vapor products.

MESH ASSESSMENT PROGRAM

Strict Quality Controls: All ingredients of high purity and produced in-house in Europe. Quality checks on every product.

Standard Toxicology: Vapor formers show no respiratory toxicity. Flavor study complete, results being evaluated.

Systems Toxicology: Assessment framework for e-vapor products developed.

Clinical Studies: Program expected to start in Q3 with pharmacokinetic study; reduced exposure study anticipated.

REFERENCES

4 Iskandar, A. R., I. Gonzalez-Suarez, S. Majeed, D. Marescotti, A. Sewer, Y. Xiang, P. Leroy, E. Guedj, C. Mathis, J. P. Schaller, P. Vanscheeuwijck, S. Frentzel, F. Martin, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2016). A framework for *in vitro* systems toxicology assessment of e-liquids. Toxicol Mech Methods 26(6): 389-413. (@PMI Science) (PMID: 27117495) - doi:10.3109/15376516.2016.1170251

5 Gonzalez Suarez I, Marescotti D, Martin F, Scotti E, Guedj E, Acali S, Dulize R, Baumer K, Peric D, Frentzel S, Ivanov NV, Hoeng J and Peitsch MC (2017) In vitro systems toxicology assessment of non-flavored e-cigarette liquids in primary lung epithelial cells. Applied In Vitro Toxicology, 3:41-55.) - doi:10.1089/aivt.2016.0040

PEER-REVIEWED PUBLICATION HIGHLIGHTS

The following peer-reviewed publications reflect PMI's product assessment activities. They demonstrate our leading role in shaping the scientific approach to the product assessment of products with the potential to reduce the harm of smoking.

SYSTEMS TOXICOLOGY: FOUR STUDIES INCORPORATING 21ST CENTURY TOXICOLOGY INTO *IN VITRO*-BASED TESTING METHODOLOGIES

PMI recently published four papers that demonstrate the potential of novel *in vitro*-based testing procedures to help in replacing animal testing with 21st century toxicology methods. Both the U.S. National Research Council and the U.S. FDA have specified their vision for the replacement of animal studies with modern toxicological methodologies comprising *in vitro* procedures on human cells, followed by computational systems biology modelling to determine toxicological risk. PMI's studies looked at the biological impact of *EHTP* aerosol on human oral, nasal, bronchial and gingival epithelium tissue cultures.

These studies used human cells grown in three-dimensional culture systems. The cultures were grown on top of an artificial membrane at the air-liquid interface, allowing them to develop "organotypic" tissue complexity closely resembling conditions found in human tissue. Cultures were then exposed to either *EHTP* aerosol or cigarette smoke at various concentrations with comparable nicotine concentrations.

PMI's scientists assessed the biological impact of exposure over time, using a combination of well-established *in vitro* testing and measurement procedures and novel computational techniques. The adopted computational techniques involved the analysis of the collected data in the context of a set of literature-supported biological network models that are known to relate to respiratory disease, such as oxidative stress, osmotic stress and hypoxic stress.

Taken together, the results of the four studies demonstrate that the biological impact of *EHTP* aerosol is not significantly different from the air control exposure and much reduced when compared with the impact of cigarette smoke exposure. It was possible to induce findings of toxicity with *EHTP*, but only at significantly higher exposure concentrations of the aerosol than cigarette smoke. There were also no additional or new findings of toxicity following *EHTP* aerosol exposure when compared with the toxicity elicited following cigarette smoke exposure.

Zanetti, F., A. Sewer, C. Mathis, A. R. Iskandar, R. Kostadinova, W. K. Schlage, P. Leroy, S. Majeed, E. Guedj, K. Trivedi, F. Martin, A. Elamin, C. Merg, N. V. Ivanov, S. Frentzel, M. C. Peitsch and J. Hoeng (2016). Systems toxicology assessment of the biological impact of a candidate Modified-Risk Tobacco Product on human organotypic oral epithelial cultures. Chem Res Toxicol 29(8): 1252–1269. (@PMI Science) (PMID: 27404394) - doi:10.1021/acs.chemrestox.6b00174

Zanetti, F., B. Titz, A. Sewer, G. Lo Sasso, E. Scotti, W. K. Schlage, C. Mathis, P. Leroy, S. Majeed, L. O. Torres, B. R. Keppler, E. Ashraf, K. Trivedi, E. Guedj, F. Martin, S. Frentzel, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). Comparative systems toxicology analysis of cigarette smoke and aerosol from a candidate modified-risk tobacco product in organotypic human gingival epithelial cultures: A three-day repeated exposure study. Food Chem Toxicol 29(8): 1252-1269. (@PMI Science) (PMID: 28025120) - doi:10.1016/j.fct.2016.12.027

Iskandar, A. R., C. Mathis, F. Martin, P. Leroy, A. Sewer, S. Majeed, D. Kuehn, K. Trivedi, D. Grandolfo, M. Cabanski, E. Guedj, C. Merg, S. Frentzel, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). 3-D nasal cultures: systems toxicological assessment of a candidate modified-risk tobacco product. Altex 34: 23-48. (@PMI Science) (PMID: 27388676) - doi:10.14573/altex

Iskandar, A. R., C. Mathis, W. K. Schlage, S. Frentzel, P. Leroy, Y. Xiang, A. Sewer, S. Majeed, L. Ortega-Torres, S. Johne, E. Guedj, K. Trivedi, G. Kratzer, C. Merg, A. Elamin, F. Martin, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). A systems toxicology approach for comparative assessment: biological impact of an aerosol from a candidate modified-risk tobacco product and cigarette smoke on human organotypic bronchial epithelial cultures. Toxicol In Vitro 39: 29-51. (@PMI Science) (PMID: 27865774) - doi:10.1016/j.tiv.2016.11.009

PMI SHARES AWARD FROM PETA FOR ITS WORK TOWARDS PHASING OUT ANIMAL STUDIES

PMI and British American Tobacco (BAT) recently shared an award from the PETA International Science Consortium Ltd. for their contributions to research that can be used by scientists and regulators to predict adverse effects and protect human health using non-animal approaches. Dr Amy Clippinger, Associate Director of the PETA Consortium, noted that this research "not only provides a framework for toxicological strategies that do not use animals, they also unlock the potential for deeper levels of scientific understanding than could be achieved with animal tests." Details of the award are available at http://www.piscltd.org.uk/aop-prize/.

