SCIENTIFIC UPDATE

PMI SCIENCE – PHILIP MORRIS INTERNATIONAL

OCTOBER 2024 | ISSUE 20

TOBACCO HEATING SYSTEM:

PAST, PRESENT, AND FUTURE

CONTENTS

INTRODUCTION

It has been a decade since the initial pilot launch of our Tobacco Heating System (THS), marketed as *IQOS*. In some ways, this product—and the scientific evidence that supports it—has become a symbol of the transformation that our company has undergone: we've developed the drive, the culture, and the experience necessary to lead the tobacco industry into a smoke-free future. In fact, we have already declared our ambition to become a predominantly smoke-free company by the year 2030, by which time we aim to have more than two-thirds of our net revenues come from the smoke-free category.

In this issue, we revisit the history of product development that led to the launch of THS, and we examine the research milestones we have met along the way. Also in this issue, we take a closer look at the clinical research Philip Morris International (PMI) conducts and how measuring biomarkers in these studies helps us understand the potential positive impact that switching to THS can have on adult smokers who would otherwise continue to smoke.

We invite you to learn more about the research and development of our leading heated tobacco product and gain a better understanding of the kind of company we've become.

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EVENTS

OPEN SCIENCE

Innovation drives progress: The evolution of smoke-free products

Warsaw, Poland/Online
June 12, 2024

In our fourteenth Open Science event, our panel of experts discussed how Philip Morris International (PMI) continues to improve and innovate its smoke-free products while ensuring that newly launched product versions maintain a comparable reduced-risk profile to the previous versions. Participants held a lively discussion about the future of smoke-free products, using the development of our Tobacco Heating System (THS) as a primary example.

During this event, the panelists explained the evolution of our smoke-free products and the differences between THS 2.2, a heated tobacco product which uses ceramic blade heating technology, and the next generation of tobacco heating, THS 3.0, which uses an inductive heating element. Our experts demonstrated that the totality of evidence on our THS shows that its risk profile remains significantly lower than that of cigarettes regardless of which version is studied.

They also showcased how the evidence gathered on both versions of THS was comparable across various studies conducted, including our clinical trials.

Watch the replay here.

GLOBAL FORUM ON NICOTINE

Global Forum on Nicotine

Warsaw, Poland/Online
June 13-15, 2024

PMI participated in the eleventh edition of the Global Forum on Nicotine (GFN), which convened stakeholders from around the world to discuss and explore the evolving landscape of safer nicotine products. The theme of this year's conference was "Economics, Health and Tobacco Harm Reduction."

During this 3-day event, PMI scientists submitted several Science Lab and GFN Fives videos—short, prerecorded multimedia presentations. Ondrej Koumal discussed the "Evolution of Tobacco Sales and Use Following Introduction of Heated Tobacco Products in Japan," Carrie Wade presented "Estimating HPHC Exposure on a Per-Stick Basis," Anna Masser, from Swedish Match, discussed "Why Nicotine Content is not Nicotine Exposure," Gizelle Baker served as a panelist on a discussion on "Rating the Evidence - Good and Bad Science," and Nevena Crjenko participated in a panel discussion entitled "Legislation and Regulation: Health and Economic Consequences."

Watch the presentations from PMI scientists at GFN here.

In the International Symposium on Nicotine Technology (ISoNTech), which runs alongside the GFN, Guido Janeke gave a presentation titled, "Innovation of Smoke-Free Products: Tobacco-Leaf free, Nicotine Containing Heat-not-Burn (HNB) Proposition."

Watch the presentation here.

SOCIETY OF TOXICOLOGY ANNUAL MEETING

Society of Toxicology Annual Meeting

Salt Lake City, USA
March 10-14, 2024

PMI participated in the sixty-third annual Society of Toxicology meeting and ToxExpo. This annual conference is an important yearly forum for sharing knowledge and collaborating in the science of toxicology. It is aimed at scientists, academics, researchers, healthcare professionals, regulators, and industry experts.

During the 5-day event, PMI scientists presented a poster titled "Comparative Toxicity Assessment of the Harm Reduction Potential of Tobacco-Free Oral Nicotine Products." This illustrated PMI's research assessing the ability of the ToxTracker® reporter assay to characterize the toxicological profiles of nicotine pouches and Swedish snus compared with reference tobacco products.

Researchers also presented a poster titled "Evaluation of Chronic Toxicity and Carcinogenicity of Flavored E-Vapor Aerosols in an 18-Month Inhalation Study in A/J Mice." This detailed PMI's research on local and systemic toxicity following lifetime exposure to cigarette smoke and aerosols from a prototype e-liquid formulation.

SCIENTIST **PROFILE**

Dr. Hélène Karcher

Hélène joined PMI as Global Head, Real World Evidence (RWE) in March 2024. Her team's mission is to conduct epidemiology and health economics studies to quantify differences in health outcomes in people who switch to smoke-free products compared with those who continue to smoke cigarettes as well as research to understand the long-term health effects and outcomes of nicotine per se, including through non-clinical and real-world evidence studies.

Hélène has more than 15 years' experience in economic modelling, epidemiology, and RWE study, having previously worked at Novartis as Global Head of RWE for Ophthalmology, Respiratory, and Allergy. An experienced evidence generation executive, she has designed and analyzed more than 100 randomized control trials and RWE studies.

