PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

SCIENTIFIC UPDATE FOR SMOKE-FREE PRODUCTS

MARCH 2019 ● ISSUE 07
Past issues can be found here

This Scientific Update provides an overview of the most recent **scientific developments behind PMI's approach to achieving a smoke-free future** through a range of alternatives to cigarettes that do not burn tobacco.
The following pages include our **product development and assessment efforts, our initiatives to share** our methodologies and results, as well as independent research and government reports.
More detailed information can be found at www.pmiscience.com.

WHAT'S INSIDE:

IMPORTANT INFORMATION
This Scientific Update is issued for the purpose of publishing and disseminating scientific information and not for advertising or marketing purposes regarding tobacco or nicotine-containing products. The content of this Scientific Update is not and should not be regarded as an offer to sell, or a solicitation of an offer to buy, any product of PMI or its affiliates. The content in this Scientific Update is also not and should not be regarded as a promise, warranty, characterization or guarantee regarding any product of PMI or its affiliates.

INTRODUCTION

There is something called the streetlight effect. The story goes that a man is walking home at night, and he drops his keys along the way. Not remembering where he dropped his keys, he searches in the circle of light under a nearby streetlight, because this is the only place he can see the ground clearly.

Searching under the streetlight can make sense when it is applied to research – if great progress is being made using existing techniques and protocols, then the work should of course continue. But when we assess our smoke-free products, we often find that we need to make our own light and shine it where there is reason to believe the keys can be found.

In the case of our product development, we've even shined the light where we already knew the keys could be found. We've designed our electrically heated tobacco system (EHTS)[1] to avoid burning tobacco because the literature shows that heating tobacco to these lower temperatures reduces the number and levels of harmful chemicals generated and inhaled. We didn't just leave it at that assumption – we measured the levels of harmful chemicals in the aerosol, and we conducted additional studies on the tobacco and the aerosol just to make sure. We found that smoke is in fact not generated by EHTS, as reviewed in this issue.

Confident in these reduced levels of harmful chemicals, we've conducted extensive toxicity tests in the laboratory. As described in the feature article on page 6 of this issue, we employ some of the most advanced techniques available in the field of toxicology. We have even developed many new techniques uniquely suited to collecting and analyzing large and complex data sets, and shared them with the scientific community in the process for validation purposes. The sbv IMPROVER program is one such example.

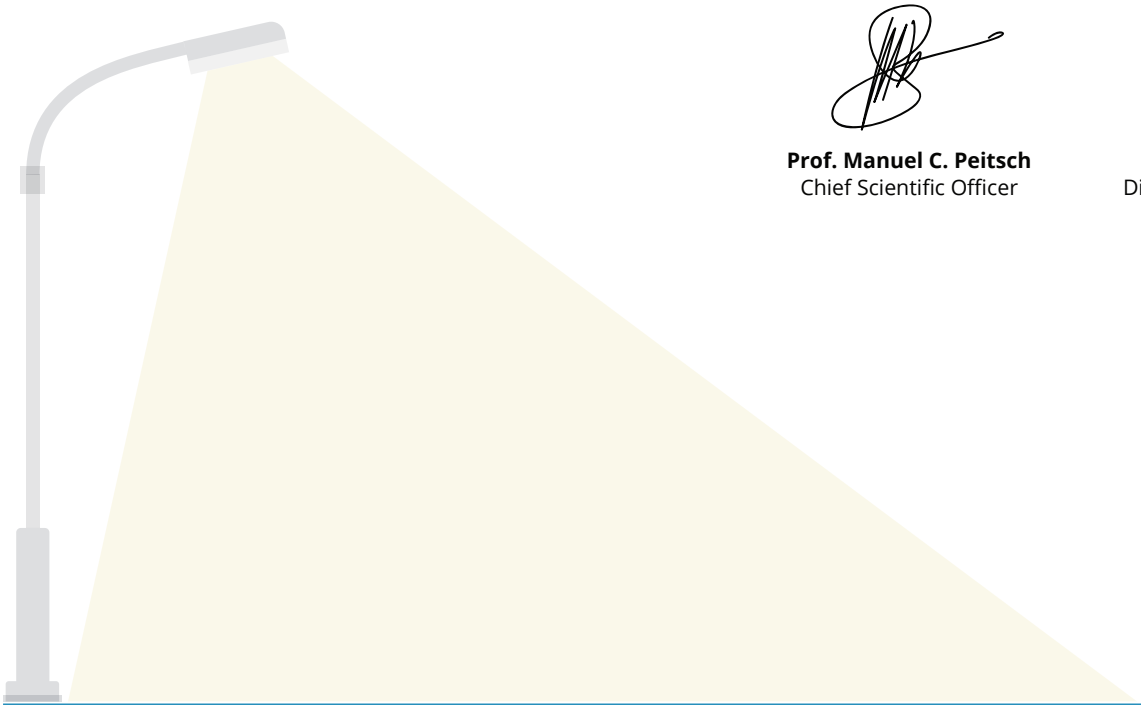
We've previously discussed the caliber of our clinical research, and we shared the results of our latest clinical study in December. We continue to hold those high standards for our clinical research and all the research we conduct here at PMI R&D.

It can seem complicated and overwhelming, at first glance, to see all the data and results we've collected as part of our assessment program. We didn't just make sure there was a significant reduction in levels of harmful chemicals. We made sure this would actually result in a significantly reduced toxicity in laboratory setting. Our goal is not to overwhelm, but to fully inform, as transparently and accurately as possible. We collect, analyze, and share this data with the scientific community because we take our job seriously.

We have a duty to be sure of our smoke-free products, a duty to conduct not just the easy or convenient studies but all the necessary ones and share our results. We have a duty to be confident when we say that these products can help current smokers lead better lives if they switch completely. And our latest count shows that 6.6 million people have agreed with that message by switching to EHTS.

Prof. Manuel C. Peitsch
Chief Scientific Officer

Dr. Julia Hoeng
Director System Toxicology



1 The EHTS is not authorized for commercialization in the US. Outside the US, the EHTS is commercialized under the *IQOS* brand.

ASSESSMENT PROGRESS OF OUR PRODUCT PORTFOLIO

HEATED TOBACCO PRODUCTS

One approach to significantly reducing the levels of emitted and inhaled toxicants is to heat tobacco (instead of burning it) to temperatures well below 400°C – the temperature where combustion can occur. Our heated tobacco products closely approximate the taste, sensory satisfaction, and ritual of cigarettes and therefore have the potential to be acceptable for people who would otherwise continue to smoke but are interested in switching to better alternatives.

PLATFORM 1
ELECTRICALLY HEATED TOBACCO SYSTEM (EHTS, REFERRED TO AS TOBACCO HEATING SYSTEM [THS] IN RESEARCH)

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, whichever comes first, similar to that of a cigarette. This device also exists in a version supporting consecutive uses without recharging between experiences.

ASSESSMENT PROGRESS

We have conducted 18 non-clinical and 10 clinical studies for this platform with results consistently showing that, whilst not risk-free, the EHTS presents less risk of harm compared to continuing to smoke. We also completed an oral health study, with the last patient completing the study in December last year, and we plan to share the top line results in 2019.