These studies have demonstrated not just the potential of *EHTP* to present less risk of harm in comparison with cigarettes, but also the ability to generate comprehensive and meaningful insights using complex human cell-based *in vitro* systems in combination with computational assessment strategies. These methods have important implications for industries such as pharmaceuticals and biotechnology, as well as for regulatory bodies responsible for the scientific oversight of chemicals, drugs and a range of consumer products.

CLINICAL STUDIES: PUBLICATION OF 90-DAY REDUCED EXPOSURE JAPAN STUDY FOR *EHTP*

PMI published findings from one of its two three-month studies to measure the extent to which smokers who switch to *EHTP* lower their exposure to harmful chemicals. The study was conducted by the well-known clinical research organization Osaki Hospital Tokyo Heart Center. Results showed that smokers who switched to *EHTP*:

- Reduced their exposure to 15 harmful chemicals to levels that approached those observed in smokers who abstained from smoking for the duration of the study.
- Showed improvements in clinical risk markers related to lung and heart disease. In all cases, the clinical risk markers improved in the same direction as seen in smokers who abstained from smoking. These clinical risk markers are being further tested through a longer-term study that is underway.
- Found the product satisfying and may completely switch to it, eliminating their use of cigarettes.

The research was conducted in line with internationally respected guidelines for clinical trials, such as Good Clinical Practice. The trial's 160 smokers were randomly divided into three groups: continued smoking, smoking abstinence, and completely switching to *EHTP*. After this, participants spent five days at a clinic and continued the study from home for an additional 85 days, during which time biological samples and measurements were regularly taken.

Lüdicke, F., P. Picavet, G. Baker, C. Haziza, V. Poux, N. Lama and R. Weitkunat (2017). Effects of switching to the Tobacco Heating System 2.2 menthol, smoking abstinence, or continued cigarette smoking on biomarkers of exposure: a randomized, controlled, open-label, multicenter study in sequential confinement and ambulatory settings (Part 1). Nicotine Tob Res, in press. (@PMI Science) (PMID: 28177489) - doi:10.1093/ntr/ntw287

Lüdicke, F., P. Picavet, G. Baker, C. Haziza, V. Poux, N. Lama and R. Weitkunat (2017). Effects of switching to the menthol Tobacco Heating System 2.2, smoking abstinence, or continued cigarette smoking on clinically relevant risk markers: a randomized, controlled, open-label, multicenter study in sequential confinement and ambulatory settings (Part 2). Nicotine Tob Res, in press. (@PMI Science) (PMID: 28177498) - doi:10.1093/ntr/ntx028

MULTI-STEP ASSESSMENT PROGRAM STUDIES: AN EVALUATION OF *EHTP*, FROM REDUCED FORMATION THROUGH CLINICAL CONFIRMATION AND SYSTEMS TOXICOLOGY

PMI published a series of nine publications describing our smoke-free assessment program and sharing results from the non-clinical assessment and initial clinical studies of *EHTP*, referred to in the papers as THS2.2. This includes studies demonstrating that the lack of combustion greatly reduces the formation of harmful and potentially harmful constituents (HPHCs) compared with cigarette smoke. *In vitro* and *in vivo* assessments of the aerosol reveal reduced toxicity and no new hazards. Additional mechanistic endpoints, measured as part of the *in vivo* studies, are described and confirm a reduced impact on smoking-related disease networks. A clinical study described in one of the papers confirmed the reduced exposure to HPHCs in smokers switching to *EHTP*. This research forms the core of our application to the U.S. FDA for *EHTP* as an MRTP.

Smith, M. R., B. Clark, F. Luedicke, J.-P. Schaller, P. Vanscheeuwijck, J. Hoeng and M. C. Peitsch (2016). Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the system and the scientific assessment program. Regul Toxicol Pharmacol 81 Suppl 2: S17-S26. (@PMI Science) (PMID: 27450400) - doi:10.1016/j.yrtph.2016.07.006 (For the remaining parts, see the bibliography below)

A GENE EXPRESSION SIGNATURE FOR CIGARETTE SMOKE EXPOSURE RESPONSE IN WHOLE BLOOD: FROM SIGNATURE IDENTIFICATION TO CLINICAL APPLICATION AND INDEPENDENT VERIFICATION

PMI scientists have previously identified a gene expression signature in whole blood that can distinguish current smokers from either non-smokers or former smokers with high specificity and sensitivity.[6] They then tested the small signature consisting of only 11 genes on the blood transcriptome of subjects enrolled in a five-day reduced exposure clinical study conducted in Poland.[7] The study showed a reduced exposure response in subjects that either stopped smoking or switched to *EHTP*, compared with subjects who continued smoking.[8] To verify the conclusions from such studies, it is important to conduct unbiased evaluations by independent third parties. This was achieved through crowdsourcing a computational challenge (sbvIMPROVER),[9] which aimed to evaluate computational methods for the development of blood-based gene expression signature classification models with the ability to predict smoking exposure status. Participants first created and trained models on blood gene expression datasets, including smokers and non-current smokers, and then applied their models to unseen datasets to predict whether subjects classify closer to smokers or non-smokers. The unseen datasets also comprised the data from the five-day reduced exposure clinical study conducted in Poland as well as a similar study conducted in Japan.[10] The results confirmed our own data analysis outcomes with regards to the classification of the clinical study participants. Furthermore, these studies confirmed a species-independent signature that can be applied across human and mouse blood samples.

Poussin C, Belcastro V, Martin F, Boué S, Peitsch MC and Hoeng J. (2017) Crowd-sourced verification of computational methods and data in systems toxicology: a case study with a heat-not-burn candidate modified-risk tobacco product. Chemical Research Toxicology, e-pub ahead of print. (PMID: 28085253) - doi: 10.1021/acs.chemrestox.6b00345.

REFERENCES

6 Martin F, Talikka M, Hoeng J and Peitsch MC (2015) Identification of gene expression signature for cigarette smoke exposure response - from man to mouse. Human and Experimental Toxicology, 34:1200-1211. (PMID: 26614807).