She earned her bachelor and master's degree in Mathematics and Physics from the École Polytechnique in France and completed a PhD in Computer Science and Cell Biology from Massachusetts Institute of Technology in the U.S.

Hélène has also published extensively, having authored more than 70 peer-reviewed articles in health economics, epidemiology, and medical journals. She is currently the Editor in Chief of the *Epidemiologic Methods* journal.

HISTORY OF THS

FROM EARLY DEVELOPMENT THROUGH DECADES OF INNOVATION

Discover how the technology and science behind our Tobacco Heating System (THS) have evolved since its commercial launch as *IQOS* in 2014, and what the future holds.

A continuing journey

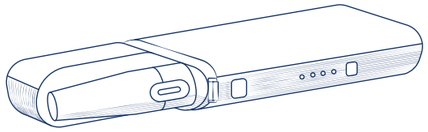
THS was first released commercially 10 years ago, but its development began long before that, with the concept of providing smoke-free alternatives to cigarettes that would reduce the risk of smoking-related disease for adult smokers who switch rather than continuing to smoke.

Development began by addressing questions such as what kind of product would best meet consumer needs, how to eliminate combustion and generate an aerosol with far fewer toxicants than cigarette smoke, and what type of heating technology should be used to achieve this. Hundreds of experiments were conducted to address these and other questions.

Between 1990 and 2011, prototype products were constructed and tested to understand the aerosol chemistry involved, measure the levels of harmful and potentially harmful constituents (HPHCs) in the aerosol, and see if consumers would use the product. The lessons learned were critical to the eventual development and success of THS, which would go on to be commercialized under the *IQOS* brand.

Read on to explore the timeline of key developments in the history of THS.

FROM CONCEPT TO COMMERCIALIZATION



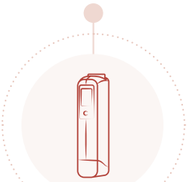
Foundations of innovation

The development of a completely new category of product—one which does not burn tobacco or produce smoke—can be traced back decades. In the years between 1990 and 1998, Philip Morris International (PMI) developed and commercialized the Electrically Heated Cigarette Smoking System (EHCSS) Series E. This used an array of eight blades, or pins, to heat a specially designed cigarette. Over the next 8 years, the EHCSS was updated to include larger blades, more sensitive puff detection and improved filtration, airflow, and heat control, to reduce the formation of combustion products.

Development and launch of THS

Advances in heating technology made with EHCSS were built on in the following years with the development of THS 2.0. This was a lab prototype that used a single, 3 mm blade to heat a specially designed tobacco stick which was fundamentally different from those used in previous versions. The new stick contained a tobacco plug made from tobacco leaves which had been ground and reconstituted into a tobacco sheet that was then crimped and folded, combined with a new filter element.

The two-piece design of the THS 2.0 featured a separate holder and charger, making it lighter and less bulky than the EHCSS, and a temperature control for the heating blade. This prototype was not commercialized, but it represented a major advance in heat-not-burn technology. It was followed by the THS 2.1 prototype, designed specifically for consumer research.



1990-1995

Product development of the EHCSS Series E

1995-2000

Product development of the EHCSS Series JLI

1998

EHCSS Series E commercialized as *Accord* (U.S.), and in 1999 as *Oasis* (Japan)

2000-2004

Product development of the EHCSS Series K6

2002

EHCSS Series JLI commercialized as *Accord* (U.S.) and *Oasis* (Japan)

2006

EHCSS Series K6 commercialized as *HeatBar* (Australia, Germany, and Switzerland)

2007-2009

THS 2.0 developed as lab prototype

2009-2011

Development of the THS 2.1 consumer research prototype

The technological and scientific learnings from the prototype research were then incorporated into the development of THS 2.2, which would be commercialized as the first *IQOS* model ever launched. This featured a 5 mm heating blade, increased battery life, and improved puff-to-puff consistency. In the following years, the design of the THS 2.2 was further refined for large-scale manufacturing and to make the device lighter and easier to hold.

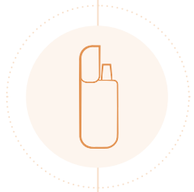
Expansion and innovation

In the years following the commercial launch of THS 2.2, PMI continued to update and improve its technology and to substantiate its reduced risk potential with scientific studies. This included the publication, in 2016, of a series of nine peer-reviewed papers supporting PMI's submission to the U.S. Food and Drug Administration (FDA) of a modified-risk tobacco product (MRTP) application.

In 2020, the FDA issued its decision, finding that the issuance of exposure modification orders with reduced exposure claims for THS and three variants of our tobacco sticks would be appropriate to promote the public health and is expected to benefit the health of the population as a whole.

"... for adults who do not quit, switching completely to THS can potentially reduce the risk of smoking related harm compared with continued smoking."

Technological improvements were made to THS 2.2 over the following years in order to improve key elements of the user experience. This included adding holder vibration, Bluetooth, holder autocleaning, a doubling of the battery lifetime, and greatly improved device robustness and reliability.