PLATFORM 2
CARBON-HEATED TOBACCO PRODUCT (CHTP)

DESCRIPTION

A carbon heat source heats the tobacco to temperatures below 350°C. The heat source is fully separated from the tobacco by a proprietary design to prevent the tobacco from burning.

ASSESSMENT PROGRESS

The non-clinical assessment on CHTP is well advanced. The results of our pharmacokinetic study and our reduced exposure studies indicate that CHTP could be an acceptable substitute for adult smokers who seek an alternative to cigarettes. The reduced exposure study showed a substantial reduction in relevant biomarkers of exposure to the measured harmful chemicals in those who switched to Platform 2 compared to those who continued to smoke during the study. We will share the conclusions in scientific forums and submit them for inclusion in peer-reviewed journals in 2019.

PRODUCTS WITHOUT TOBACCO

Another approach to reduce the levels of toxicants emitted by novel products is to produce a nicotine-containing aerosol without the use of tobacco. We precisely design the composition of the aerosol-producing components and the conditions of the aerosol generation. This provides control over the resulting aerosol in terms of quality and consistency. These platforms may be best suited for people who smoke and are open to different taste and sensory experiences, or are already using e-vapor products.

PLATFORM 3
E-VAPOR PRODUCT USING NICOTINE SALT

DESCRIPTION

Includes products in which nicotine (a weak base) reacts with a weak organic acid to generate a respirable nicotine salt. We have explored two routes for this platform: one with electronics and one without.

ASSESSMENT PROGRESS

We finished the clinical phase of a nicotine pharmacokinetic study for the version without electronics, and the associated report was finalized in 2018. The results indicate that Platform 3 has the potential to be an acceptable alternative to continued cigarette smoking in terms of product satisfaction. We will also initiate a clinical product use and adaptation study.

PLATFORM 4
E-VAPOR PRODUCTS (COMMERCIALIZED UNDER VARIOUS TRADEMARKS)

DESCRIPTION

Battery-powered devices that vaporize a liquid nicotine solution (also known as e-cigarettes). Included among our Platform 4 products is our proprietary *MESH* technology designed to improve the quality and consistency of the generated aerosol, and increasing the content delivery and avoiding "dry puffing".

ASSESSMENT PROGRESS

The non-clinical assessment on our e-liquids is well advanced. The results of our recent nicotine pharmacokinetic study indicate that MESH products are an effective means of nicotine delivery while being a satisfying e-vapor product. We will also initiate a clinical reduced exposure study to measure selected biomarkers of exposure to harmful chemicals and assess changes in clinical risk markers.

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.

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 continuing to smoke. Our *Idea Submission Portal* offers innovators an opportunity to provide technical solutions that can enhance our product portfolio.

PMI STEP BY STEP ASSESSMENT PROGRAM

To learn more about the steps of our assessment program, please visit pmiscience.com Colored blocks indicate progress completed.

NEW LONG-TERM ASSESSMENT TOOLS OFFICIALLY LAUNCHED

Many health authorities recognize that risks associated with smoke-free products lie somewhere between cigarettes and cessation. This leads to the conclusion of tobacco harm reduction: making smoke-free products available to current smokers who would otherwise continue to smoke could reduce individual and population harms. But researchers are still missing the tools to learn how people – consumers – understand those risks, and the choices they make as a result.

This is why a team of PMI scientists led by Dr. Christelle Chrea, Manager Behavioral Science, developed and launched the ABOUT™ toolbox. The ABOUT™ toolbox contains a set of surveys, also known as self-report instruments, that are specially developed to measure how people think about or use smoke-free products compared to other nicotine or tobacco-containing products. Accordingly, ABOUT stands for **a**ssessment of **b**ehavioral **ou**tcomes related to **t**obacco and nicotine products.

Ultimately, the toolbox will cover five different categories of surveys: Perceived Risk, Use History, Product Experience, Dependence, and Health and Functioning. The first available instrument, called ABOUT™ – Perceived Risk, measures peoples' perceptions of the risks associated with a range of tobacco and nicotine-containing products.

The development of the toolbox is detailed in an article in F1000Research, an open access journal whose peer review process is open to the public. The article is still undergoing peer-review but is already available for anyone to read.[2] We have also already published two papers related to the instrument ABOUT™ – Perceived Risk,[3,4] and will keep our readers updated as more news on this exciting toolbox becomes available.

Researchers can explore the ABOUT™ Toolbox here.

ABOUTToolbox
Assessment of Behavioral OUtcomes related to Tobacco and nicotine products

2 Chrea et al. Developing fit-for-purpose self-report instruments for assessing consumer responses to tobacco and nicotine products: the ABOUT™ Toolbox initiative.[version 1; referees: awaiting peer review]. *F1000Research*. 2018. 7:1878. (Link)
3 Cano et al. Development and validation of a new instrument to measure perceived risk associated with the use of tobacco and nicotine-containing products. *Health Quality of Life Outcomes*. 2018. 16(1):192. (Link)
4 Salzberger et al. Perceived risks associated with the use of tobacco and nicotine-containing products: Findings from qualitative research. *Tobacco Science & Technology*. 2017. 50(13):32-42. (Link)

HOW EHTS AVOIDS CREATING SMOKE

Scientists know that the greatest cause of harm from cigarettes stems from the combustion of the tobacco, which creates the high level and number of harmful chemicals, as well as the solid particles found in cigarette smoke. So, our approach to build alternative products to cigarettes is based on minimizing the creation of those harmful components. How would this be done? By not burning the tobacco.

This is how we developed our smoke-free product portfolio. We created EHTS to heat specially designed tobacco consumables to temperatures far below tobacco's burning point. Then, we conducted several studies to ensure that our product met the expectations we set for it: that it doesn't burn tobacco, it doesn't create smoke, and thus doesn't expose bystanders to secondhand smoke.

This shouldn't be news to most readers, because we've been vocal about these conclusions for a while now. But, since our scientists recently published all the evidence together in one convenient location,[5] it seems an opportune time to discuss.

THERE IS NO BURNING IN EHTS

EHTS contains an electronically controlled heating blade that heats the tobacco stick at the same time as it measures the temperature of the tobacco. The temperature of the tobacco remains below 350 °C, so it doesn't even come close to burning. We've seen that the aerosol EHTS produces is comparable in an oxygen-containing environment as what it produces in an all-nitrogen environment, where burning is impossible.[6]

We've also studied the contents of the tobacco stick after use, finding that only a tiny portion of the tobacco close to the heater experiences certain heat-related processes like torrefaction or low-temperature pyrolysis. None of these findings indicated that the tobacco was burnt. Most of the tobacco only experiences drying and evaporation. These are the processes that allow EHTS to produce a satisfying nicotine-containing aerosol with lower levels of the harmful chemicals found in smoke produced by burning tobacco.

EHTS DOESN'T CREATE SMOKE

Smoke is an aerosol, but not all aerosols are smoke. Smoke is created by burning or high-temperature pyrolysis, but neither of these processes happens when EHTS heats specially designed tobacco sticks. So, by definition, EHTS doesn't create smoke. Even so, we conducted studies that demonstrate this fact.

