PMI SCIENCE
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An Assessment Strategy for Modified-Risk Tobacco Products (MRTP)

February 3rd, 2017

Presented by Carine Poussin, PhD

This research was funded by Philip Morris International

Tobacco Harm Reduction

- Smoking is addictive and causes a number of serious diseases

- Worldwide it is estimated that more than one billion people will continue to smoke in the foreseeable future*

- Successful harm reduction requires that current adult smokers be offered a range of Reduced Risk Products so that consumer acceptance can be best fulfilled

- Our ambition is to lead a full-scale effort to ensure that non-combustible products ultimately replace cigarettes to the benefit of adult smokers, society, our company and our shareholders

* http://www.who.int/tobacco/publications/surveillance/reportontrendstobaccosmoking/en/index4.html
Figure adapted from Clive Bates presentation to E-Cigarette Summit (19 Nov 2013)
Note: Reduced-Risk Products ("RRPs") is the term we use to refer to products that have the potential to reduce individual risk and population harm in comparison to smoking combustible cigarettes

Introduction to PMI R&D

Technology and Scientific Network (2014)
- 2 R&D Centers
- Major partners and service providers

Extensive and rigorous scientific assessment studies using quality and regulatory standards

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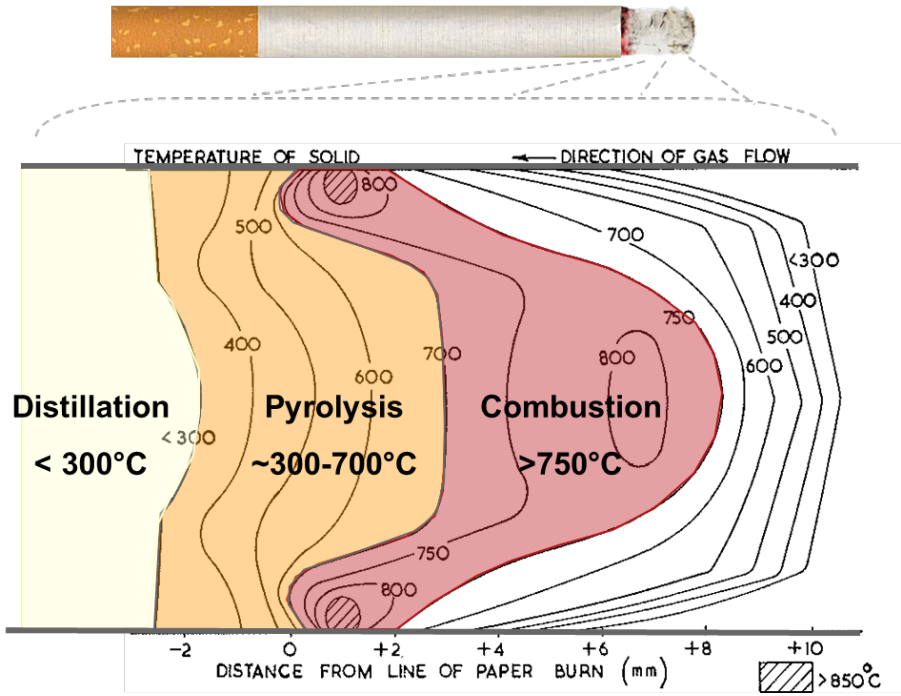
Eliminating combustion is key…

- More than 6,000 constituents identified in cigarette smoke
- About 100 of these constituents are categorized as **harmful or potentially harmful constituents** ("HPHCs")
- Most of the HPHCs are formed when the tobacco burns



Electrically Tobacco Heating System (THS) 2.2 – Operating Principles

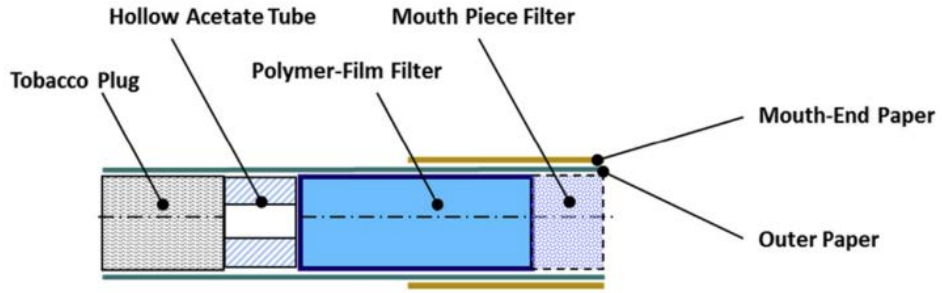


Fig. 3. Cross-sectional view of the *tobacco stick*.

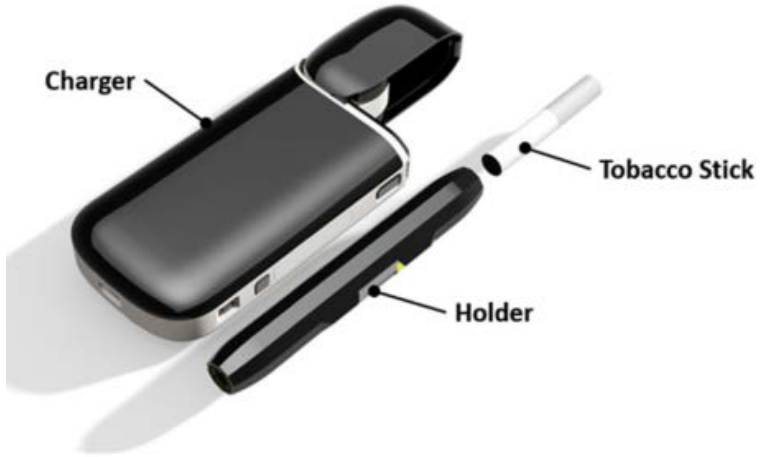


Fig. 2. The three components of the THS 2.2 product.