2011-2013

Development of the THS 2.2

2014

Pilot launch of THS 2.2, commercialized as *IQOS 2.2* in Milan, Italy and Nagoya, Japan

2015

THS 2.2 launched in additional cities in Japan, Italy, Portugal, Romania, Russia, and Switzerland

2016

THS 2.2 commercialized as *IQOS 2.4*

2018

THS 2.2 updated and commercialized as *IQOS 2.4 Plus*

THS 2.2 updated and commercialized as *IQOS 3* and all-in-one, pocket-sized version of THS 2.2 commercialized as *IQOS 3 Multi*

2019

Development and commercialization of the *IQOS 3 DUO*

Recent advancements

More recently, PMI has developed an all-new, bladeless SMARTCORE INDUCTION SYSTEM™ (THS 3.0), commercialized as *IQOS ILUMA*. This system heats tobacco without any direct contact between the electronics and the heating element.

The THS 3.0 induction system relies on specially designed heated tobacco units (TEREA SMARTCORE STICKS™), each with their own internal heating element at their core. These use a magnetic field to heat the surrounding tobacco, offering improved draw and taste consistency without the need for cleaning. PMI has also developed tobacco-free nicotine-containing sticks, commercialized as *LEVIA*, which generate a nicotine-containing aerosol when heated in THS 3.0.

This was followed by the development of another new heated tobacco system—the bladeless ROUNDHEAT TOBACCO SYSTEM™ (THS 6.0), commercialized as *BONDS by IQOS*. This uses a flexible heater that surrounds the tobacco stick and heats its external surface. The all-in-one design focuses on robustness, low maintenance, and ease-of-use. A new tobacco stick was also developed specifically for the system.

In 2023, PMI submitted premarket tobacco product applications (PMTAs) and MRTPAs to the FDA for *IQOS ILUMA*. The following year, PMI launched the *IQOS ILUMA* i series, with advanced features such as a touch screen, FlexPuff, and pause mode. PMI also published a clinical study demonstrating that product acceptance is similar between *IQOS ILUMA* and the previous *IQOS* version and a pharmacokinetic study showing that the reduction of exposure to toxicants is similar between the two versions of *IQOS*.

2018-2020

SMARTCORE INDUCTION SYSTEM™ (THS 3.0) is developed

2021

THS 3.0 is commercialized as the *IQOS ILUMA*

ROUNDHEAT TOBACCO SYSTEM™ and THS 6.0 are developed

2022

THS 6.0 commercialized as *BONDS by IQOS*

2023

Introduction of *LEVIA* nontobacco sticks

There are an estimated 28.6 million *IQOS* users

2024

Launch of the *IQOS ILUMA i*

What's next for THS?

In the decade since its launch, research studies have demonstrated that, for adults who do not quit, switching completely to THS can potentially reduce the risk of smoking related harm compared with continued smoking. However, quitting tobacco and nicotine products altogether is the best way to reduce the risk from smoking.

Scientifically substantiated harm reduction is only part of the equation, however. To reduce harm at a population level, adult smokers who do not quit need to switch to using smoke-free products such as PMI's THS. Many of the technological advances made in the development of THS are designed to make it easier for adult smokers to switch and to provide a variety of options to choose from.

There is also independent research suggesting that THS may be having an impact. In Japan, cigarette sales began declining after the introduction of THS. Data from the 2022 Japan National Health and Nutrition Survey indicated that 86% of consumers who use heated tobacco products like *IQOS* do so exclusively, and do not smoke cigarettes.

By 2030, PMI aims to be a substantially smoke-free business, replacing cigarettes with smoke-free products that are scientifically substantiated to be less harmful than smoking. THS will continue to be a cornerstone of this commitment to advancing smoke-free technology and improving public health outcomes.

MILESTONES IN TOBACCO HEATING SYSTEM RESEARCH

Research to substantiate harm reduction potential has been the cornerstone of the development of Philip Morris International's (PMI's) Tobacco Heating System (THS), commercialized as *IQOS*. In this article, PMI answers questions about some of the key scientific research relating to THS and what this can tell us about the harm reduction potential of the THS.

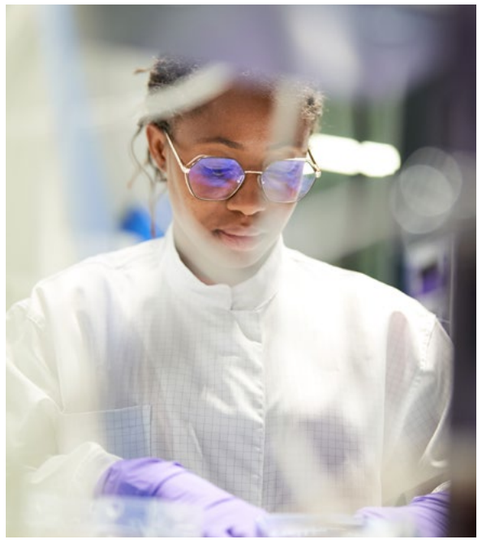
PLATFORM DEVELOPMENT · TOXICOLOGICAL ASSESSMENT · CLINICAL ASSESSMENT · PERCEPTION AND BEHAVOIR · LONG-TERM ASSESSMENT

What are the key research takeaways for THS?

Each of the conclusions from our research is important, but the finding that THS doesn't burn tobacco (and thus, doesn't produce smoke) is critical as a foundation of all our other research. Almost equally foundational is the fact that THS emits, on average, 95% lower levels of harmful chemicals.