By defintion, smoke contains carbon-based nanoparticles that are created by burning, while the aerosol of EHTS does not. To demonstrate this fact, we used an experimental setup consisting of a thermodenuder (a heated tube to remove the more easily evaporated species in smoke/aerosols), and collected samples that were analyzed by electron microscopy and X-ray to study what elements were present in the remaining particles. This work[7] showed that, while smoke from a single cigarette contains approximately one trillion (10^{12} in scientific notation) solid carbon-based particles with a median diameter of 75 nm, the aerosol of EHTS does not contain any solid particles. This is yet another piece of evidence showing that the EHTS does not produce smoke.

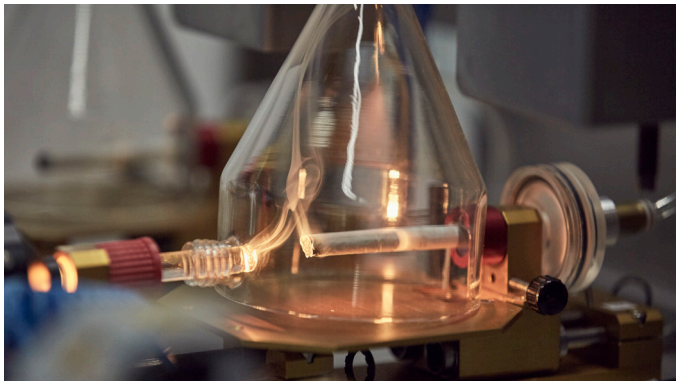
Smoke also contains high levels of many harmful chemicals, including carbon monoxide, formaldehyde, and benzene, as examples. The aerosol generated by EHTS contains significantly fewer harmful chemicals, and the levels of the harmful chemicals that are present are reduced by an average of 95% compared to those found in cigarette smoke.[8,9] Many independent researchers who studied the composition of the aerosol generated by EHTS have come to similar conclusions.[10,11,12]

EHTS DOESN'T CREATE SECONDHAND SMOKE

If EHTS doesn't create smoke, then clearly it cannot create secondhand smoke either. Even so, we investigated whether EHTS impacts the environment, to gather data providing the scientific support for evidence-based policy-making. We conducted studies in a dedicated air quality assessment room with adequate ventilation, and found that only three compounds (nicotine, acetaldehyde, and glycerol) could be detected above background levels when EHTS was in use. Glycerol isn't considered to be an air pollutant, and the concentrations of the other two chemicals were much lower than the permissible limits set in existing air quality guidelines.[13]

To better understand the impact of EHTS in a real-life setting, we conducted a study in collaboration with a restaurant in Japan where EHTS use, but not cigarette use, was allowed.[14] We found that EHTS use in the properly ventilated space had no negative impact on either the bystanders' exposure levels or the air quality in the restaurant. We have since conducted similar studies in a nightclub and a catering establishment and obtained similar results.

Importantly, although EHTS is not risk-free, and delivers nicotine, which is addictive, the facts explained here are an important part of the totality of evidence that shows EHTS presents less risk of harm for smokers who switch completely rather than continue smoking.



5 View the full summary on PMIScience.com (Link)
6 Mc Grath. What is combustion and why is the absence of combustion important for heat not burn products. Presentation, GFN 2017. (Link)
7 Pratte et al. Investigation of solid particles in the mainstream aerosol of the Tobacco Heating System THS2.2 and mainstream smoke of a 3R4F reference cigarette. *Human & Experimental Toxicology*, 36(11):1115–1120. (Link)
8 Values obtained using the Health Canada Intense Puffing Regime. Link to data on PMIScience.com: (Link)
9 Schaller et al. Evaluation of the Tobacco Heating System 2.2 Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. *Regulatory Toxicology and Pharmacology*. 2016. 81(S2):S27-S47. (Link)
10 Li et al. Chemical Analysis and Simulated Pyrolysis of Tobacco Heating System 2.2 Compared to Conventional Cigarettes. *Nicotine & Tobacco Research*, 2018. 21(1):111-118. (Link)
11 Bekki et al. Carbonyl Compounds Generated from Electronic Cigarettes. *International Journal of Environmental Research and Public Health*, 2014. 11(11): 11192-11200. (Link)
12 Mallock et al., Levels of selected analytes in the emissions of "heat not burn" tobacco products that are relevant to assess human health risks. *Archives of Toxicology*, 2018. 92(6):2145-2149. (Link)
13 Mitova et al. Comparison of the impact of the Tobacco Heating System 2.2 and a cigarette on indoor air quality. *Regulatory Toxicology and Pharmacology*. 2016. 80:91-101. (Link)
14 Information on this study can be found on PMIScience.com (Link)

RECENT MILESTONES IN PMI'S RESEARCH

FOCUS ON SYSTEMS TOXICOLOGY: DRAWING CONCLUSIONS FROM A MOUNTAIN OF DATA

TELL US ABOUT YOUR WORK

The systems toxicology group is made of researchers in chemistry, biology, physics, and computational science. All these researchers examine how exposure leads to disease by studying each step in the causal chain of events. And one busy person is trying to manage it all.

I've spent some time working in many of those disciplines during my career, and I love that I can rely on all those experiences in my work at PMI. Our team works together to build new approaches to product assessment, and these approaches give us a highly detailed picture of how the body responds when it is exposed to certain stimuli. With the combined effort of our team and others in the field of toxicology, testing strategies are radically transforming to become more effective, more efficient, and more reliable.

Rather than taking the standard approach of simply understanding whether something is toxic and at what dose, we dig deeper to see how exposure leads to toxicity or disease outcomes. We're interested in learning what systems of the body are affected, and how they're affected by exposure.

To do this, we are developing and supporting new technologies and research techniques, like organs-on-a-chip, 3D cell cultures, and high-performance computing. The INTERVALS.science platform gives researchers access to industry data and the methods behind the published results. We are also creating community engagement initiatives to advance the field of toxicology, like the sbv IMPROVER program that encourages crowdsourcing approaches to verifying research methods used in industry.

All this work begins within the context of product assessment for smoke-free products, but it is relevant to other research too. Like nutraceuticals, pharmaceuticals, nanoparticle toxicity, environmental studies on chemicals and pesticides, and more.

SYSTEMS TOXICOLOGY IN SIMPLE WORDS

Systems toxicology combines standard toxicology methods with new and diverse techniques that give us a bigger and more detailed picture of how toxic substances affect the body. Standard toxicological tests for products are required before they can be allowed on the market so that regulators can learn how those products compare to others. These tests are about reporting whether or not something is toxic and just how toxic it is, but they don't provide the reason why the exposure leads to that observation.

These tests are important, because they support estimations of the relative toxicity of the products in question compared with the toxicity of the reference product. These standard tests are also a foundation we can build on using systems toxicology approaches. These new approaches incorporate more quantitative analyses that examine all the observations from more scientific angles. They focus on the impact of chemicals on cells to organs to a whole organism.