Heating engine precisely controlled using built-in software

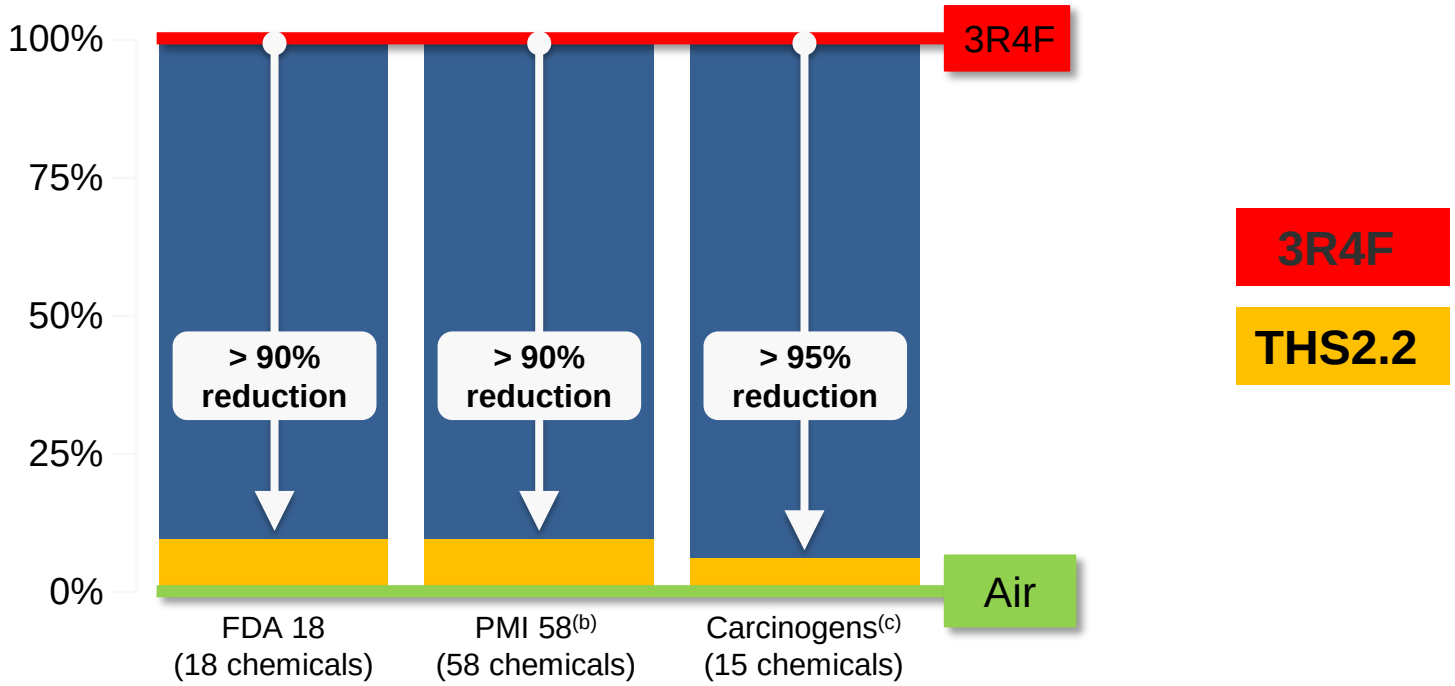
- Heater maintains tobacco temperature in the distillation range (< 350 ° C)
- Heater also acts as a temperature sensor

Smith MR, Regul Toxicol Pharmacol, 81:17-26 (2016)

PMI RESEARCH & DEVELOPMENT

Aerosol Chemistry

Average reductions in formation of harmful or potentially harmful constituents for **THS2.2** compared to levels measured in smoke from the **3R4F reference cigarette***



*Aerosol collection with Intense Health Canada's Smoking Regime (55 mL puff volume, 2 second puff duration, 30 second interval puff); Comparison on a per-stick basis Reduction calculations exclude Nicotine, Glycerin and Total Particulate Matter
The PMI 58 list includes the FDA 18 and the 15 carcinogens of the IARC Groups 1