7 Haziza C, De La Bourdonnaye G, Skiada D, Ancerewicz J, Baker G, Picavet P and Luedicke F (2016) Evaluation of the Tobacco Heating System 2.2. Part 8: 5-day randomized reduced exposure clinical trial in Poland. Regulatory Toxicology and Pharmacology, 81 Suppl 2:S139-S150. (PMID: 27816672).

8 Martin F, Ivanov NV, Haziza C, Hoeng J and Peitsch MC (2016) Evaluation of the Tobacco Heating System 2.2. Part 9: Application of systems pharmacology to identify exposure response markers in peripheral blood of smokers switching to THS2.2. Regulatory Toxicology and Pharmacology, 81 Suppl 2:S151-S157. (PMID: 27845159).

9 https://sbvimprover.com/challenge-4/the-computational-challenge

10 Haziza C, de La Bourdonnaye G, Merlet S, Benzimra M, Ancerewicz J, Donelli A, Baker G, Picavet P and Luedicke F. (2016) Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: a randomized controlled study in confinement. Regulatory Toxicology and Pharmacology, 81:489-499. (PMID: 27693654).

LATEST EVENTS & OTHER MILESTONES

In 2016, PMI presented at 79 conferences around the world, at international, regional and local levels in a variety of areas. Below are some highlights since the last issue.

U.S. FDA: RISK ASSESSMENT OF TOBACCO PRODUCTS – A PUBLIC WORKSHOP 2016

HYATTSVILLE, MARYLAND, USA

15-16 NOVEMBER 2016

The U.S. FDA's Center for Tobacco Products hosted a workshop designed to open the discussion regarding available data and approaches to inform the risk assessment of tobacco products and to support tobacco regulatory science. The objectives of the workshop included identifying available and novel approaches to risk assessment methods to characterize exposures and health risks associated with tobacco. Participants included scientific and medical experts, academia, public health organizations, and government agencies. PMI's Julia Hoeng, Director Systems Toxicology, participated in a panel on Hazard Characterization along with academics and industry representatives.

Learn more about the event here:
http://www.fda.gov/TobaccoProducts/NewsEvents/ucm515442.htm

SOCIETY OF TOXICOLOGY ANNUAL MEETING 2017

The Society of Toxicology is the largest global gathering of toxicologists, with over 6,500 participants from more than 50 countries. This year PMI featured eight presentations covering studies from our standard and systems toxicology program. We also delivered an oral presentation on best practices for data sharing.

Learn more about PMI's presence at the event here: https://www.pmiscience.com/events/society-toxicology-2017

SOCIETY FOR RESEARCH ON NICOTINE & TOBACCO ANNUAL MEETING 2017

FLORENCE, ITALY

8-11 MARCH 2017

The Society for Research on Nicotine and Tobacco (SRNT) is the leading association focused on tobacco-specific research. The event features a transdisciplinary collection of cutting-edge science across the full spectrum of basic and applied research on nicotine and tobacco. This year PMI presented eight posters, including clinical studies, behavioral research and population modeling.

Learn more about PMI's participation at the event here: https://www.pmiscience.com/events/society-research-nicotine-and-tobacco-2017

The booth for PMI's crowd sourcing systems toxicology initiative "sbvIMPROVER" at EUROTOX.

EUROTOX 2016

SEVILLE, SPAIN

4-7 SEPTEMBER 2016

EUROTOX is a Federation of 34 European National Societies of Toxicology, with more than 7,000 members. At the EUROTOX 2016 Seville Congress, PMI presented 10 posters, which included a variety of standard and systems toxicology studies, as well as a study on indoor air quality where e-cigarettes are used and a 90-day reduced exposure study on *EHTP*.

Learn more about PMI's participation at the event here: https://www.pmiscience.com/events/eurotox-2016

R&D MILESTONES

REGULATORY DEVELOPMENT: PMI SUBMITTED MRTP AND PMT APPLICATIONS FOR *EHTP* TO THE U.S. FDA

On December 5, 2016, PMI submitted a Modified Risk Tobacco Product Application (MRTPA) for its *EHTP* (referred to as THS or the Tobacco Heating System in this application), to the U.S. FDA Center for Tobacco Products. The FDA has the authority to regulate tobacco products and to authorize claims of reduced, or "modified", risk and exposure. This filing is consistent with the company's stated goal of submitting its MRTPA in 2016. On March 30, 2017, PMI also submitted a Premarket Tobacco Product Application (PMTA) for *EHTP* to the FDA. A PMTA marketing order is a prerequisite to commercializing a New Tobacco Product in the U.S. A decision on the PMTA would allow the marketing of PMI's *EHTP* without any modified risk or exposure claims, and it would be independent of a decision on the MRTPA.

PMI MAKES EU SCIENTIFIC DOSSIER FOR *EHTP* PUBLICLY AVAILABLE

The European Union's updated Tobacco Product Directive requires manufacturers to submit a notification to the appropriate Member State authorities for each Novel Tobacco Product six months before placing the product on the market in that Member State. PMI has prepared a scientific dossier for *EHTP* which has been submitted to several EU member states in 2016, a redacted version of which has now been made publicly available on pmiscience.com. The required information includes a description of the product and its intended use, as well as available data regarding toxicity, addictiveness, attractiveness and consumer preferences. The scientific dossier and its appendices summarize the above based on PMI's scientific assessment program and include the results of that assessment program for *EHTP* as of May 2016.

The scientific dossier is available here:
https://www.pmiscience.com/news/platform-1%E2%80%99s-scientific-dossier-submitted-line-eu%E2%80%99s-tobacco-products-directive

POSTDOCTORAL FELLOWSHIP PROGRAM

PMI has launched a postdoctoral fellowship program to provide aspiring scientists an opportunity to work on interdisciplinary and innovative research projects that have a direct impact on the development of our smoke-free products. Based in the Cube, our main R&D center in Neuchatel, the fellows will conduct their respective research projects with the support and guidance of a mentor in their field of research. Fellows will have the opportunity to publish the outcome of their research in leading scientific publications and be part of major research conferences.

For more information and how to apply:
https://www.pmiscience.com/pmi-postdoctoral-fellowship-research-program

GLOSSARY

AEROSOL

A gaseous suspension of fine solid or liquid particles. Cigarette smoke is the result of combustion and contains carbon-based solid particles, whereas PMI's smoke-free products produce an aerosol either by heating or other technologies which do not involve combustion and do not contain solid particles. Both cigarette smoke and smoke-free emissions are aerosols, but with very different profiles. Given the lack of a term for a liquid-only aerosol to distinguish smoke-free emissions from smoke, we refer to the emissions of our smoke-free products as aerosol.