Together, these findings demonstrate how different THS is from cigarettes. When smokers puff on a cigarette, the temperature reaches more than 800°C at the tip, which results in burning the tobacco and creating smoke, which contains high levels of toxicants.

However, the tobacco in THS is heated to a much lower temperature, and no combustion takes place. So, no smoke is created, and much lower levels of harmful chemicals are emitted. In addition, the lower levels of harmful chemicals in THS aerosol lead to other observable differences between THS and cigarettes, and these are further studied in other areas of our smoke-free product assessment program.



How does the evidence of reduced emissions relate to other conclusions from PMI's assessment program?

PMI's assessment program involves a number of different steps in addition to aerosol research, including toxicology, clinical studies, perception and behavior research, and long-term studies. Each of these steps builds up the picture of lower harm from THS compared with cigarettes. As we have seen, aerosol research demonstrated that THS does not produce smoke. Following this, toxicology studies have shown that the absence of combustion leads to lower toxicity on cells from the THS aerosol, compared with cigarette smoke.

Some of the most important findings from these studies demonstrate that the levels of selected toxicants in THS aerosols were, on average, 95% lower than those found in cigarette smoke. On top of this, PMI's research has demonstrated that THS aerosol contains fewer chemicals than cigarette smoke, and that, although some of the chemicals in THS aerosol are genotoxic or cytotoxic, they are present at very low levels.

> *"Smokers who switched completely from cigarettes to THS saw improvements in biomarkers of disease, along the lines of those seen in smokers who quit altogether."*

How do conclusions based on laboratory research translate to clinical study results?

There are two main outcomes from our clinical program, which are aligned with the results of our chemical analysis and with our toxicology data.

1. We see a reduction in exposure to harmful chemicals for participants who switch to THS as compared with those who continued to smoke.
2. And we see a reduction in levels of biomarkers of potential harm (BoPH) in participants who switch to THS as compared with those who continue to smoke.

We conduct a wide range of clinical studies with the help of contract research organizations, including interventional studies and cross-sectional studies.

Clinical studies measuring biomarkers associated with exposure to chemicals found in cigarette smoke and THS aerosol show that levels of these chemicals in the body are significantly reduced with THS use compared with continued smoking. In fact, the levels with THS were almost the same as those in people who quit tobacco and nicotine altogether.

Research measuring biomarkers of potential harm for smoking-related diseases, such as cardiovascular disease, chronic obstructive pulmonary disease, and cancer, have also shown positive results. Smokers who switched completely from cigarettes to THS saw improvements in biomarkers of disease, along the lines of those seen in smokers who quit altogether.

These studies also showed that, for THS users who also smoked cigarettes, the fewer cigarettes smoked, the more positive the results. This illustrates the importance of switching completely in order to see the greatest benefit.

It is important to note that THS is not risk free and contains nicotine which is addictive, but based on the available science to date presents a better choice for adults smokers who would otherwise continue to smoke.

Based on PMI's research, can we expect to see a positive impact on public health?

Harm reduction has two parts:

- The smoke-free product has to be scientifically substantiated to have lower risk of harm than cigarette smoking.
- Existing adult smokers have to switch to the smoke-free product at scale while the amount of unintended use (including people under legal age, former and never smokers) should be minimized.

We have already seen, from the results of PMI's aerosol, toxicology, and clinical studies, that our smoke-free products meet the first part of this equation—THS use shows a potential to lower the risk of harm compared with cigarettes smoking.

However, to have a positive impact on public health, we also need the second part of that harm reduction equation—we need to confirm that existing adult smokers who would otherwise continue to smoke will switch at scale. This has been demonstrated in separate studies in Germany, Italy, Japan, South Korea, Switzerland, and the U.S. These studies found that, not only do a percentage of existing smokers switch from cigarettes to THS, but also that total tobacco product consumption among the study groups did not increase. This means that smokers are, on average, not adding THS use on top of smoking but are switching from smoking to THS use.

There is also evidence from multi-country perception and behavior studies that smokers are more likely to switch completely to THS when they are aware that THS presents a lower risk of harm than continued smoking. This indicates that providing accurate and nonmisleading information to adult smokers can facilitate switching, and thus availability of this information can increase the prospect of long-term positive impact on public health.

"Studies in Japan... suggest that the growth in smoke-free product usage was largely coming from adult smokers who would otherwise have continued to smoke switching to THS."

Are we seeing a long-term impact on public health from smoke-free products?

It is important to remember that smoke-free products are relatively novel, for example, THS has only been commercially available for around 10 years and therefore it is still early to assess if a large-scale public health change has emerged.

However, studies in Japan, where THS was introduced in 2014, found that in the years 2016 to 2022, the rate of cigarette smoking continued to decrease (at an accelerated pace), while the use of smoke-free products increased. Even more interesting is that smoke-free product use among nonsmokers, as well as the number of THS users moving back to cigarette smoking, were very low over this period. This suggests that the growth in smoke-free product usage was largely coming from adult smokers who would otherwise have continued to smoke switching to THS.

What research studies are coming next from PMI?

PMI is currently conducting clinical studies to further understand the potential that THS could play in lowering the risk of harm from smoking. One of these is a cross-sectional, multi-country study to see if switching from cigarettes to THS reduces exposure to key toxicants, oxidative stress, and inflammation.