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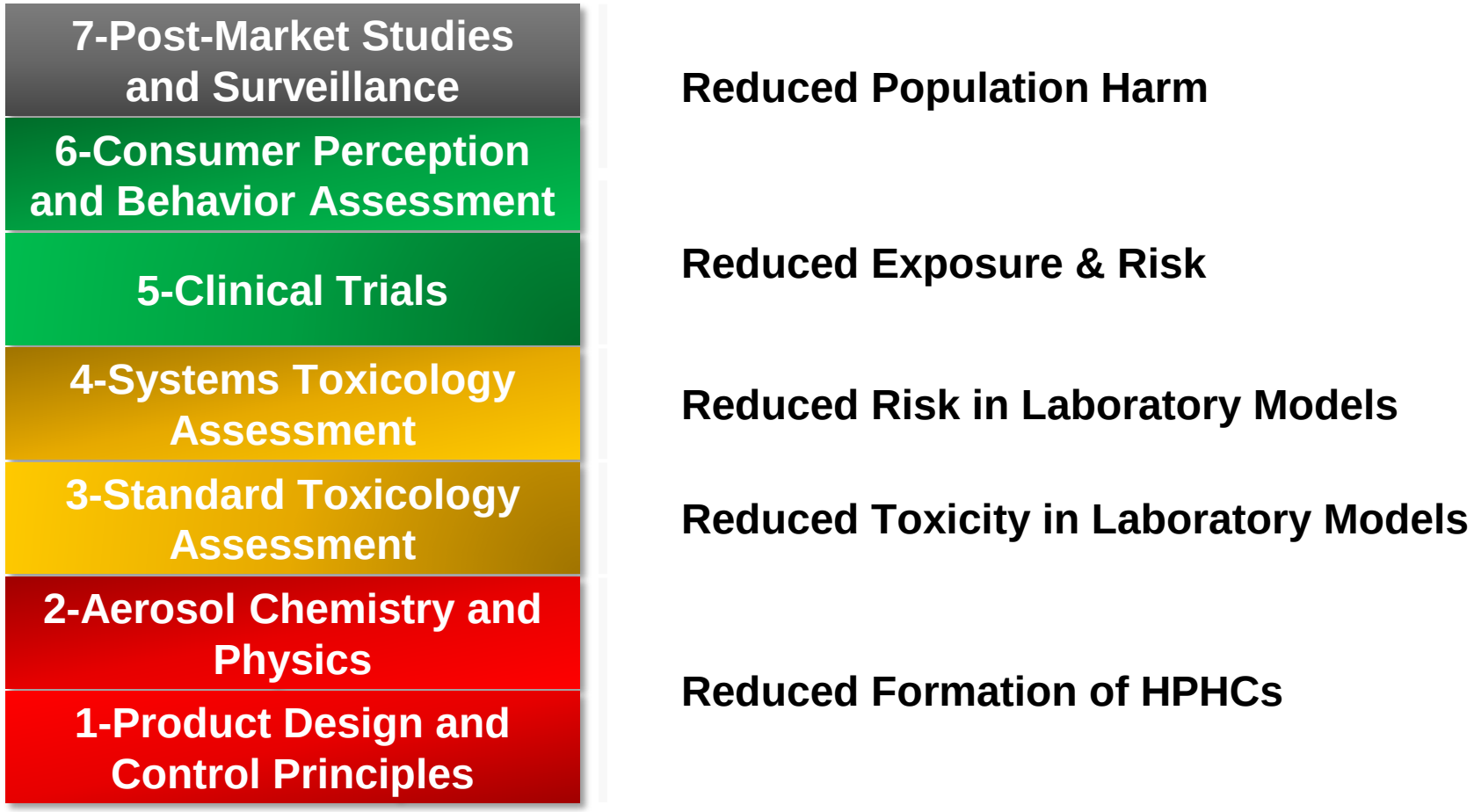
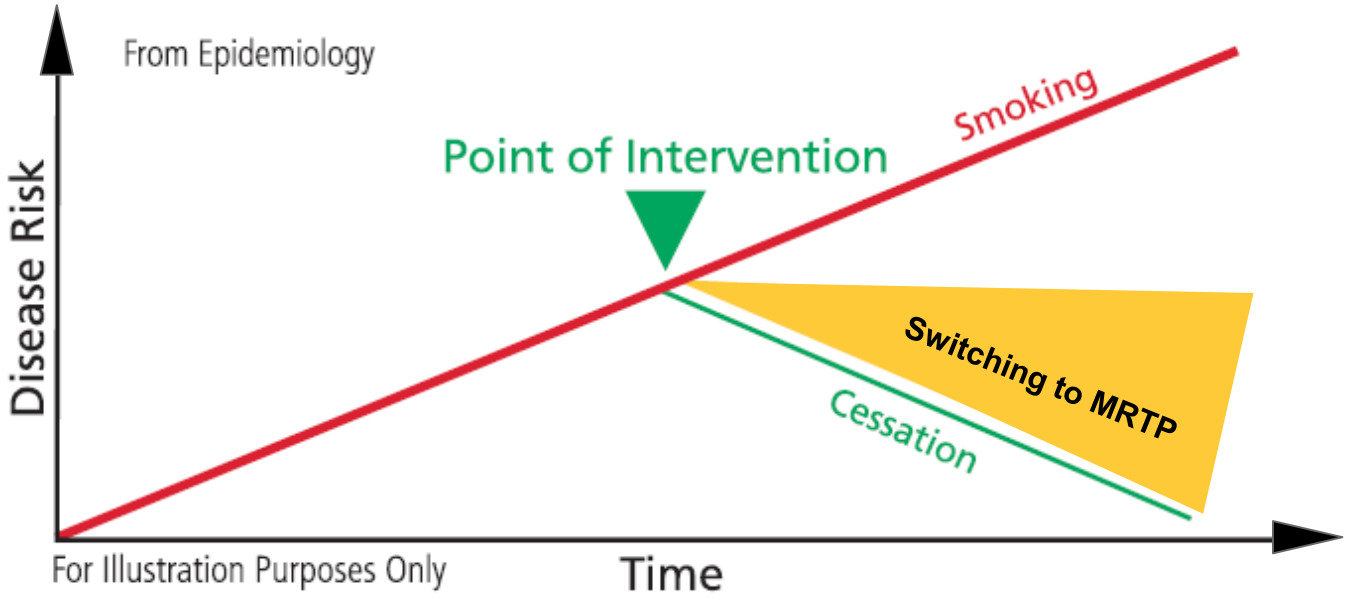
Developing Scientific Evidence: MRTP Assessment Program



Smith MR, Regul Toxicol Pharmacol, 81:17-26 (2016)