BIOMARKERS

Biomarkers can be classified into biomarkers of exposure and clinical risk markers.

- Biomarkers of exposure: indicate exposure to a potentially hazardous substance. In our case, the biomarker may be a cigarette smoke constituent or metabolite that is measured in a biological fluid or tissue and that can provide a measure of internal dose (i.e., the amount of the constituent taken up into the body).
- Clinical risk markers: a measurable biochemical, physiological, behavioral, or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease.

CLINICAL RISK MARKERS

See Biomarkers.

COMBUSTION

Combustion is the process of burning a substance in oxygen. 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. The combustion of tobacco results in the formation of smoke (which contains a range of chemical constituents), heat and ash. The high heat associated with combustion leads to the thermal breakdown of the tobacco when it is burned, resulting in the production of many of the toxicants found in cigarette smoke.

MODIFIED-RISK TOBACCO PRODUCTS (MRTPs)

The term used to classify potentially less harmful products by the U.S. Tobacco Control Act, which granted to the FDA authority to regulate tobacco products and to authorize claims of reduced risk.

PHARMACOKINETIC STUDIES

Measure how a substance, such as nicotine, is absorbed by the body. This helps in determining the extent to which adult smokers would find the alternative product an acceptable substitute for cigarettes, although other factors such as taste and product design are important elements in determining consumer acceptability. In addition to the kinetic profile of nicotine, we also monitor the safety of the users of the product under investigation (e.g., data on vital signs, clinical biochemistry and adverse events).

REFERENCE CIGARETTE (3R4F)

A standard cigarette for laboratory testing provided by the University of Kentucky. The current version is known as 3R4F and is used for non-clinical investigations by tobacco manufacturers, contract and government laboratories, and academic institutions.

STANDARD TOXICOLOGY

To compare whether the reduction in generated harmful chemicals reduces the toxicity of the aerosol generated by our smoke-free products, we perform a range of standard toxicological assays. For example, we have conducted a number of widely used *in vitro* assays comparing the toxicity of our smoke-free products to cigarette smoke. These include, but are not limited to:

- The Neutral Red Uptake cytotoxicity assay (measuring mammalian cell toxicity)
- The Ames bacterial mutagenicity assay (measuring bacteria cell mutations)
- The Mouse Lymphoma mammalian mutagenicity assay (measuring mutations in mammalian cells)

We have also conducted *in vivo* assays of different durations, including acute and repeated dose inhalation studies in accordance with Organization for Economic Co-operation and Development (OECD) Test Guidelines.

SYSTEMS TOXICOLOGY

Systems toxicology[11] integrates standard toxicology with advanced experimental and computational methods (including large-scale molecular measurements, imaging technologies, mathematical modeling and computational biology) to identify the biological mechanisms triggered by exposure to toxic substances and quantify their biological impact.

REFERENCES

11 Sturla, S. J., A. R. Boobis, R. E. Fitzgerald, J. Hoeng, R. J. Kavlock, K. Schirmer, M. Whelan, M. F. Wilks and M. C. Peitsch (2014). Systems toxicology: From basic research to risk assessment. Chem Res Toxicol 27(3): 314-329. (@PMI Science) (PMID: 24446777) - doi:10.1021/tx400410s

BIBLIOGRAPHY

ALL PUBLICATIONS IN 2016 AND 2017 YEAR TO DATE

ASSESSMENT OVERVIEW

Smith, M. R., B. Clark, F. Luedicke, J.-P. Schaller, P. Vanscheeuwijck, J. Hoeng and M. C. Peitsch (2016). Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the system and the scientific assessment program. Regul Toxicol Pharmacol 81 Suppl 2: S17-S26. (@PMI Science) (PMID: 27450400) - doi:10.1016/j.yrtph.2016.07.006

AEROSOL CHEMISTRY AND PHYSICS

Platform 1

1. Mitova, M. I., P. B. Campelos, C. G. Goujon-Ginglinger, S. Maeder, N. Mottier, E. G. R. Rouget, M. Tharin and A. R. Tricker (2016). Comparison of the impact of the Tobacco Heating System 2.2 and a cigarette on indoor air quality. Regul Toxicol Pharmacol 80: 91-101. (@PMI Science) (PMID: 27311683) - doi:10.1016/j.yrtph.2016.06.005

2. Nordlund, M. and A. K. Kuczaj (2016). Modeling aerosol formation in an electrically heated tobacco product. WASET Int J Chem Mol Nucl Mater Metall Eng 10(4): 326-338. (@PMI Science) - doi:scholar.waset.org/1999.2/10004053

3. Nordlund, M. and A. K. Kuczaj (2016). Modeling flow, heat and mass transfer in a porous biomass plug – when used in an electrically heated tobacco system. In Sixth International Conference on Porous Media and Its Applications in Science, Engineering and Industry, Eds, ECI Symposium Series. (@PMI Science) - http://dc.engconfintl.org/porous_media_vi/19/

4. Pratte, P., S. Cosandey and C. Goujon Ginglinger (2017). Investigation of solid particles in the mainstream aerosol of the Tobacco Heating System THS2.2 and mainstream smoke of a 3R4F reference cigarette. Hum Exp Toxicol, in press. (PMID: 27932538) - doi:10.1177/0960327116681653

5. Schaller, J. P., J. P. Pijnenburg, A. Ajithkumar and A. R. Tricker (2016). Evaluation of the Tobacco Heating System 2.2. Part 3: Influence of the tobacco blend on the formation of harmful and potentially harmful constituents of the Tobacco Heating System 2.2 aerosol. Regul Toxicol Pharmacol 81 Suppl 2: S48-S58. (@PMI Science) (PMID: 27793747) – doi:10.1016/j.yrtph.2016.10.016

Platform 4

1. Pratte, P., S. Cosandey and C. Goujon-Ginglinger (2016). A scattering methodology for droplet sizing of e-cigarette aerosols. Inhal Toxicol 28(12): 537-545. (@PMI Science) (PMID: 27644268) - doi:10.1080/08958378.2016.1224956