PMI has also recently completed a clinical trial examining nine biomarkers in longer-term smokers who switched completely to THS use for 2 years, compared with those who continued to smoke cigarettes. Results showed that switching was associated with favorable differences in biomarkers related to biological pathways impacted by cigarette smoke (such as inflammation and oxygen transport), as well as with better cardiovascular and respiratory function compared with current smokers.

The study also provided some of the first evidence that arterial elasticity—the decrease in which is a sign of atherosclerosis—was greater in THS users than in smokers.

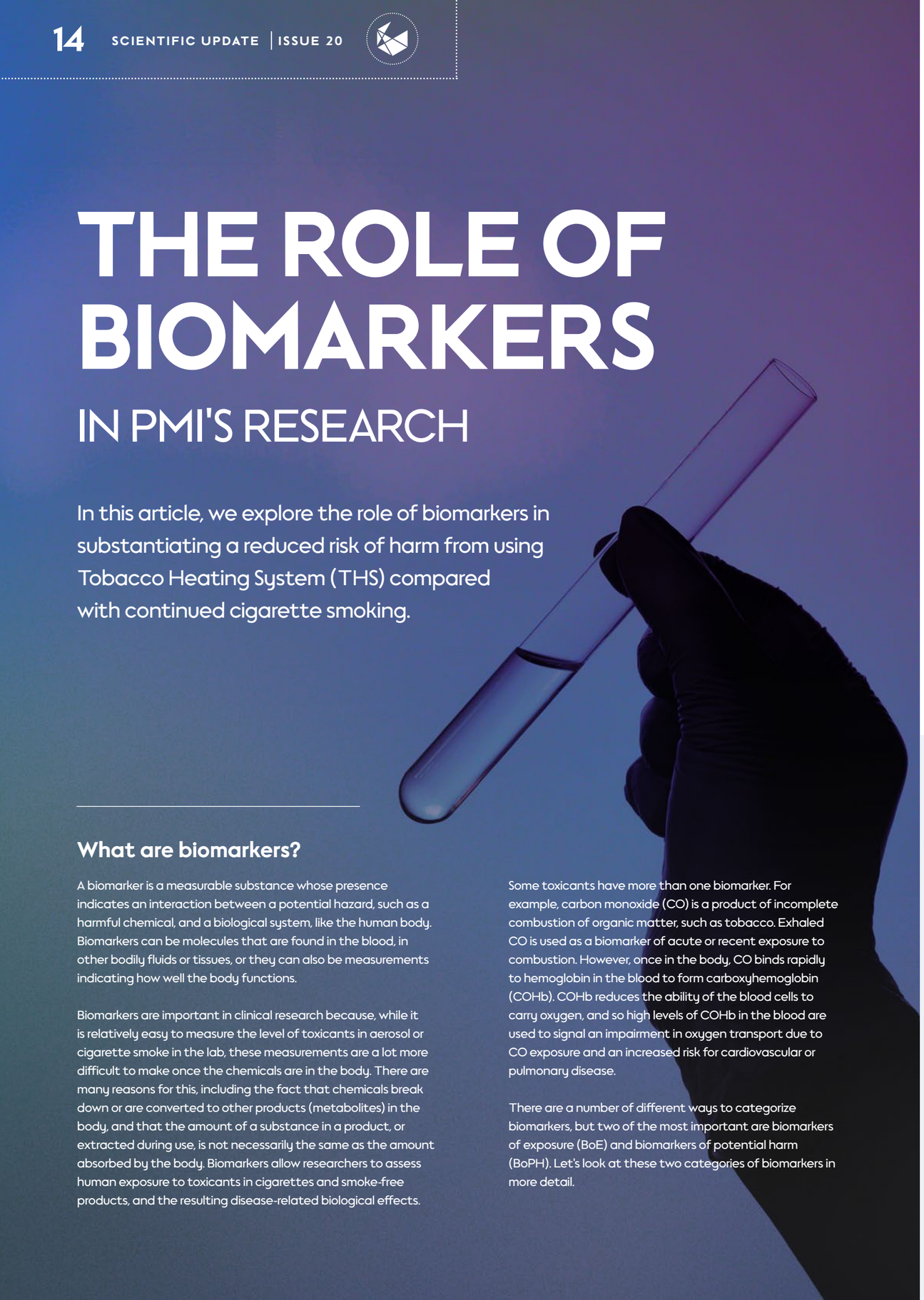
Based on the totality of evidence to date, THS has the potential to reduce the risk of smoking related disease for those adults who switch completely away from cigarettes.

The best choice any smoker can make is to quit tobacco and nicotine altogether. And while smoke-free products are not risk free and contain nicotine, which is addictive, scientifically substantiated smoke-free alternatives, like the THS, can play a significant role in tobacco harm reduction by providing acceptable alternatives to adult smokers who would otherwise continue to smoke.

THE ROLE OF BIOMARKERS

IN PMI'S RESEARCH

In this article, we explore the role of biomarkers in substantiating a reduced risk of harm from using Tobacco Heating System (THS) compared with continued cigarette smoking.



What are biomarkers?

A biomarker is a measurable substance whose presence indicates an interaction between a potential hazard, such as a harmful chemical, and a biological system, like the human body. Biomarkers can be molecules that are found in the blood, in other bodily fluids or tissues, or they can also be measurements indicating how well the body functions.