Risk Framework for MRTP Assessment

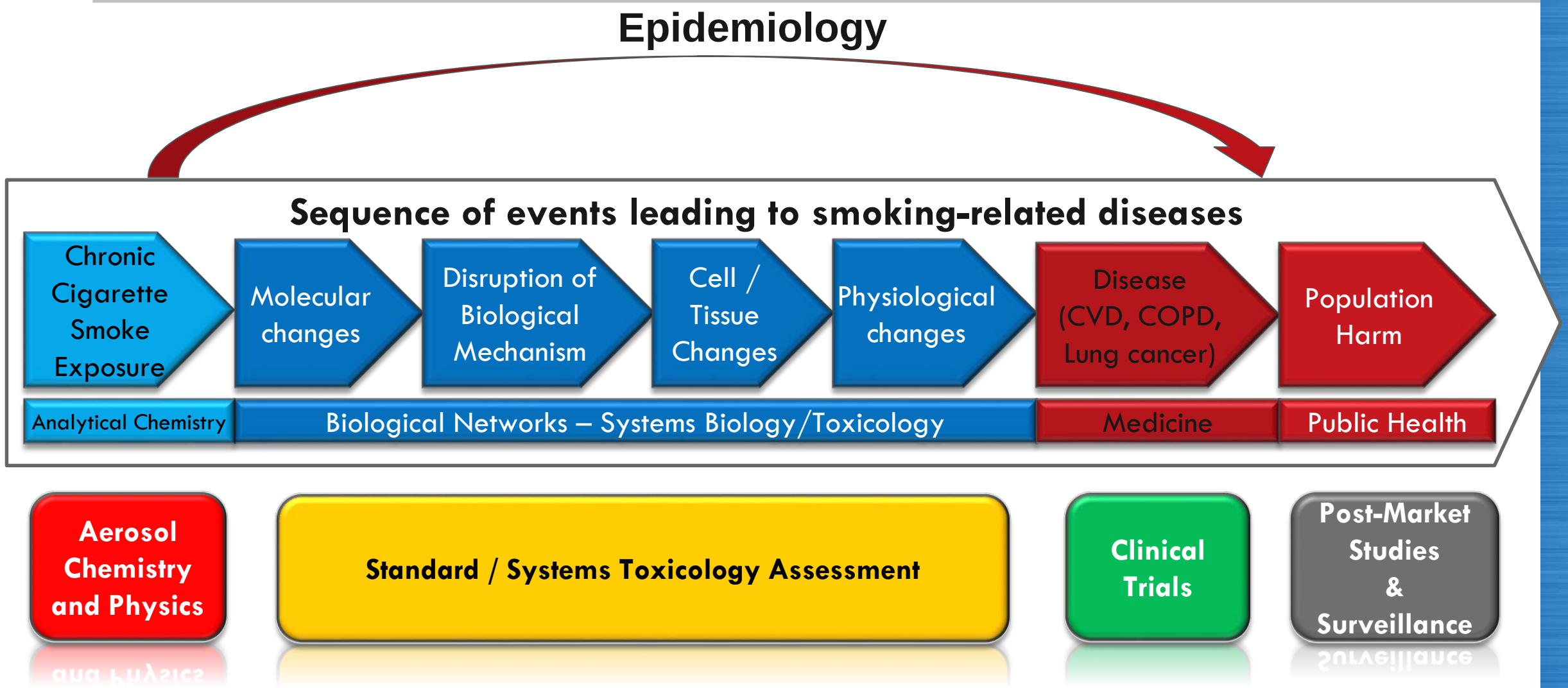


MRTP: Modified-Risk Tobacco Product

- Compare **switching to a candidate MRTP** with continued smoking and benchmark against **smoking cessation** (= “gold standard” as defined by US Institute of Medicine state)
- Assess **how close switching to candidate MRTP** is to smoking cessation

Source: IOM (Institute of Medicine), 2012, Scientific Standards for Studies on Modified Risk Tobacco Products. Washington, DC: The National Academies Press

From Chronic Exposure to Population Harm: A Causal Chain of Events



Smith MR, Regul Toxicol Pharmacol, 81:17-26 (2016)

PMI RESEARCH & DEVELOPMENT

Developing Scientific Evidence: MRTP Assessment Program

Reduced Population Harm

Reduced Exposure & Risk

Reduced Risk in Laboratory Models

Reduced Toxicity in Laboratory Models

Reduced Formation of HPHCs

Smith MR, Regul Toxicol Pharmacol, 81:17-26 (2016)

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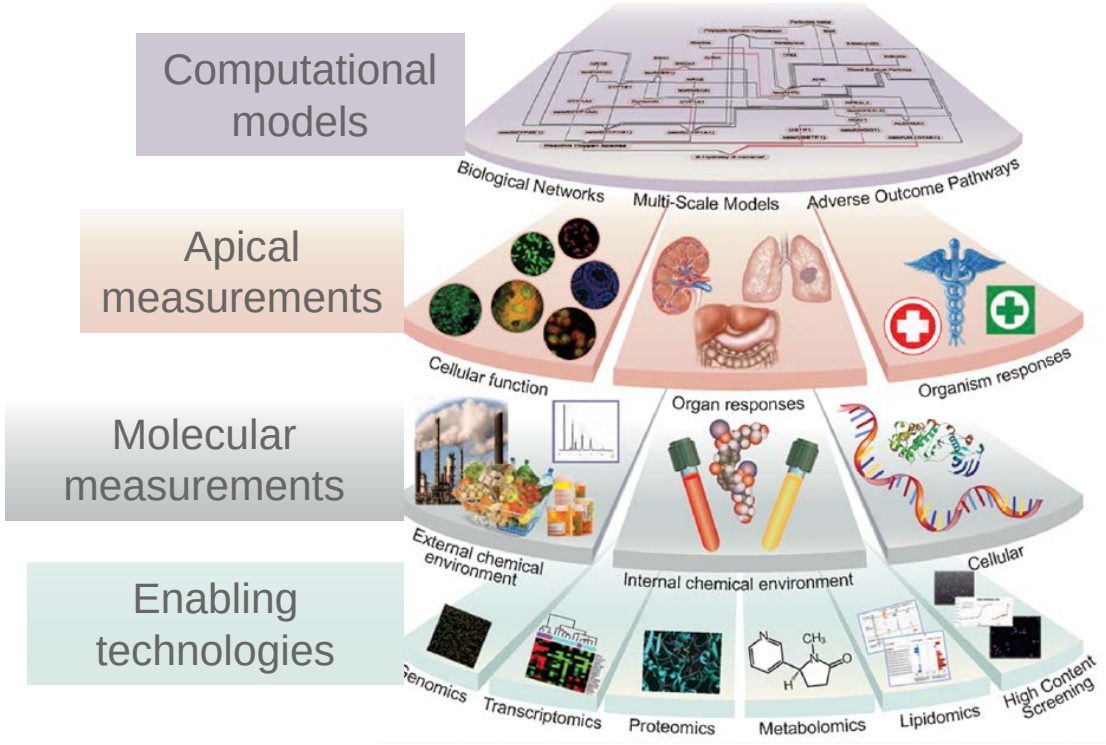
Systems Toxicology for Comparative Product Testing - Background