STANDARD TOXICOLOGY ASSESSMENT

Platform 1

1. Oviedo, A., S. Lebrun, U. Kogel, J. Ho, W. T. Tan, B. Titz, P. Leroy, G. Vuillaume, M. Bera, F. Martin, G. Rodrigo, M. Esposito, R. Dempsey, N. V. Ivanov, J. Hoeng, M. C. Peitsch and P. Vanscheeuwijck (2016). Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with mentholated and non-mentholated cigarette smoke. Regul Toxicol Pharmacol 81 Suppl 2: S93-S122. (@PMI Science) (PMID: 27818348) - doi:10.1016/j.yrtph.2016.11.004

2. Schaller, J.-P., D. Keller, L. Poget, P. Pratte, E. Kaelin, D. McHugh, G. Cudazzo, D. Smart, A. R. Tricker, L. Gautier, M. Yerly, R. R. Pires, S. Le Bouhellec, D. Ghosh, I. Hofer, E. Garcia, P. Vanscheeuwijck and S. Maeder Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. Regul Toxicol Pharmacol 81 Suppl 2: S27-S47. (@PMI Science) (PMID: 27720919) - doi:10.1016/j.yrtph.2016.10.001

3. Wong, E. T., U. Kogel, E. Veljkovic, F. Martin, Y. Xiang, S. Boue, G. Vuillaume, P. Leroyb, E. Guedj, G. Rodrigo, N. V. Ivanov, J. Hoeng, M. C. Peitsch and P. Vanscheeuwijck (2016). Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarette smoke. Regul Toxicol Pharmacol, in press. (@PMI Science) (PMID: 27793746) - doi:10.1016/j.yrtph.2016.10.015

SYSTEMS TOXICOLOGY ASSESSMENT

Platform 1

1. Gonzalez Suarez, I., F. Martin, D. Marescotti, E. Guedj, S. Acali, S. Johne, R. Dulize, K. Baumer, D. Peric, D. Goedertier, S. Frentzel, N. V. Ivanov, C. Mathis, J. Hoeng and M. C. Peitsch (2016). In vitro systems toxicology assessment of a candidate modified-risk tobacco product shows reduced toxicity compared to a conventional cigarette. Chem Res Toxicol 29 (1): 3-18. (@PMI Science) (PMID: 26651182) - doi:10.1021/acs.chemrestox.5b00321

2. Iskandar, A. R., C. Mathis, F. Martin, P. Leroy, A. Sewer, S. Majeed, D. Kuehn, K. Trivedi, D. Grandolfo, M. Cabanski, E. Guedj, C. Merg, S. Frentzel, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). 3-D nasal cultures: systems toxicological assessment of a candidate modified-risk tobacco product. Altex 34: 23-48. (@PMI Science) (PMID: 27388676) - doi:10.14573/altex

3. Iskandar, A. R., C. Mathis, W. K. Schlage, S. Frentzel, P. Leroy, Y. Xiang, A. Sewer, S. Majeed, L. Ortega-Torres, S. Johne, E. Guedj, K. Trivedi, G. Kratzer, C. Merg, A. Elamin, F. Martin, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). A systems toxicology approach for comparative assessment: biological impact of an aerosol from a candidate modified-risk tobacco product and cigarette smoke on human organotypic bronchial epithelial cultures. Toxicol In Vitro 39: 29-51. (@PMI Science) (PMID: 27865774) - doi:10.1016/j.tiv.2016.11.009

4. Kogel, U., B. Titz, W. K. Schlage, C. Nury, F. Martin, A. Oviedo, S. Lebrun, A. Elamin, E. Guedj, K. Trivedi, N. V. Ivanov, P. Vanscheeuwijck, M. C. Peitsch and J. Hoeng (2016). Evaluation of the Tobacco Heating System 2.2. Part 7: Systems toxicological assessment of a mentholated version revealed reduced cellular and molecular exposure effects compared with mentholated and non-mentholated cigarette smoke. Regul Toxicol Pharmacol 81 Suppl 2: S123-S138. (@PMI Science) (PMID: 27818347) - doi:10.1016/j.yrtph.2016.11.001

5. Lo Sasso, G., B. Titz, C. Nury, S. Boué, B. Phillips, V. Belcastro, T. Schneider, S. Dijon, K. Baumer, D. Peric, R. Dulize, A. Elamin, E. Guedj, A. Buettner, P. Leroy, S. Kleinhans, G. Vuillaume, E. Veljkovic, N. V. Ivanov, F. Martin, P. Vanscheeuwijck, M. C. Peitsch and J. Hoeng (2016). Effects of cigarette smoke, cessation and switching to a candidate modified-risk tobacco product on the liver in Apoe−/− mice – a systems toxicology analysis. Inhal Toxicol 28(5): 226-240. (@PMI Science) (PMID: 27027324) - doi:10.3109/08958378.2016.1150368

6. Phillips, B., E. Veljkovic, S. Boue, W. K. Schlage, G. Vuillaume, F. Martin, B. Titz, P. Leroy, A. Buettner, A. Elamin, A. Oviedo, M. Cabanski, E. Guedj, T. Schneider, M. Talikka, N. V. Ivanov, P. Vanscheeuwijck, M. C. Peitsch and J. Hoeng (2016). An eight-month systems toxicology inhalation/cessation study in Apoe-/- mice to investigate cardiovascular and respiratory exposure effects of a candidate modified-risk tobacco product, THS2.2, compared with conventional cigarettes. Toxicol Sci 149(2): 411-432. (@PMI Science) (PMID: 26609137) - doi:10.1093/toxsci/kfv243

7. Poussin, C., A. Laurent, M. C. Peitsch, J. Hoeng and H. De Leon (2016). Systems toxicology-based assessment of the candidate modified-risk tobacco product THS2.2 for the adhesion of monocytic cells to

human coronary arterial endothelial cells. Toxicology 339: 73-86. (@PMI Science) (PMID: 26655683) - doi:10.1016/j.tox.2015.11.007

8. Sewer, A., U. Kogel, M. Talikka, E. T. Wong, F. Martin, Y. Xiang, E. Guedj, N. V. Ivanov, J. Hoeng and M. C. Peitsch (2016). Evaluation of the Tobacco Heating System 2.2 (THS2.2). Part 5: microRNA expression from a 90-day rat inhalation study indicates that exposure to THS2.2 aerosol causes reduced effects on lung tissue compared with cigarette smoke. Regul Toxicol Pharmacol 81 Suppl 2: S82-S92. (@PMI Science) (PMID: 27866933) - doi:10.1016/j.yrtph.2016.11.018