JULIA HOENG
DIRECTOR OF SYSTEMS TOXICOLOGY AT PHILIP MORRIS INTERNATIONAL RESEARCH & DEVELOPMENT

After earning her PhD at Cambridge University in 2007, Julia joined PMI R&D to lead a cross-functional team to define biomarkers to be used for clinical assessment. She has continued to lead multidisciplinary research throughout her career at PMI.

Our stepwise Systems Toxicology approach has been successfully used for *in vitro* and *in vivo* studies to assess smoke-free products. It builds a holistic understanding of the impact of the exposure, often helping us to discover new mechanisms along the way.

1. **DESIGN AND CONDUCT A SYSTEMS TOXICOLOGY EXPERIMENT.**
2. **COLLECT BIOLOGICAL SAMPLES, MEASURE THE RESPONSE OF THE SYSTEM.**
3. **IDENTIFY OR DEFINE THE BIOLOGICAL MECHANISMS** *that are affected by the exposure.*
4. **QUANTIFY THE EFFECT OF THE EXPOSURE** *on each biological mechanism by computing a Network Perturbation Amplitude (NPA) score.*
5. **QUANTIFY THE OVERALL BIOLOGICAL IMPACT** *caused by exposure by combining the NPA scores: the Biological Impact Factor (BIF).*

All this work results in terabytes of data that are used to map the chain of events inside the body that are set off by exposure. Accordingly, we have developed high performance computing infrastructure to store and analyze that data. Researchers working on all applications of systems toxicology, not just tobacco, have been searching for successful ways to work with such giant blocks of data. So, we share our data and methods transparently, not just because it's in our best interests, but also because it fills that important need in the scientific community.

RESULTS ON EHTS

The totality of our evidence shows that switching completely to EHTS presents less risk to health than continuing to smoke cigarettes. Our toxicology findings[15] indicate that EHTS aerosol causes minimal or even no toxic effects compared to cigarette smoke. These results were very consistent across different mechanisms of disease causation including inflammation, cell stress and growth, tissue repair, and cell fate.

The mechanistic findings were remarkably consistent across animal studies conducted in vivo and studies conducted with human-derived cells and organotypic tissues in vitro. All the evidence from the systems toxicology assessment studies is consistent with the hypothesis that reduced exposure, as seen with switching from cigarette smoke to EHTS aerosol, leads to reduced toxicity, which, in turn, leads to reduced risk of harm and tobacco-related disease. Furthermore, we did not detect any new toxicological effect with THS, in either classical or systems toxicology studies.

In the few tests where there is some measured toxicity, it's when the concentration of EHTS aerosol is much higher than cigarette smoke – generally at twice the nicotine concentration compared to that delivered by cigarette smoke. Our *in vitro* work has also shown that EHTS doesn't discolor teeth as badly as cigarettes,[16] minimally affects oral health,[17] and doesn't disrupt the function of mitochondria.[18]

Our findings[19] also indicate that there is no increase in atherosclerosis nor development of emphysema in mice who were switched from cigarette smoke exposure to EHTS aerosol. What I find really striking is that these results are comparable to the results of the cessation group, a result that agrees with the clinical studies from our product assessment program. We also saw only mild toxicity, no lung inflammation or emphysema, and no increases in lung tumor development in mice with lifelong exposure to EHTS aerosol compared to lifelong exposure to cigarettes.[20]

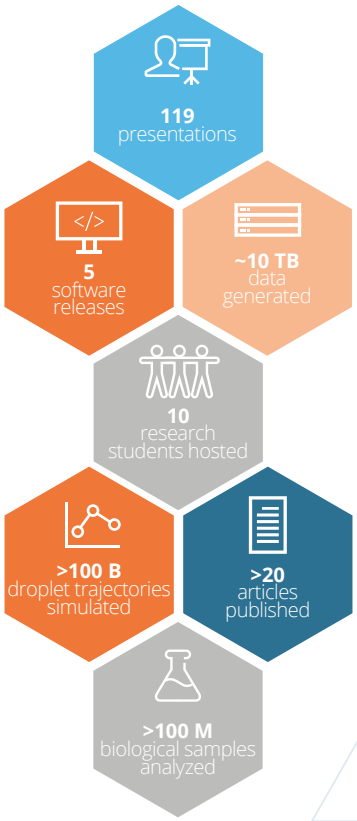
And don't think we're focusing only on EHTS – we're performing many of these studies on each of our four platforms.

WHY USE SYSTEMS TOXICOLOGY METHODS?

The range of disciplines and the sheer size of the data we collect can be intimidating, but it's necessary to give us the highly detailed picture we're looking for. We gain information on what dose makes a substance toxic, what chemical interactions occur, how a chemical interacts with DNA, or how it impacts the immune system, just to name a few examples. All this information helps us to learn more about the biological systems we're studying, not just the products being tested.

The standard toxicology tests that are required for tobacco products includes studies on rodents. Despite these requirements, we were recognized by PETA (People for the Ethical Treatment of Animals) in 2017 for our work toward replacing animal testing protocols,[21] and continue to work toward non-animal assessment approaches to toxicology.[22] We follow the 3Rs guidelines: **R**eplacement of methods that require animal research, **R**eduction of the number of animals needed to obtain robust results, and **R**efinement of methods to enhance animal welfare.

STATISTICS FROM 2018



15 Our Findings on PMIScience.com (Link)
16 Zhao et al. Effects of cigarette smoke on color stability of dental resin composites. *American Journal of Dentistry*. 2018. 30(6):316-322. (Link)
17 Zanetti et al. Comparative systems toxicology analysis of cigarette smoke and aerosol from a candidate modified risk tobacco product in organotypic human gingival epithelial cultures: A 3-day repeated exposure study. *Food and Chemical Toxicology*. 2017. 101:15-35. (Link)
18 Malińska et al. Assessment of mitochondrial function following short- and long-term exposure of human bronchial epithelial cells to total particulate matter from a candidate modified-risk tobacco product and reference cigarettes. *Food and Chemical Toxicology*. 2018. 115:1-12 (Link)
19 Our Findings on PMIScience.com (Link)
20 Luettich et al. Chronic Toxicity and Lung Tumorigenesis in A/J Mice following lifetime exposre to aerosol from the tobacco heating system 2.2 in comparison with exposure to 3R4F reference cigarette smoke. Presentation at APTox Porto, 7 February 2019. (Link)
21 PMI and BAT receive PETA award for their contributions to a public knowledge base. 20 Feb 2017. (Link)
22 Clippinger et al. Pathway-based predictive approaches for on-animal assessment of acute inhalation toxicity. *Toxicology in Vitro*. 2018. 52: 131-145. (Link)

Systems toxicology makes it easier to meet the first two of these guidelines. For example, we have conducted *in-vitro* studies using human oral cells,[23] 3D nasal cell cultures,[24] and gingival epithelial cells,[25] giving us results that are more relevant to human health than rodent studies might have given us. And when we must use rodents in our studies, we collect as much data as we can from the smallest reasonable number of rodents, while teasing as much information as possible from the data.