This research was funded by Philip Morris International

Systems Toxicology

- Decoding the toxicological blueprint of active substances that interact with living systems
- Integrates classic toxicology approaches with network models and quantitative measurements of molecular and functional changes occurring across multiple levels of biological organization



Detailed mechanistic understanding of toxicology

Prediction of adverse outcomes

New paradigm for risk assessment

Environmental protection

Safe drugs

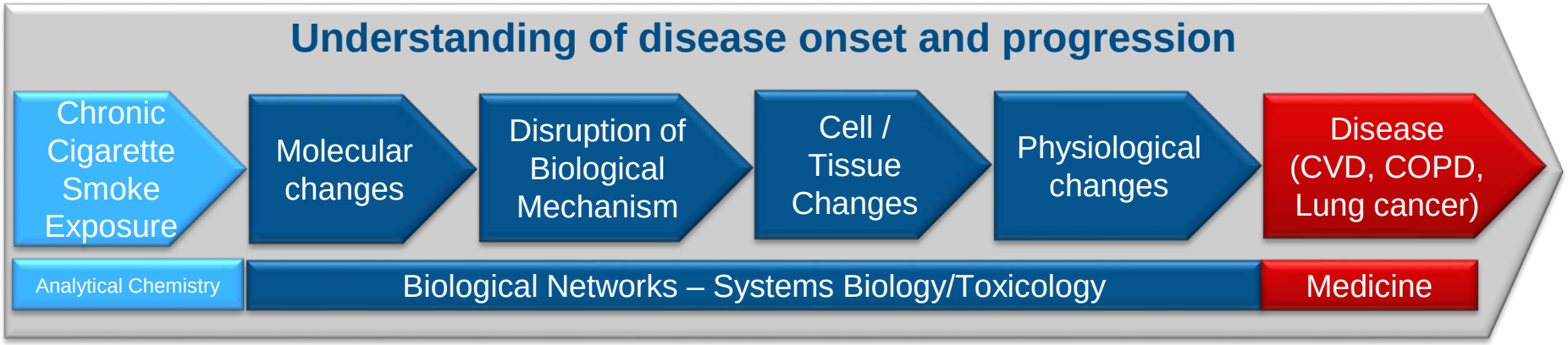
Green chemistry

Safe food

PMI RESEARCH & DEVELOPMENT

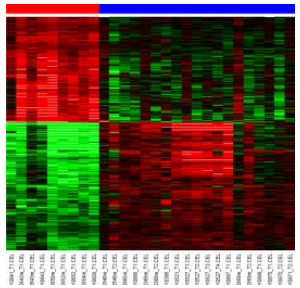
Sturla SJ, Boobis AR, Fitzgerald RE et al. (2014) Systems Toxicology: from basic research to risk assessment. Chemical research in toxicology 27:314-329

System Toxicology Research
Identify and Represent Disease Mechanisms

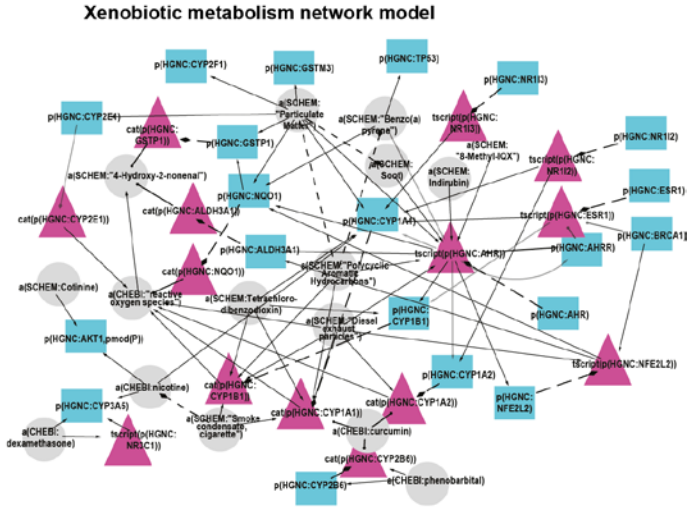


Literature-derived knowledge

Data-derived knowledge



Identify & Build Biological Network models



Hoeng et al., Case Study: The Role of Mechanistic Network Models in Systems Toxicology. **Drug Discovery Today**, 2013, 19:183-192. (PMID: 23933191)

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Methods - Systems Toxicology Assessment
Use Disease Mechanism Understanding for Product Assessment