9. Szostak, J., S. Boué, M. Talikka, E. Guedj, F. Martin, B. Phillips, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). Aerosol from Tobacco Heating System 2.2 has reduced impact on mouse heart gene expression compared with cigarette smoke. Food Chem Toxicol 101: 157-167. (@PMI Science) (PMID: 28111298) - doi:10.1016/j.fct.2017.01.013

10. Titz, B., S. Boue, B. Phillips, M. Talikka, T. Vihervaara, T. Schneider, C. Nury, A. Elamin, E. Guedj, M. J. Peck, W. K. Schlage, M. Cabanski, P. Leroy, G. Vuillaume, F. Martin, N. V. Ivanov, E. Veljkovic, K. Ekroos, R. Laaksonen, P. Vanscheeuwijck, M. C. Peitsch and J. Hoeng (2016). Effects of cigarette smoke, cessation and switching to two heat-not-burn tobacco products on lung lipid metabolism in C57BL/6 and Apoe-/- mice - an integrative systems toxicology analysis. Toxicol Sci 149(2): 441-457. (@PMI Science) (PMID: 26582801) - doi:10.1093/toxsci/kfv244

11. Zanetti, F., A. Sewer, C. Mathis, A. R. Iskandar, R. Kostadinova, W. K. Schlage, P. Leroy, S. Majeed, E. Guedj, K. Trivedi, F. Martin, A. Elamin, C. Merg, N. V. Ivanov, S. Frentzel, M. C. Peitsch and J. Hoeng (2016). Systems toxicology assessment of the biological impact of a candidate Modified-Risk Tobacco Product on human organotypic oral epithelial cultures. Chem Res Toxicol 29(8): 1252–1269. (@PMI Science) (PMID: 27404394) - doi:10.1021/acs.chemrestox.6b00174

12. Zanetti, F., B. Titz, A. Sewer, G. Lo Sasso, E. Scotti, W. K. Schlage, C. Mathis, P. Leroy, S. Majeed, L. O. Torres, B. R. Keppler, E. Ashraf, K. Trivedi, E. Guedj, F. Martin, S. Frentzel, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2017). Comparative systems toxicology analysis of cigarette smoke and aerosol from a candidate modified-risk tobacco product in organotypic human gingival epithelial cultures: A three-day repeated exposure study. Food Chem Toxicol 29(8): 1252-1269. (PMID: 28025120) - doi:10.1016/j.fct.2016.12.027

Platform 2

1. Elamin, A., B. Titz, S. Dijon, C. Merg, M. Geertz, T. Schneider, F. Martin, W. K. Schlage, S. Frentzel, F. Talamo, B. Phillips, E. Veljkovic, N. V. Ivanov, P. Vanscheeuwijck, M. C. Peitsch and J. Hoeng (2016). Quantitative proteomics analysis using 2D-PAGE to investigate the effects of cigarette smoke and aerosol of a prototypic modified-risk tobacco product on the lung proteome in C57BL/6 mice. J Proteomics 145: 237-245. (@PMI Science) (PMID: 27268958) - doi:10.1016/j.jprot.2016.05.037

Platform 4

1. Gonzalez Suarez I, Marescotti D, Martin F, Scotti E, Guedj E, Acali S, Dulize R, Baumer K, Peric D, Frentzel S, Ivanov NV, Hoeng J and Peitsch MC (2017) In vitro systems toxicology assessment of non-flavored e-cigarette liquids in primary lung epithelial cells. Applied In Vitro Toxicol, 3:41-55. (@PMI Science) (PMID: - doi:10.1089/aivt.2016.0040

CLINICAL TRIALS

Platform 1

1. Haziza, C., G. de La Bourdonnaye, S. Merlet, M. Benzimra, J. Ancerewicz, A. Donelli, G. Baker, P. Picavet and F. Lüdicke (2016). Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: A randomized controlled study in confinement. Regul Toxicol Pharmacol 81: 489-499. (@PMI Science) (PMID: 27693654) - doi:10.1016/j.yrtph.2016.09.014

2. Haziza, C., G. de La Bourdonnaye, D. Skiada, J. Ancerewicz, G. Baker, P. Picavet and F. Lüdicke (2016). Evaluation of the Tobacco Heating System 2.2. Part 8: Five-Day randomized reduced exposure clinical study in Poland. Regul Toxicol Pharmacol 81 Suppl 2: S139-S150. (@PMI Science) (PMID: 27816672) - doi:10.1016/j.yrtph.2016.11.003

3. Haziza, C., G. de La Bourdonnaye, D. Skiada, J. Ancerewicz, G. Baker, P. Picavet and F. Lüdicke (2017). Biomarker of exposure level data set in smokers switching from conventional cigarettes to Tobacco Heating System 2.2, continuing smoking or abstaining from smoking for five days. Data Brief 10: 283-293. (@PMI Science) (PMID: 27995164) - doi:10.1016/j.dib.2016.11.047

4. Lüdicke, F., G. Baker, J. Magnette, P. Picavet and R. Weitkunat (2017). Reduced exposure to harmful and potentially harmful smoke constituents with the Tobacco Heating System 2.1. Nicotine Tob Res 19: 168-175 (@PMI Science) (PMID: 27613951) - doi:10.1093/ntr/ntw164

5. Martin, F., M. Talikka, N. V. Ivanov, C. Haziza, J. Hoeng and M. C. Peitsch (2016). Evaluation of the Tobacco Heating System 2.2. Part 9: Application of systems pharmacology to identify exposure response markers in peripheral blood of smokers switching to THS2.2. Regul Toxicol Pharmacol 81 Suppl 2: S151-S157. (@PMI Science) (PMID: 27845159) - doi:10.1016/j.yrtph.2016.11.011

6. Picavet, P., C. Haziza, N. Lama, R. Weitkunat and F. Lüdicke (2016). Comparison of the pharmacokinetics of nicotine following single and ad libitum use of a Tobacco Heating System or combustible cigarettes. Nicotine Tob Res 18(5): 557-563. (@PMI Science) (PMID: 26438645) - doi:10.1093/ntr/ntv220