SYSTEMS TOXICOLOGY AND THE FUTURE

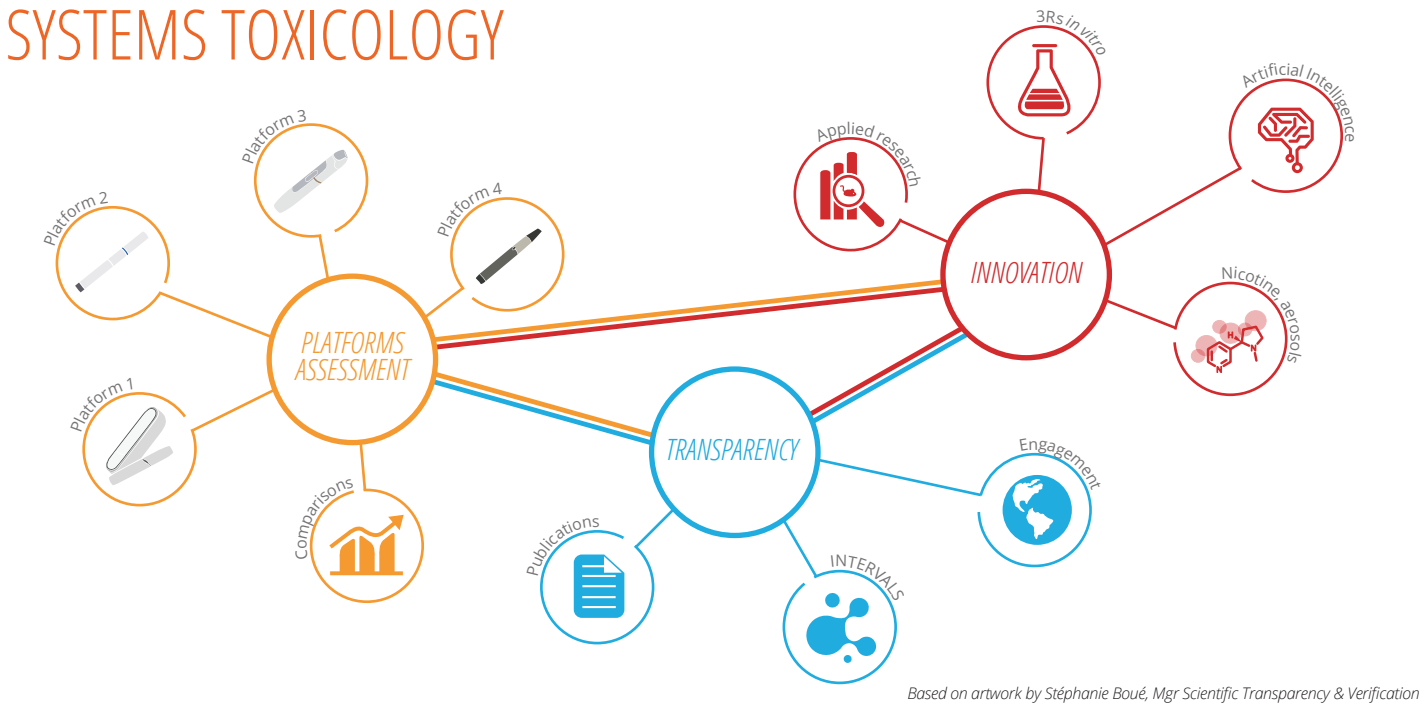
Transparency in research – and by that I mean fully disclosing the methods and data so others can replicate the results – is necessary to help make scientists' conclusions credible. We researchers in systems toxicology often deal with large, multi-faceted, and highly complex datasets, and so it is important that these data are shared in a way that is practical for other scientists to use.

That's a complex task that can be supported by a solid quality management system, good laboratory practices, and the existence of the sharing platform accessible to the scientific community. Everything is documented, reported, and validated. Our team is even co-developing protocols together with our suppliers, ensuring that we take full advantage of the capabilities of our research instruments. We are in the process of writing up these efforts to share with the community.

One area of research that I think will benefit most greatly from all these advancements in systems toxicology is what's called "P4 medicine": medicine that is **p**redictive, **p**reventative, **p**ersonalized, and **p**articipatory. P4 medicine is aimed at treating the causes of disease rather than the symptoms, something systems toxicology is clearly uniquely suited for. Systems toxicology helps us understand the mechanisms behind smoking-related diseases, but that's just a taste of the good this field of research can do for public health.

CONCLUSIONS

A system like the human body is, as the saying goes, more than the sum of its parts. All those parts interact with each other in different ways, and that's what makes it so complicated to answer questions of how certain chemicals affect the body. By building up new methods to answer those questions for our own products, we are at the same time collaborating and creating new tools to help other researchers in their work too. I feel incredibly lucky to be working for a company with the courage to invest in such thorough scientific study of these products, and to be working with people who are generous enough to share our work so openly with the scientific community.



Based on artwork by Stéphanie Boué, Mgr Scientific Transparency & Verification

23 Zanetti et al. Systems toxicology assessment of the biological impact of a candidate modified risk tobacco product on human organotypic oral epithelial cultures. *Chemical Research in Toxicology.* 2016. 29(8):1252-1269. (Link)
24 Iskandar et al. 3-D Nasal Cultures: System toxicological assessment of a candidate modified-risk tobacco product. *Alternatives to Animal Experimentation.* 2017. 34(1):23-48. (Link)
25 Zanetti et al. Comparative systems toxicology analysis of cigarette smoke and aerosol from a candidate modified risk tobacco product in organotypic human gingival epithelial cultures: A 3-day repeated exposure study. *Food and Chemical Toxicology.* 2017. 101:15-35. (Link)

PMI'S PEER-REVIEWED PUBLICATION HIGHLIGHTS

Organs-on-a-chip: liver and lung working together

Organ-on-a-chip devices are a rapidly-evolving technology that has taken *in vitro* testing to the next level. The technology works by growing 3D cell cultures of human tissues in wells of a polymer chip, allowing them to mimic key physiological aspects of organs, such as the lungs and liver in our recent study. We placed the cell cultures in separated compartments, but they were connected through a fluid channel system that could be controlled via Bluetooth from a researcher's cell phone. This fluid channel system allows the two different kinds of cells to share an environment without directly interfering in each others' growth. The cells continued to thrive for the full 28 days. This experimental device could be useful in the future to study chemicals implicated in COPD, asthma, lung cancer, and more.

Read more about this publication on PMIScience.com.[26]

A proposed approach to measuring the risk of lung cancer

The assessment of the risk of lung cancer is complicated by the fact that lung cancer is typically found after decades of smoking, and even when a person does quit, the risk of lung cancer decreases slowly, not all at once. Until now, only long-term studies for products already on the market were expected to provide clear answers to the risk reduction potential of smoke-free products. In this peer-reviewed publication, we proposed a new approach to the assessment of risk reduction based on three questions: Does switching from cigarettes to smoke-free products reduce genetic damage? Does switching reduce inflammation? Does switching reduce the risk of lung cancer in the laboratory? This approach is based on a causal chain of events that leads from smoking to disease and leverages both non-clinical and clinical studies as well as the principles of systems toxicology. The three questions can be answered *before* the product is widely available, not years or decades later. If evidence supports the answer "yes" to these three question, it should be reasonably likely that switching from cigarettes to the product in question would reduce the risk of lung cancer.