Biomarkers are important in clinical research because, while it is relatively easy to measure the level of toxicants in aerosol or cigarette smoke in the lab, these measurements are a lot more difficult to make once the chemicals are in the body. There are many reasons for this, including the fact that chemicals break down or are converted to other products (metabolites) in the body, and that the amount of a substance in a product, or extracted during use, is not necessarily the same as the amount absorbed by the body. Biomarkers allow researchers to assess human exposure to toxicants in cigarettes and smoke-free products, and the resulting disease-related biological effects.

Some toxicants have more than one biomarker. For example, carbon monoxide (CO) is a product of incomplete combustion of organic matter, such as tobacco. Exhaled CO is used as a biomarker of acute or recent exposure to combustion. However, once in the body, CO binds rapidly to hemoglobin in the blood to form carboxyhemoglobin (COHb). COHb reduces the ability of the blood cells to carry oxygen, and so high levels of COHb in the blood are used to signal an impairment in oxygen transport due to CO exposure and an increased risk for cardiovascular or pulmonary disease.

There are a number of different ways to categorize biomarkers, but two of the most important are biomarkers of exposure (BoE) and biomarkers of potential harm (BoPH). Let's look at these two categories of biomarkers in more detail.

What are biomarkers of exposure?

Biomarkers of exposure are used to measure the levels of particular substances, such as potentially harmful chemicals in cigarette smoke or smoke-free product aerosol, which a user has been exposed to.

In research on cigarette and smoke-free product use, BoE are used to confirm exposure to specific nicotine or tobacco products, and to indicate changes in levels of exposure to specific chemical compounds when users switch between products—such as switching from cigarette smoking to THS use. To be useful, a BoE must be related to a specific chemical or exposure and must be capable of being reliably measured.

Nicotine, for example, rapidly breaks down into cotinine and several other metabolites once inside the body. So, in order to determine nicotine levels precisely, the presence of cotinine and several other metabolites of nicotine are assessed to give a measurement of nicotine equivalents (NEQ) or total nicotine equivalents (TNE).

In addition to nicotine, biomarkers signaling exposure to tobacco-specific nitrosamines (TSNAs) are used in many of PMI's clinical studies. TSNAs are a group of carcinogens formed from nicotine and related tobacco alkaloids. Important TSNA biomarkers include 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and N-nitrosonornicotine (NNN). Other important BoE include those for chemicals formed during the burning of cigarettes, such as benzo[a]pyrene and 1,3-butadiene.

What are biomarkers of potential harm?

Biomarkers that are used to measure an effect, such as those due to exposure to a chemical or substance are referred to as biomarkers of potential harm (BoPH). The effect measured may be changes in physiological structure or function, or clinical symptoms that can signal an increased risk of disease.

For example, changes in white blood cell count, high-density lipoprotein cholesterol levels (i.e., "good" cholesterol), or how much air a person can forcefully exhale in one second (FEV_1) can provide an indication of a person's health status or potential risk of disease.

Because chronic diseases associated with smoking, such as cancer, chronic obstructive pulmonary disease (COPD), and cardiovascular disease (CVD) take a long time to develop, BoPH are also used by regulatory bodies as surrogate indicators for assessing health risks when new products are introduced onto the market.

It is also important to keep in mind that a single biomarker can be both a BoE and a BoPH. For example, NNAL can signal both exposure to tobacco smoke and an increased risk of cancer.

How does PMI choose which biomarkers to study?

When planning a clinical study, researchers will often choose to measure a set of biomarkers which are generally representative of different chemical or toxicological groups, such as carcinogens, cardiovascular, or respiratory toxicants.

Additional criteria for choosing BoE for study may include:

- Biomarkers for substances that are specific to cigarette smoke and sensitive to smoking cessation.
- Biomarkers are measurable using validated, reliable, reproducible, and precise analytical methods.
- Biomarkers are clinically relevant.
- Biomarkers for substances that reflect a specific toxic exposure or are a reliable surrogate of exposure to those substances.
- Feasibility of observing an effect based on the study duration.

Some criteria for choosing BoPH for study may include:

- Biomarkers that signal the risk of developing a smoking-related disease, such as cardiovascular disease or emphysema.
- Biomarkers that signal the onset or presence of a specific smoking-related disease.
- Biomarkers linked to multiple aspects of pathogenesis associated with smoking-related diseases.
- Biomarkers for chemicals from a broad variety of organ toxicity classes, such as carcinogens, cardiovascular toxicants, respiratory toxicants, and substances that can interfere with fetal development (teratogens).

Researchers also often select biomarkers for study that are linked to chemicals recommended for lowering by regulatory organizations. For example, the U.S. Food and Drug Administration (FDA) has a list of 20 harmful and potentially harmful constituents (HPHCs) for which testing methods are well established and for which they require reporting. In addition, a group of nine toxicants has been proposed by the World Health Organization (WHO) Study Group on Tobacco Product Regulation (TobReg) for mandated lowering in cigarette smoke.

Examples of some of the biomarkers used in PMI studies and the toxicants of exposure or potential harm they are used to test for.