Exposure

Test items

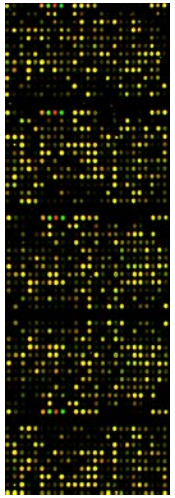
Test system

3R4F

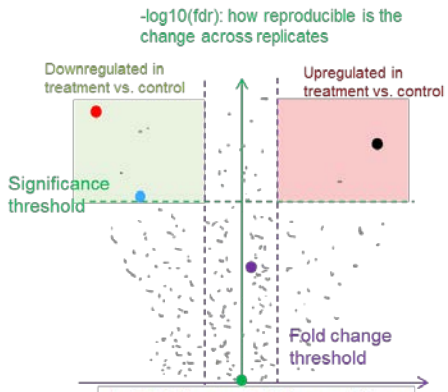
Sham

MRTP

Multi-Omics Data



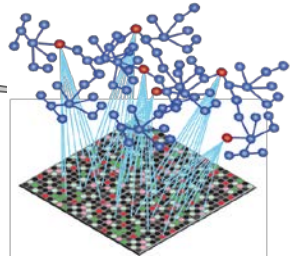
Systems Response Profile



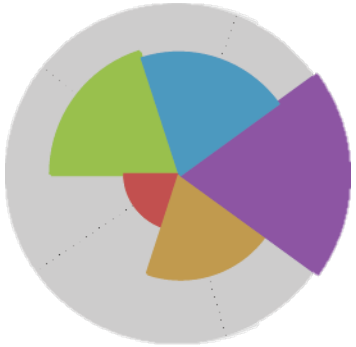
1 per condition vs. control

Biological Network models

- Inflammation (IPN)
- Cell Proliferation (CPR)
- Cell Stress (CST)
- Cell Fate (CFA)
- Tissue repair and angiogenesis (TRA)

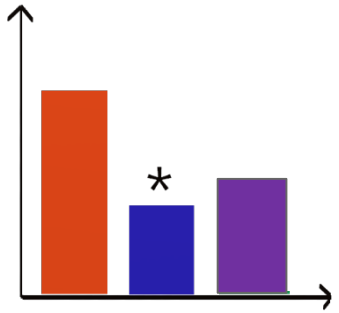


Network Perturbation Amplitudes



1 per condition vs. control

Biological impact factor (BIF)



comparing conditions

Σ

Hoeng J *et al*. A network-based approach to quantifying the impact of biologically active substances. **Drug Discovery Today,** 2012, 17:413-418 (PMID: 22155224)

Product Items and Mode of Exposure for Comparative Assessment

Conventional cigarettes:

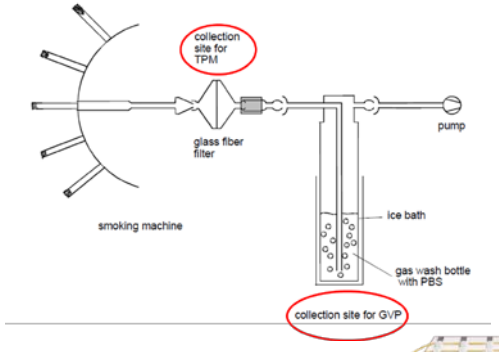
University of Kentucky Standard Reference Cigarette 3R4F

Generation of smoke using a standard smoking protocol: Health Canada Intense

Potentially modified-risk tobacco product:

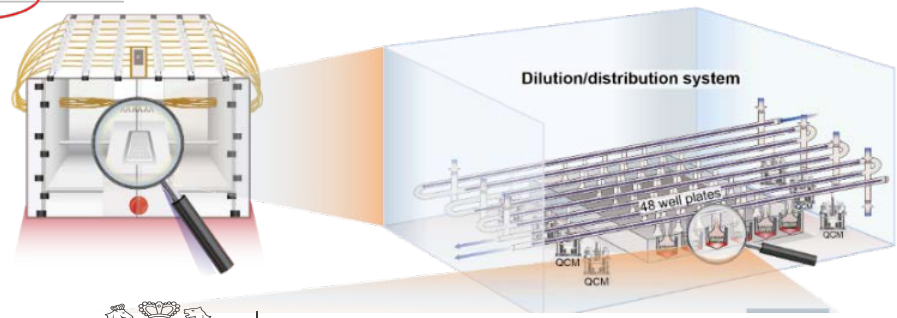
Heatsticks and Tobacco Heating System, THS 2.2

Trapping of aerosol for in vitro studies



Mode of Exposure

- **Smoke / Aerosol** (*in vivo* / *in vitro*)
- **Smoke/ Aerosol Fractions** (*in vitro*)
  - Gas vapor phase (GVP)
  - Total particulate matter (TPM)
  - Smoke/Aerosol bubbled in aqueous solution (Aqueous extract)



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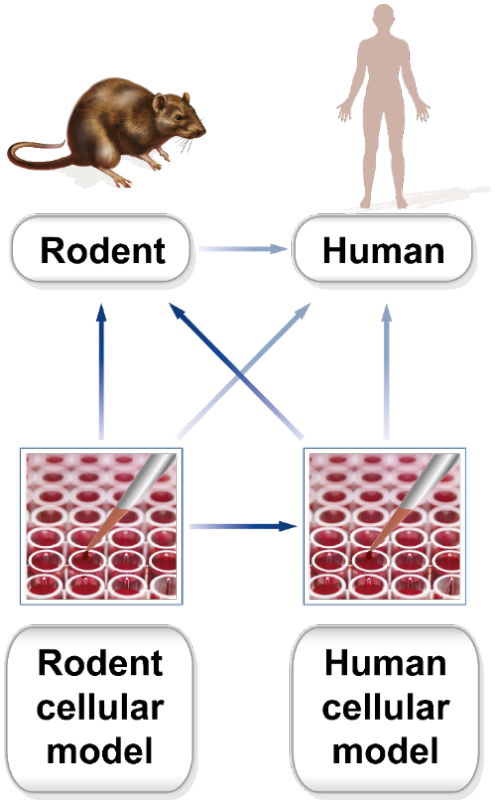
In vitro Systems Toxicology

This research was funded by Philip Morris International

Choice of *In vitro* Systems

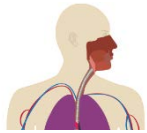
Translation between species and experimental systems