Read more about this publication on PMIScience.com.[27]

Reduced DNA modifications after switching to EHTS

Cigarette smoke is known to cause changes to DNA, including one kind of change called methylation. This is a process where the DNA is modified in a way that changes how it is read, but doesn't change the underlying sequence of base pairs. These kinds of changes can play a fundamental role in the development of cancer. This systems toxicology study involving laboratory mice was designed to further understand smoke's effect on DNA methylation, and to compare its impact on methylation to EHTS aerosol. We found that DNA is methylated by cigarette smoke in the lungs but not in the liver, and that only certain parts of the DNA are methylated by cigarette exposure. The alterations we observed were strongly reduced after either complete cessation of smoking, or after switching to EHTS. When mice were exposed to EHTS only, minimal DNA methylation was observed.

Read more about this publication on PMIScience.com.[28]

Reduced exposure: EHTS with menthol variant vs menthol cigarettes

While this research was already shared with the FDA some time ago, we are excited to see it now published and more readily available to the public. The study included 160 smokers and measured the biomarkers of exposure for 16 harmful chemicals in adult participants who continued smoking menthol cigarettes, switched to menthol EHTS, or quit smoking altogether. The participants spent the first 5 days of the study in the clinic in a controlled environment, followed by an ambulatory period of 85 days when participants were back home in their usual setting. On average, the biomarkers of exposure for those who switched to menthol EHTS were reduced by 51% to 96% on day 5 of the study compared to the cigarette smoking group. These reductions exclude nicotine and were sustained for most biomarkers over the ambulatory period of the study. The average levels of the biomarkers for people who switched to menthol EHTS were similar to the levels of biomarkers found in participants who quit smoking.

Read more about this publication on PMIScience.com.[29]

EHTS doesn't stain teeth as much as cigarettes

Smoking cigarettes can discolor teeth and stain dental resins. The goal of this study was to compare the level of discoloration, if any, caused by EHTS versus cigarette smoke as well as whether any color mismatches between the resins and the teeth would develop. Our researchers created cavities and applied dental resins to twenty-two *ex vivo* human teeth, which were then separated into two equal groups. One group was exposed to EHTS aerosol, and the other was exposed to cigarette smoke. Both groups of teeth underwent exposure for four days a week, over three weeks. Each week, the teeth were brushed following a strict protocol, and the color of the teeth was assessed. At the end of the three weeks, cigarette smoke caused marked discoloration of the enamel and dentin, and caused color mismatches between the resins and the teeth. EHTS aerosol, on the other hand, had a minimal effect on the color of the enamel or dentin, and caused no mismatch in color between the resins and the teeth.

Read more about this publication on PMIScience.com.[30]

IN THE RESEARCH LITERATURE, EHTS IS REFERRED TO AS TOBACCO HEATING SYSTEM (THS OR THS 2.2)

26 Bovard et al. A lung/liver-on-a-chip platform for acute and chronic toxicity studies. *Lab on a Chip.* 2018. 18(24):3814-3829. (Link)
27 Hoeng et al. Assessing the lung cancer risk reduction potential of candidate Modified Risk Tobacco Products. *Internal and Emergency Medicine.* 2019. Accepted Manuscript. (Link)
28 Choukrallah et al. Tobacco heating system 2.2 has a limited impact on DA methylation of candidate enhancers in mouse lung compared with cigarette smoke. *Food and Chemical Toxicology.* 2019. 123:501-510. (Link)
29 Haziza et al. Reduction In Exposure To Selected Harmful And Potentially Harmful Constituents Approaching Those Observed Upon Smoking Abstinence In Smokers Switching To The Menthol Tobacco Heating System 2.2 For Three Months. *Nicotine and Tobacco Research.* 2019. Accepted Manuscript. (Link)
30 Zanetti et al. Effects of cigarette smoke and tobacco heating aerosol on color stability of dental enamel, dentin, and composite resin restorations. *Quintessence International.* 2019. 18:2-12. (Link)

LATEST EVENTS & OTHER MILESTONES

MORE MRTP PRODUCTS ON US FDA'S DOCKET

We presented our evidence on EHTS last year at the Tobacco Products Scientific Advisory Committee (TPSAC).

Since then, and especially now that the comment period on our application is closed, we're waiting eagerly for news on our applications. While we wait, it's great to see that there are more candidate Modified Risk Tobacco Products (MRTPs), developed by U.S. Smokeless Tobacco Company, Swedish Match North America, and R.J. Reynolds Tobacco Company, now under review with the US FDA.[31] We look forward to seeing these and other products go through the review process, and we can't wait for current smokers to have many more options that are better than cigarettes to choose from.

INTERVALS NEARLY DOUBLES NUMBER OF AVAILABLE STUDIES IN 3 MONTHS

December 2018 – February 2019

This winter, the INTERVALS team nearly doubled the number of published studies on our data-sharing platform, and added almost 50 associated study protocols. Among these studies, to cite just a few, we can find a 5-day clinical study aimed at a comparative understanding of how a smoker's body reacts to cessation or to switching to THS 2.2, a study which looks at effect of either THS 2.2 aerosol or cigarette smoke on the coloration of teeth and dental resins, and a collaboration between PMI and Altria on the impact of cigarette smoke versus electronic cigarette aerosol on human lung and oral epithelium. The INTERVALS team remains resolute to add more studies and associated data sets, and they are looking for researchers committed to scientific transparency and interested in increasing the visibility of their research.

Find out more about the Intervals platform on INTERVALS.science.

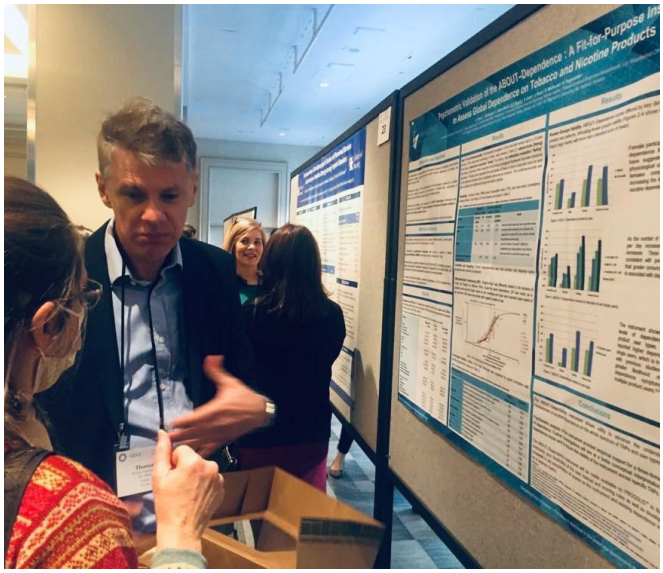
PMI RESEARCHERS HOST WOMEN IN SCIENCE DAY

Neuchatel, Switzerland
11 February 2019

Monday, February 11th marked the annual celebration of women and girls in science. To signify the day's importance, Dr. Christelle Chrea and Maxime Magnier organized an event at our Research & Development center in Neuchâtel. Students from Swiss universities were invited for the day; they received an introductory lecture explaining what our scientists do, a tour of our laboratories, and participated in an open forum with senior and junior female scientists at PMI, all sharing their personal anecdotes and stories. The conversations covered academia vs. industry, social status in science, opportunities for women in the field, and more. The purpose was to start a conversation about and among women in science, from different backgrounds/fields of expertise and across different levels of experience.