Biomarker	Toxicant/Chemical	Potential harm
Carbon monoxide (exhaled breath) Carboxyhemoglobin (COHb) (blood)	Carbon monoxide	COPD, CVD
FEV_1		Values lower than average can indicate reduced lung function
White blood cell count (WBC) (blood)		High values can indicate activated inflammatory pathways that can lead to risk of CVD, COPD, cancer, or infection
8-iso-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) (urine)		High levels can signal oxidative stress, which indicates risk of diseases including COPD and CVD
11-dehydrothromboxane B2 (11-DTX-B2) (blood)		Signals platelet activation, which can indicate risk of CVD
Soluble intercellular adhesion molecule-1 (sICAM-1) (plasma or serum)		High levels can indicate risk of CVD, diabetes, peripheral arterial disease, and cancer
Nicotine (plasma) TNE (urine) Cotinine (urine)	Nicotine	
NNAL (urine)	NNK	Cancer
2-Cyanoethylmercapturic acid (2-CyEMA) (urine)	Acrylonitrile	A TSN used to assess tobacco exposure, can indicate risk of cancer

Findings from PMI's clinical biomarker studies

PMI's clinical biomarker studies are designed to address questions related to the potential for harm reduction for those switching from cigarette smoking to THS use. These include whether the magnitude of biological changes seen in those who switch from cigarettes to THS is relevant and continues over time, and whether the reduction in exposure to harmful chemicals found in THS users is similar to that observed in those who quit smoking completely.

LOWER LEVELS OF BOPH SEEN 6 MONTHS AND 12 MONTHS AFTER SWITCHING TO THS

PMI researchers conducted a 6-month exposure response study, followed by a 6-month extension, to understand if the switching from cigarettes to predominately THS use is associated with favorable changes in eight BoPH. The studies assigned healthy adults who had been smoking for at least 10 years to either continue smoking their own brand of cigarettes or to switch to predominately THS use for the duration of the study.

After 6 months, all eight BoPH showed favorable changes in the THS users in the same direction as seen in those who quit smoking altogether, with five out of the eight showing statistically significant improvements compared with continued smoking. For participants who predominantly used THS, the favorable biological responses observed at 6 months were overall maintained at 12 months. The extension study also found that the fewer cigarettes smoked per day in addition to THS, the greater the magnitude of beneficial effects observed among healthy participants.

REDUCED EXPOSURE TO HARMFUL CHEMICALS WITH THS VERSUS CIGARETTES

PMI conducted a series of studies examining levels of biomarkers of exposure in existing adult smokers who switched to THS use, compared with continuing smoking and quitting smoking altogether. These three-arm studies compared groups of healthy participants who had not used THS before and switched to THS for the study, continued smoking their normal brand of cigarettes, or quit altogether for the duration of the study.

Together, these studies demonstrated that switching completely to THS resulted in significantly lower levels of 15 biomarkers representing exposure to harmful chemicals compared with those who continued smoking. The reductions among those who switched to THS were in the same direction observed among those who quit for the duration of the studies.

CROSS-SECTIONAL STUDY CONFIRMS EARLIER FINDINGS

Our most recently completed clinical study, which has not yet been published, demonstrated favorable improvements in nine BoPH among healthy participants who switched completely from cigarettes to THS for at least 2 years (on average 4.5 years), compared with those who continued to smoke. The magnitude of changes seen were larger than those observed in the exposure response study of recently started THS users, where participants could use cigarettes along with THS. This sustained effect among former smokers who had switched to THS demonstrates the importance of fully replacing cigarettes with THS to achieve maximum harm reduction potential.

Conclusion

The use of biomarkers makes it make it possible for researchers to measure exposure to harmful chemicals and the effects of that exposure in real life. This makes them an important tool for comparing the effects of exposure to THS aerosol and cigarette smoke.

Overall, the data from PMI's biomarker studies provide evidence that users of THS have lower exposure to HPHCs and reduced signs of negative health impacts related to smoking, including emphysema, cancer, COPD, and CVD, compared with cigarette smokers.

Importantly, the data indicates that the fewer cigarettes smoked, the higher the beneficial impact, with the biggest improvements seen in those who switch completely to THS.

For existing smokers, the best way to maximize the reduction of risk is to quit tobacco and nicotine altogether, but for those adults who don't quit altogether, smoke-free products can be a better choice than continued smoking.

PMI PUBLICATIONS

Applying new approach methodologies to assess next-generation tobacco and nicotine products

This review explored the use of new approach methodologies (NAMs), such as *in silico*, human *in vitro* tissue systems, and computational screening approaches, in testing novel tobacco and nicotine products (TNPs), including smoke-free products. The study examined NAMs used for comparing TNP emissions with cigarette smoke, including dosimetry, high-content screening, genotoxicity flow cytometric assays, organ-on-a-chip models, and others.

Overall, results indicated reduced bioactivity of TNPs when compared with cigarettes. The researchers also highlighted key considerations for the effective use of NAMs for testing TNPs, including the use of appropriate in vitro model systems, deploying screening approaches for hazard identification, the importance of fit-for-purpose testing and method standardization, and the value of industry and cross-industry collaboration. They conclude that supporting the development of NAMs that are accepted by regulatory bodies could aid in TNP testing.