Platform 2

1. Lüdicke, F., C. Haziza, R. Weitkunat and J. Magnette (2016). Evaluation of biomarkers of exposure in smokers switching to a carbon-heated tobacco product: a controlled, randomized, open-label five-day exposure study. Nicotine Tob Res 18: 1606-1613. (@PMI Science) (PMID: 26817490) - doi:10.1093/ntr/ntw022

METHODS DEVELOPED FOR RRP ASSESSMENT

Aerosol Chemistry and Physics

1. Dossin, E., E. Martin, P. Diana, A. Castellon, A. Monge, P. Pospisil, M. Bentley and P. A. Guy (2016). Prediction models of retention indices for increased confidence in structural elucidation during complex matrix analysis: application to gas chromatography coupled with high-resolution mass spectrometry. Anal Chem 88(15): 7539-7547. (@PMI Science) (PMID: 27403731) - doi:10.1021/acs.analchem.6b00868

2. Frederix, E. M. A., M. Stanic, A. K. Kuczaj, M. Nordlund and B. J. Geurts (2016). Characteristics-based sectional modeling of aerosol nucleation and condensation. J Comput Phys 326: 499-515. doi:10.1016/j.jcp.2016.09.005

3. Frederix, E. M. A., A. K. Kuczaj, M. Nordlund, A. E. P. Veldman and B. J. Geurts (2017). Application of the characteristics-based sectional method to spatially varying aerosol formation and transport. J Aerosol Sci 104: 123-140. (@PMI Science) - doi:10.1016/j.jaerosci.2016.10.008

4. Kuczaj, A. K., M. Nordlund, S. Jayaraju, E. Komen, T. Krebs, M. C. Peitsch and J. Hoeng (2016). Aerosol flow in the Vitrocell 24/48 exposure system: flow mixing and aerosol coalescence. Appl In Vitro Toxicol 2(3): 165-174. (@PMI Science) - doi:10.1089/aivt.2016.0009

5. Mottier, N., M. Tharin, C. Cluse, J.-R. Crudo, M. G. Lueso, C. G. Goujon-Ginglinger, A. Jaquier, M. I. Mitova, E. G. R. Rouget, M. Schaller and J. Solioz (2016). Validation of selected analytical methods using accuracy profiles to assess the impact of a Tobacco Heating System on indoor air quality. Talanta, 158: 165-178. (@PMI Science, Infographic) (PMID: 27343591) - doi:10.1016/j.talanta.2016.05.022

6. Nordlund, M., M. Stanic, A. K. Kuczaj, E. M. A. Frederix and B. J. Geurts (2016). Improved PISO algorithms for modeling density varying flow in conjugate fluid--porous domains. J Comput Phys 306: 199-215. (@PMI Science) - doi:10.1016/j.jcp.2015.11.035

7. Stanic, M., M. Nordlund, E. M. A. Frederix, A. K. Kuczaj and B. J. Geurts (2016). Evaluation of oscillation-free fluid-porous interface treatments for segregated finite volume flow solvers. Comput Fluids 131: 169-179. doi:10.1016/j.compfluid.2016.03.019

PRE-CLINICAL TOXICOLOGY

1. Roemer, E., T. Meisgen, J. Diekmann, L. Conroy and R. Stabbert (2016). Heterocyclic aromatic amines and their contribution to the bacterial mutagenicity of the particulate phase of cigarette smoke. Toxicol Lett 243: 40-47. (PMID: 26724587) - doi:10.1016/j.toxlet.2015.12.008

SYSTEMS TOXICOLOGY

Concepts, Tools and Algorithms

1. Fluck, J., S. Madan, S. Ansari, A. T. Kodamullil, R. Karki, M. Rastegar-Mojarad, N. L. Catlett, W. Hayes, J. Szostak, J. Hoeng and M. Peitsch (2016). Training and evaluation corpora for the extraction of causal relationships encoded in biological expression language (BEL). Database, in press. (PMID: 27554092) - doi:10.1093/database/baw113

2. Hirschman, L., K. Fort, S. Boué, N. Kyrpides, R. Islamaj Doğan and K. B. Cohen (2016). Crowdsourcing and curation: perspectives from biology and natural language processing. Database 2016: baw115. (@PMI Science) (PMID: 27504010) - doi:10.1093/database/baw1115

3. Iskandar, A. R., I. Gonzalez-Suarez, S. Majeed, D. Marescotti, A. Sewer, Y. Xiang, P. Leroy, E. Guedj, C. Mathis, J. P. Schaller, P. Vanscheeuwijck, S. Frentzel, F. Martin, N. V. Ivanov, M. C. Peitsch and J. Hoeng (2016). A framework for *in vitro* systems toxicology assessment of e-liquids. Toxicol Mech Methods 26(6): 389-413. (@PMI Science) (PMID: 27117495) - doi:10.3109/15376516.2016.1170251

4. Luettich K, Talikka M, Lowe FJ, Haswell LE, Park J, Gaca MD and Hoeng J (2017) The Adverse Outcome Pathway for Oxidative Stress-Mediated EGFR Activation Leading to Decreased Lung Function free access. Applied In Vitro Toxicology, 3:99-109. - doi: 10.1089/aivt.2016.0032

5. Lowe FJ, Luettich K, Talikka M, Hoang V, Haswell LE, Hoeng J and Gaca MD (2017) Development of an Adverse Outcome Pathway for the Onset of Hypertension by Oxidative Stress-Mediated Perturbation of Endothelial Nitric Oxide Bioavailability free access. Applied In Vitro Toxicology, 3:131-148. - doi: 10.1089/aivt.2016.0031

6. Madan, S., S. Hodapp, P. Senger, S. Ansari, J. Szostak, J. Hoeng, M. Peitsch and J. Fluck (2016). The BEL information extraction workflow (BELIEF): evaluation in the BioCreative V BEL and IAT track. Database (Oxford) 2016: baw136. (@PMI Science) (PMID: 27694210) - doi:10.1093/database/baw136

7. Martin, F. (2017). Frustration and isoperimetric inequalities for signed graphs. Discrete Appl Math 217, Part 2: 276-285. (@PMI Science) - doi:10.1016/j.dam.2016.09.015