To find out more about the event and its significance, read Dr Chrea's article on PMIScience.com.

31 U.S. Food & Drug Administration website. Modified Risk Tobacco Products. MRTP Applications Currently Under Scientific Review. Updated 1/14/2019. (Link)



SOCIETY FOR RESEARCH ON NICOTINE AND TOBACCO (SRNT) ANNUAL MEETING

San Francisco, California, USA
20-23 February, 2019

PMI R&D attended the SRNT Annual Meeting, a conference that covers topics related to nicotine and tobacco from diverse fields including preclinical, clinical, public health policy, regulatory science, global health research and more. PMI scientists presented our original research results in 15 posters and counted themselves among the more than 1,100 international attendees from more than 40 countries worldwide who followed or participated in the three and a half day program.

Posters included details on the first year of a repeated cross-sectional survey conducted in Japan, presented by Dr. Peter Langer, Manager Post-Market Assessment, and by Dr. Erica Spies, Senior Behavioral Scientist. Dr. Loyse Felber Medlin, Clinical Scientist, presented a poster that described the results of a 1-year clinical trial on smoking abstinence, assessing changes in many of the same clinical risk endpoints as have been previously described in our Exposure Response Study (ERS). The results of the ERS related to cardiovascular effects were also showcased at SRNT, in a poster by Dr. Calin Pater, Medical Director.

All of our smoke-free platforms made an appearance in the presented posters. Dr. Cam Tuan Tran, Manger Clinical Program, presented a poster describing the reduction in biomarkers of exposure for people who switched to Platform 2 from cigarettes over 90 days compared to those who continued to smoke cigarettes. Nicotine pharmacokinetics and subjective effects of Platform 3 were presented by Marija Bosilkovska, Clinical Scientist, and several posters covered studies with results on our e-cigarette platforms including MESH.

Learn more about the conference and our presentations on PMIScience.com.

58TH ANNUAL SOCIETY OF TOXICOLOGY MEETING

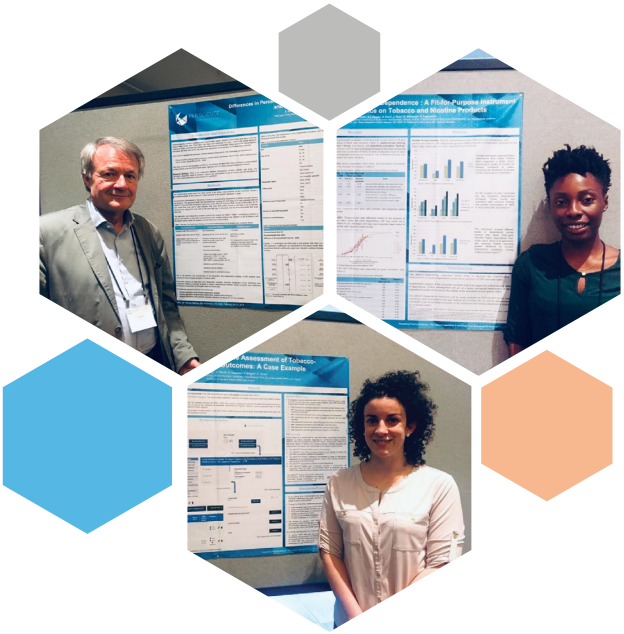
Baltimore, Maryland, USA
10-14 March, 2019

PMI researchers presented at the Society of Toxicology annual meeting at the Baltimore Convention Center, a meeting that features more than 100 scientific sessions and 2,100 individual presentations. This meeting brought more than 6,000 scientists from 50 countries. PMI delivered an oral presentation and 13 posters at this year's meeting.

Poster presentations included the development of new blood drop diagnostics to determine smoking status, presented by Dr. Vincenzo Belcastro, Scientist – Computational Biology, and another presented by Dr. Carine Poussin, Senior Scientist – Computational Biology. Dr. Stefan Frentzel, Manager Cellular Laboratories, showed a new multi organ-on-a-chip that emulates the systemic effects of inhaled substances. The PMI team presented several other posters describing our latest e-cigarette research, and enjoyed discussing their research with external colleagues.

This year, PMI researchers were excited to have the honor of giving an oral presentation about their work. Dr. Justyna Szostak, Scientist – Cell Signaling, presented results on a study comparing the effects of cigarette smoke and e-cigarette vapor on cardiovascular disease in laboratory mice. Laboratory mice were used to evaluate their effects on atherosclerosis development, cardiac function, and stiffening of the aorta, the largest artery in the body. Over the six-month study, the results showed that cigarette smoke worsened atherosclerosis, which wasn't the case with e-cigarettes. Aortic stiffness, already a natural result of aging, increased more with cigarette smoke exposure than e-cigarette vapor.

Check out our participation at the conference on PMIScience.com.



INDEPENDENT RESEARCH ON SMOKE-FREE PRODUCTS

NO BURNING IN EHTS CONFIRMED BY MEASUREMENTS OF USERS' BREATH

It is well established that many of the harmful chemicals in cigarette smoke are created by burning the tobacco. Researchers in Italy sought to learn whether heated tobacco products like EHTS burn tobacco and compare the levels of CO exposure with cigarettes, because *"Smokers will be able to make better choices if they are guided by findings of independent studies focused on the relative reduction in exposure risk after switching to [heated tobacco products]."* They conducted a small, randomized study on 12 healthy smokers, measuring exhaled carbon monoxide (eCO) after subjects used EHTS or another heated tobacco product.[32] eCO was measured as an indicator of whether the tobacco in these products was burnt or not, because increased eCO levels would indicate combustion. The authors found no statistically significant increase in eCO levels among any of the participants after using either product.

EHTS VERSUS VAPING OR SMOKING AFTER 12 HOURS OF NO SMOKING

Researchers from KU Leuven and Thomas More Universities in Belgium conducted a study[33] comparing EHTS to e-cigarettes and cigarettes with two goals in mind: to measure the eCO levels before and after product use, and to determine the ability of each product to meet the needs of the users after they refrained from smoking cigarettes overnight. The results from the 30 participants followed expectations, showing cigarettes as the best at satisfying cravings, calming withdrawal symptoms, and subjective evaluations, followed by EHTS and then e-cigarettes. The authors concluded that EHTS can satisfy cravings for cigarettes without the severe impact on eCO levels that comes from smoking a cigarette. They also concluded that users new to both heated tobacco products and e-cigarettes showed preference for EHTS over the e-cigarette that was tested.