Nicotine flux and pharmacokinetics-based considerations for early assessment of nicotine delivery systems

Nicotine flux—a metric that describes the rate of nicotine emitted from a device (microgram/second)—has been proposed as a regulatory tool for electronic nicotine delivery systems, which would limit one metric that is proposed to be associated with the abuse liability of an individual tobacco product. For this proposal to be viable regulatory target, it assumes a direct relationship between nicotine emission from a device and nicotine delivery to the user, while also assuming that a high flux product has the same abuse liability as a low flux product.

To analyze if increases in nicotine flux correspond with a rapid rise in systemic nicotine exposure, and what effect this might have on abuse liability outcomes, PMI researcher evaluated existing data from studies on clinical nicotine pharmacokinetics for products with different routes of administration.

The researchers found little relationship between nicotine flux from the device and total dose of nicotine delivered to the user, suggesting that nicotine flux may not be reliable for assessing nicotine delivery systems. When assessing the relationship between flux and 52-week quitting, as a measure of nicotine delivery that would facilitate switching, the authors found almost no relationship between nicotine flux and 52-week quitting or switching success, but a significant relationship between quitting and physiologic measures of nicotine delivery. Overall, these results indicate that nicotine flux, by itself, is a poor indicator of human exposure to nicotine and of abuse liability.

Quantitative modeling of *in vitro* data using an adverse outcome pathway for the risk assessment of decreased lung function in humans

In the absence of epidemiological data, there is a need to develop computational models that convert *in vitro* findings to human disease risk predictions following toxicant exposure. A main challenge in predicting potential disease risk from preclinical studies is that in vitro and animal studies provide only limited insight into human risk. To meet this challenge, researchers at PMI conducted a study demonstrating how a quantitative approach using adverse outcome pathways (AOPs) can be used to evaluate potential risk of reduced lung function in THS users compared with continued cigarette smoking. The study combined data from a series of experiments using advanced airway cultures with mechanistic information provided by the Adverse Outcome Pathway Oxidative Stress Leading to Decreased Lung Function (AOP 411). Mathematical models were used to address each key event relationship within the AOP.

This approach provided a plausible prediction of lung function in response to THS use compared with continued smoking. The researchers concluded that this Adverse Outcome Pathway-based approach aligns with Integrated Approaches to Testing and Assessment (IATA) principles and may also present a basis for testing and assessment of tobacco products for future regulatory decision making.

Non-targeted analytical comparison of a heated tobacco product aerosol against mainstream cigarette smoke: does heating tobacco produce an inherently different set of aerosol constituents?

Studies have found that heated tobacco product (HTP) aerosols contain lower levels of harmful and potentially harmful constituents (HPHCs) than cigarette smoke, but less is known about constituents that are of toxicological relevance and intrinsically higher in HTP aerosols. This work provides a comprehensive comparative assessment of aerosol produced by the THS and cigarette smoke with the aim of identifying compounds in the THS aerosol that are unique or increased compared with those found in cigarette smoke. To focus on differences due to heating versus burning tobacco, confounding factors were minimized by using the same tobacco in both test items and not adding flavors.

Researchers found that only 3.5% of compounds—corresponding to 31 distinctive compounds—were significantly more abundant in THS aerosol than in cigarette smoke. The results demonstrate that, using the testing methods applied in this study, heating a glycerol-containing tobacco substrate to the temperatures applied in THS does not introduce new compounds in the resulting aerosol, compared with cigarette smoke.

Impact of switching from cigarette smoking to tobacco heating system use on biomarkers of potential harm in a randomized trial

In the absence of epidemiological data, researchers at PMI conducted a clinical study to evaluate the potential benefits of switching from cigarettes to the THS, and the potential of THS to reduce the risk of developing smoking related diseases. The study was designed to verify if the reduction in exposure to HPHCs was associated with favorable changes in eight biomarkers of potential harm (BoPH), and if these changes were maintained over the course of the study. The 6-month initial study and 6-month extension were conducted in healthy adult smokers who entirely or predominantly switched to THS.

In the initial study, all eight BoPH showed favorable changes in the same direction as with smoking cessation, even though the THS group were still allowed up to 30% concomitant use of cigarettes. The extension demonstrated that favorable biological responses were overall maintained at 12 months, compared with participants who continued smoking cigarettes. The data from the extension also indicated that the fewer cigarettes smoked per day in addition to THS, the greater the magnitude of beneficial effects among healthy participants.

Overall, the results of this study confirm that, for adult smokers, switching to THS likely presents less risk of harm than continued smoking.

A comparative assessment of HPHC yields and *in vitro* toxicity for 1R6F reference cigarette smoke versus aerosol generated by Tobacco Heating System 3.0

In this paper, researchers at PMI describe the results of their investigation into the chemical composition, physical properties, cytotoxicity, genotoxicity, and mutagenicity of the aerosols produced by THS 3.0, commercialized as *IQOS ILUMA*. A standard battery of *in vitro* toxicology tests was performed, including looking for both known compounds (targeted toxicological screening) and new compounds (nontargeted toxicological screening). The results demonstrated that THS 3.0 has similar aerosol emissions and *in vitro* toxicity as THS 2.2, and substantially lower levels of HPHCs, when compared with cigarette smoke, leading to reduced in vitro toxicity and genotoxicity.

PMI SCIENCE

PHILIP MORRIS INTERNATIONAL

Important information

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 text in these pages include our product development and assessment efforts, our initiatives to share our methodologies and results, as well as our publications.

More detailed information can be found at www.pmiscience.com.