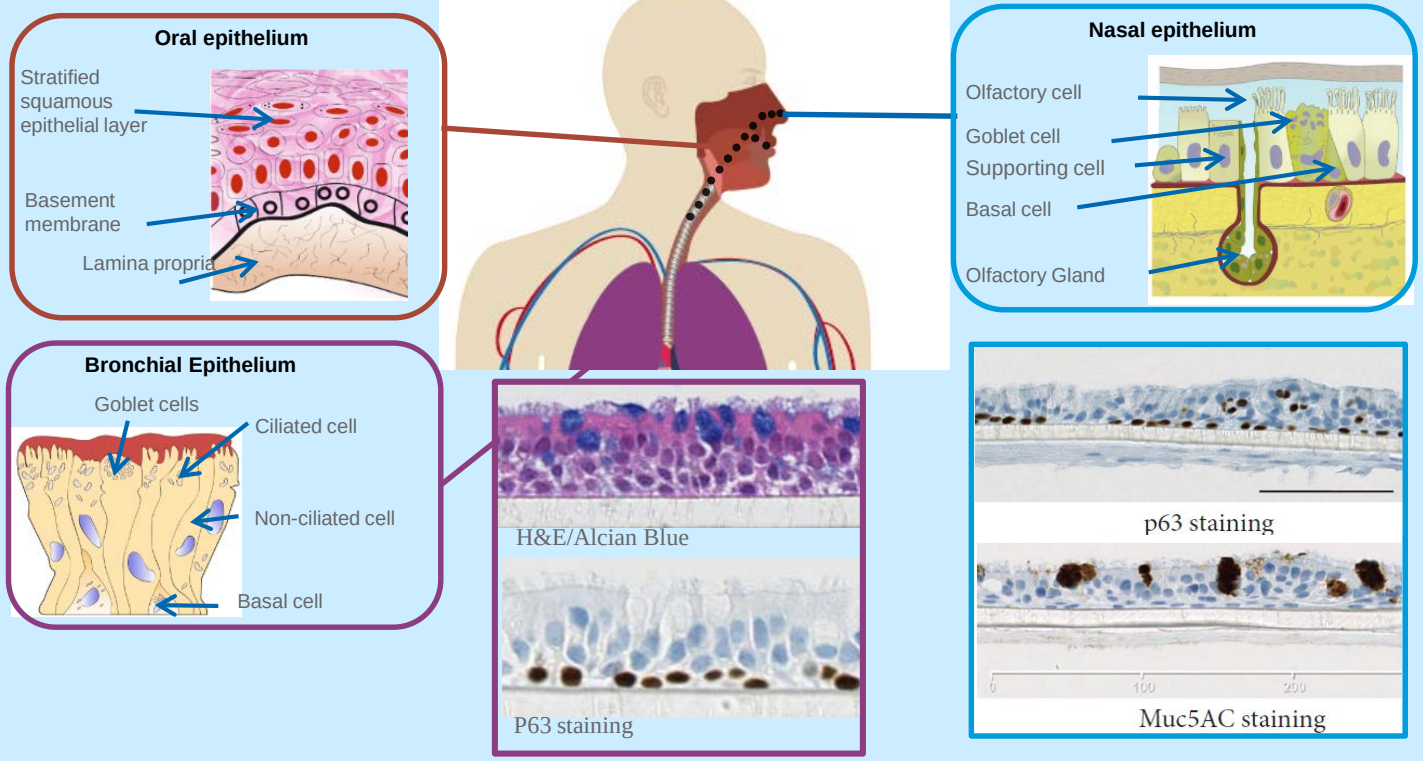
- Relevant for investigating cellular mechanisms of diseases
- Reliable for translational biology and toxicology
- Supports the principles of 3Rs (Replacing, Reducing, and Refine)
  - 2009 European Commission a report : Alternative Testing strategies for «Replacing, Reducing, and Refining» («3R») the use of animals in research (i.e. 3D cultures, organ-on-chip)

Poussin C et al (2014) The Species Translation Challenge - A Systems Biology Perspective on Human and Rat Bronchial Epithelial Cells. **Scientific Data,** 1:140009

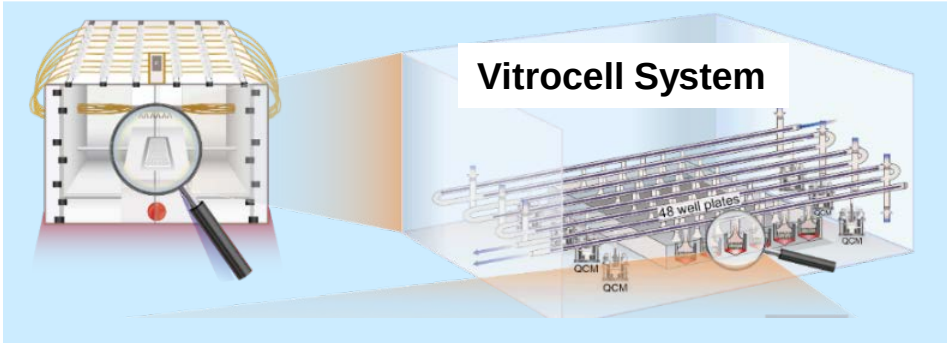
Organotypic Bronchial, Nasal, and Oral Tissue Cultures Resemble *in vivo* Respiratory Epithelium