8. Hartung T, FitzGerald RE, Jennings P, Mirams GR, Peitsch MC, Rostami-Hodjegan A, Shah I, Wilks MF and Sturla SJ (2017) Systems Toxicology – Real world applications and opportunities. Chemical Research in Toxicology, in press. (PMID: 28362102) - doi:10.1021/acs.chemrestox.7b00003

9. Sauer, J. M., A. Kleensang, M. C. Peitsch and A. W. Hayes (2016). Advancing risk assessment through the application of systems toxicology. Toxicol Res 32(1): 5-8. (PMID: 26977253) - doi:10.5487/TR.2016.32.1.005

10. Szostak, J., F. Martin, M. Talikka, M. C. Peitsch and J. Hoeng (2016). Semi-automated curation allows causal network model building for the quantification of age-dependent plaque progression in ApoE-/- mouse. Gene Regul Syst Bio 10: 95-103. (PMID: 27840576) - doi:10.4137/GRSB.S40031

EXPERIMENTAL APPROACHES

1. Ansari, S., K. Baumer, S. Boue, S. Dijon, R. Dulize, K. Ekroos, A. Elamin, C. Foong, E. Guedj, J. Hoeng, N. V. Ivanov, S. Krishnan, P. Leroy, F. Martin, C. Merg, M. J. Peck, M. C. Peitsch, B. Phillips, W. K. Schlage, T. Schneider, M. Talikka, B. Titz, P. Vanscheeuwijck, E. Veljkovic, T. Vihervaara, G. Vuillaume and C. Q. Woon (2016). Comprehensive systems biology analysis of a sevenseven-month cigarette smoke inhalation study in C57BL/6 mice. Sci Data 3: 150077. (PMID: 26731301) - doi:10.1038/sdata.2015.77

2. Behrsing, H., H. Raabe, J. Manuppello, B. Bombick, R. Curren, K. Sullivan, S. Sethi, R. Phipps, Y. Tesfaigzi, S. Yan, C. D'Ruiz, R. Tarran, S. Constant, G. Phillips, M. Gaca, P. Hayden, X. Cao, C. Mathis, J. Hoeng, A. Braun and E. Hill (2016). Assessment of *in vitro* COPD models for tobacco regulatory science: Workshop proceedings, conclusions and paths forward for *in vitro* model use. Altern Lab Anim 44(2): 129-166. (@PMI Science) (PMID: 27256455) - (link to journal)

3. Lo Sasso, G., W. K. Schlage, S. Boue, E. Veljkovic, M. C. Peitsch and J. Hoeng (2016). The Apoe-/- mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. J Transl Med 14(1): 146. (@PMI Science) (PMID: 27207171) - doi:10.1186/s12967-016-0901-1

4. Marescotti, D., I. Gonzalez Suarez, S. Acali, S. Johne, A. Laurent, S. Frentzel, J. Hoeng and M. C. Peitsch (2016). High content screening analysis to evaluate the toxicological effects of harmful and potentially harmful constituents (HPHC). J Vis Exp 111: e53987. (@PMI Science) (PMID: 27228213) - doi:10.3791/53987

5. Marx, U., T. B. Andersson, A. Bahinski, M. Beilmann, S. Beken, F. R. Cassee, M. Cirit, M. Daneshian, S. Fitzpatrick, O. Frey, C. Gaertner, C. Giese, L. Griffith, T. Hartung, M. B. Heringa, J. Hoeng, W. H. de Jong, H. Kojima, J. Kuehnl, M. Leist, A. Luch, I. Maschmeyer, D. Sakharov, A. J. Sips, T. Steger-Hartmann, D. A. Tagle, A. Tonevitsky, T. Tralau, S. Tsyb, A. van de Stolpe, R. Vandebriel, P. Vulto, J. Wang, J. Wiest, M. Rodenburg and A. Roth (2016). Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. Altex 33(3): 272-321. (PMID: 27180100) - doi:10.14573/altex.1603161

6. Steiner, S., S. Majeed, G. Kratzer, J. Hoeng and S. Frentzel (2017). A new fluorescence-based method for characterizing *in vitro* aerosol exposure systems. Toxicol In Vitro 38: 150-158. (@PMI Science) (PMID: 27664316) - doi:10.1016/j.tiv.2016.09.018

7. Titz, B., K. Luettich, P. Leroy, S. Boue, G. Vuillaume, T. Vihervaara, K. Ekroos, F. Martin, M. C. Peitsch and J. Hoeng (2016). Alterations in serum polyunsaturated fatty acids and eicosanoids in patients with mild to moderate chronic obstructive pulmonary disease (COPD). Int J Mol Sci 17(9): 1583. (@PMI Science) (PMID: 27657052) - doi:10.3390/ijms17091583

VERIFICATION AND DATA SHARING

1. Namasivayam, A. A., A. F. Morales, A. M. Lacave, A. Tallam, B. Simovic, D. G. Alfaro, D. R. Bobbili, F. Martin, G. Androsova, I. Shvydchenko, J. Park, J. V. Calvo, J. Hoeng, M. C. Peitsch, M. G. Racero, M. Biryukov, M. Talikka, M. B. Perez, N. Rohatgi, N. Diaz-Diaz, R. Mandarapu, R. A. Ruiz, S. Davidyan, S. Narayanasamy, S. Boue, S. Guryanova, S. M. Arbas, S. Menon and Y. Xiang (2016). Community-reviewed biological network models for toxicology and drug discovery applications. Gene Regul Syst Bio 10: 51-66. (@PMI Science) (PMID: 27429547) - doi:10.4137/GRSB.S39076

2. Poussin, C., V. Belcastro, F. Martin, S. Boue, M. C. Peitsch and J. Hoeng (2017). Crowd-sourced verification of computational methods and data in systems toxicology: a case study with a heat-not-burn candidate modified-risk tobacco product. Chem Res Toxicol, in press. (@PMI Science) (PMID: 28085253) - doi:10.1021/acs.chemrestox.6b00345

CLINICAL RESEARCH

1. Weitkunat, R., G. Baker and F. Lüdicke (2016). Intention-to-treat analysis but for treatment intention: how should consumer product randomized controlled trials be analyzed? Int J Stats Med Res 5: 90-98. (@PMI Science) (link to journal)

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