EFFECTS OF HEATED TOBACCO AND E-CIGARETTES ON LUNG CELLS

Researchers in Sydney, Australia recently studied the effects of EHTS, e-cigarettes, and cigarettes on lung cells.[34] The authors concluded that all three categories of products cause similar damage to the lung tissues tested. Independent researchers have spoken out publicly against this article,[35] including a comment from Dr. Ed Stephens, Senior Research Fellow at the University of St. Andres: *"E-cigarettes are certainly not harmless but the authors' conclusion is inconsistent with most published research which indicates that vaping is significantly less hazardous than smoking."* Dr. Lion Shahab, Senior Lecturer in Epidemiology & Public Health at UCL also spoke out against the publication, saying *"The claim that e-cigarettes (and heat-not-burn devices) are as toxic as cigarettes was not, in fact, tested in the analysis and is misleading."*

PUBLIC HEALTH ENGLAND: EVIDENCE UPDATE ON E-CIGARETTES

On February 27th this year, PHE published a new evidence update on e-cigarettes.[36] The report focused on the latest available information on how widely the products are used by people in the UK, and how they're using them. Among adults, 14.9% to 18.5% of current smokers are using e-cigarettes, 10.3% to 11.3% of ex-smokers are using them, and 0.4% to 0.8% of never smokers use e-cigarettes in Great Britain from 2017 to 2018. PHE reported differences in e-cigarette prevalence that correlate with socio-economic status. They also found that, among young people, experimentation with e-cigarettes has increased in recent years while regular use is still low. PHE stated that e-cigarettes combined with stop smoking support should be a recommended option for all smokers, in line with their earlier report published last year.

PUBLICATION BY UCSF SCIENTISTS CRITICIZE REDUCED EXPOSURE STUDY

Researchers from the University of California, San Francisco in the US have published a paper in Tobacco control, claiming that our *"MRTP application fails to address the important question of whether the aerosol generation process for IQOS produces toxic substances not found in the smoke of combustible cigarettes, which could have been answered through non-targeted chemical analysis."*[37] In fact, PMI researchers have already conducted a full non-targeted chemical analysis, submitted to the FDA in December 2017, which the authors referred to in their publication. Our researchers have fully characterized the aerosol of EHTS. We've demonstrated an average reduction in the levels of toxicants of 95% compared with cigarette smoke, translating to more than 90% reduced toxicity compared to cigarettes.

View our response on PMIScience.com.

E-CIGARETTES COMPARED TO NICOTINE-REPLACEMENT PRODUCTS

New research conducted in the UK aimed to study the effectiveness of e-cigarettes compared with nicotine-replacement therapy (NRT) products for smoking cessation.[38] This clinical trial included 886 adults who attended cessation clinics in the UK and were randomly assigned to either nicotine-replacement therapy (NRT) or an e-cigarette. NRTs were provided for up to 3 months for the assigned group. The e-cigarette group was given a starter pack along with one bottle of nicotine e-liquid, along with a recommendation to purchase further e-liquids of the flavor and strength of their choice. Both groups received weekly behavioral support for at least 4 weeks. The authors reported that the e-cigarette group were more likely to have abstained from cigarettes for a full year (18% vs 9.9%) and e-cigarettes were rated as more helpful to refrain from smoking than NRT.

32 Caponnetto et al. Carbon monoxide levels after inhalation from new generation heated tobacco products. *Respiratory Research.* 2018. 19:164. (Link)
33 Adriaens et al. IQOS™ vs. e-cigarette vs. Tobacco Cigarette: A direct comparison of short-term effects after overnight-abstinence. *International Journal of Environmental Research and Public Health.* 2018. 15(2):2902. (Link)
34 Sohal et al. IQOS exposure impairs human airway cell homeostasis: direct comparison with traditional cigarette and e-cigarette. *ERJ Open Research.* 2019. 5:00159-2018. (Link)
35 Science Media Centre. Expert reaction to study comparing heat-not-burn, vaping and smoking. (Link)
36 McNeill et al. Vaping in England: an evidence update February 2019. A report commissioned by Public Health England. (Link)
37 St. Helen et al. IQOS: Examination of Philip Morris International's claim of reduced exposure. *Tobacco Control.* 2018. 27:s30-s36. (Link)
38 Hajek et al. A randomized trial of e-cigarettes versus nicotine-replacement therapy. *The New England Journal of Medicine.* 2019. 380:629-637. (Link)

GLOSSARY

AEROSOL
An aerosol is a suspension of fine solid particles and/or liquid droplets in a gas (usually air). Cigarettes generate a smoke aerosol that is the result of the combustion (burning) of tobacco and contains carbon-based solid particles. While smoke is an aerosol, not all aerosols are smoke.

PMI's smoke-free products do not produce smoke because they do not burn tobacco. Instead, they generate a nicotine-containing aerosol, either by heating tobacco or through other technologies that do not involve combustion.

Consumers typically use the term "vapor" to refer to the aerosol generated from heated tobacco products or other nicotine-containing products.

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. Biomarkers of exposure can provide a measure of internal dose, which is the amount of the constituent taken up into the body.
- *Clinical risk markers:* a measurable change in biochemical, physiological (organs, tissues, cells), or behavioral function within an organism that is known to be associated with a health impairment or disease. These biomarkers indicate the body's response to exposure to harmful chemicals. While clinical risk markers do not necessarily cause these health concerns, their presence and magnitude help identify whether a person already has or is in danger of developing a health impairment or disease.
- *Clinical risk endpoints:* clinical risk markers that have been selected for measure in a clinical study.

CLINICAL RISK MARKERS OR ENDPOINTS
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.

EXPOSURE RESPONSE STUDY
Designed to assess whether switching to a smoke-free product leads to favorable changes in clinical risk markers that are benchmarked to smoking cessation. This is a longer-term study (six months + a six month extension) conducted with adults who smoke.

MODIFIED RISK TOBACCO PRODUCT (MRTP)
The US Family Smoking Prevention and Tobacco Control Act (2009) granted to the FDA authority to regulate tobacco products. MRTP is defined in that Statute 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."*

PHARMACOKINETIC STUDIES
These studies measure how a substance, such as nicotine, is absorbed by the body. This helps in determining the extent to which adults who smoke 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).

REDUCED-RISK PRODUCT (RRP)
The term PMI uses to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment, and commercialization. Because our RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.

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 the levels of harmful and potentially harmful chemicals generated by our smoke-free products reduces the toxicity of their aerosol, 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' aerosol 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 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.

One example of a systems toxicology approach is to use organotypic tissues: tissue samples which behave as if they were in the body. These tissues can make the results more complex and difficult to interpret but also more relevant to effects on the human body compared to standard toxicology methods.

PMI'S PEER-REVIEWED SYSTEMS TOXICOLOGY PUBLICATIONS ON SMOKE-FREE PRODUCTS

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Choukrallah, M. A.; Sierro, N.; Martin, F.; Baumer, K.; Thomas, J.; Ouadi, S.; Hoeng, J.; Peitsch, M. C.; Ivanov, N. V. Tobacco Heating System 2.2 has a limited impact on DNA methylation of candidate enhancers in mouse lung compared with cigarette smoke. *Food and Chemical Toxicology*. **2019**. 123: 501-510.
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