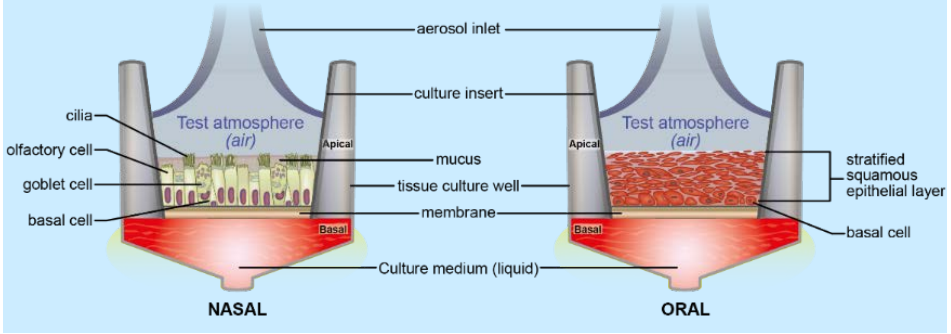
In vitro Systems



Exposure System

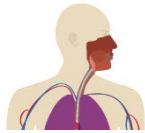


Air-Liquid Interface Organotypic Cultures



Sridhar et al, Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. **BMC genomics** 9: 259 (2008)
Iskandar et al, Systems approaches evaluating the perturbation of xenobiotic metabolism in response to cigarette smoke exposure in nasal and bronchial tissues. **BioMed research international**. 2013;2013.

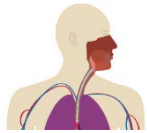
A Series of Studies Investigating Cigarette Smoke (CS) Exposure using 3D-Organotypic Upper and Lower Airway Epithelial Tissue Cultures



Author/ Year	Mode of Exposure	Duration	Cytotoxicity	Gene Expression	Cilia Frequency/ Function	Inflammatory Mediators	Epithelial Barrier Integrity	Additional endpoints
Mathis 2013	Single exposure to whole CS	7-28 min		✓		✓		miRNA profile
Iskandar 2013	Repeated exposure of whole CS	4 cigs		✓		✓		CYP activity
Talikka 2014	Repeated exposure of whole CS	4 cigs		✓		✓		Histology
Zhang 2014	Single exposure of CSE	1-4 h					✓	Glycosylation
Aufderheide 2015	Repeated exposure of whole CS	4 cig/d (8 d)	✓		✓			
Astrand 2015	Single exposure of whole CS	1 cig			✓			Epithelial sodium channel activity
Azzopardi 2015	Single exposure of whole CS	30 min	✓			✓		
Kuehn 2015	Repeated exposure of whole CS	4 cigs		✓	✓			CYP activity
Mathis 2015	Single exposure of whole CS	7-28 min		✓				miRNA profile
Iskandar 2015	Single exposure of whole CS	28 min	✓	✓		✓		Histology, CYP activity
Schamber 2015	Single exposure of CSE	7-28 d		✓	✓	✓		Protein expression

CSE: cigarette smoke extract. Studies conducted at PMI.

THS2.2 Aerosol Exposure Assessment using 3D Organotypic Cultures



Author/ Year	Mode of Exposure	Duration	Cytotoxicity	Gene Expression	Cilia Frequency/ Function	Inflammatory Mediators	Epithelial Barrier Integrity	Additional endpoints
Zanetti 2016 (Buccal)	Single exposure of whole CS and MRTP	28 min	✓	✓		✓	✓	Histology, CYP activity, miRNA profile
Iskandar 2016 (Nasal)	Single exposure of whole CS and MRTP	28 min	✓	✓	✓	✓		Histology, CYP activity, miRNA profile
Iskandar 2017 (Bronchial)	Single exposure of whole CS and MRTP	28 min	✓	✓	✓	✓		Histology, CYP activity, miRNA profile

Conclusions:

THS2.2 aerosol exposure has a reduced biological impact in human bronchial, nasal and oral cultures compared with 3R4F (reference cigarette) smoke at comparable nicotine concentrations

Atherosclerosis – Vascular Disorder

Development of atherosclerotic plaques over time

Endothelium activation and dysfunction

Leukocyte adhesion and transmigration

Foam cell formation

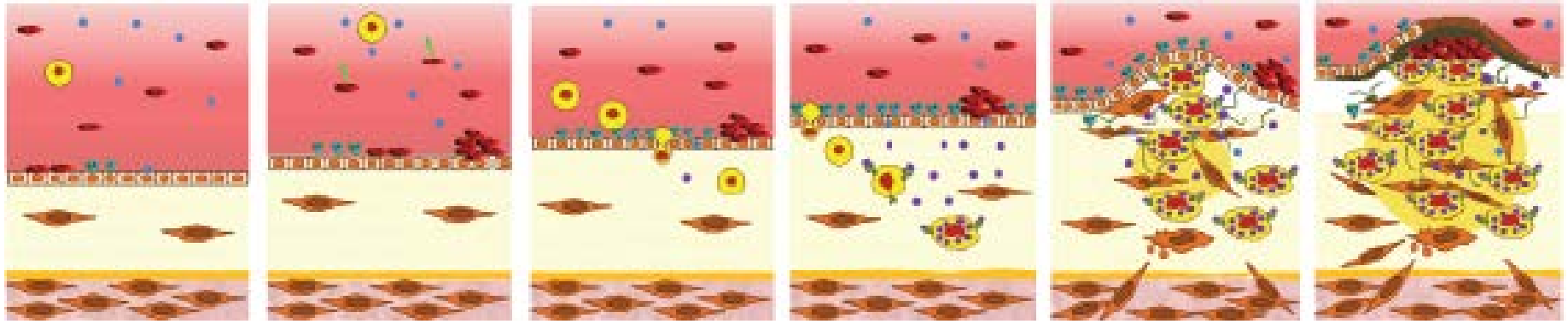
***In vitro* models**

Smooth muscle cell proliferation and migration

Coagulation

Plaque instability and rupture

Infarctus
Stroke



Libby et al, Nature 473 (7347): 317 (2011)

PMI RESEARCH & DEVELOPMENT

Case Study 1

Toxicology 339 (2016) 73–86

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Systems toxicology-based assessment of the candidate modified risk tobacco product THS2.2 for the adhesion of monocytic cells to human coronary arterial endothelial cells

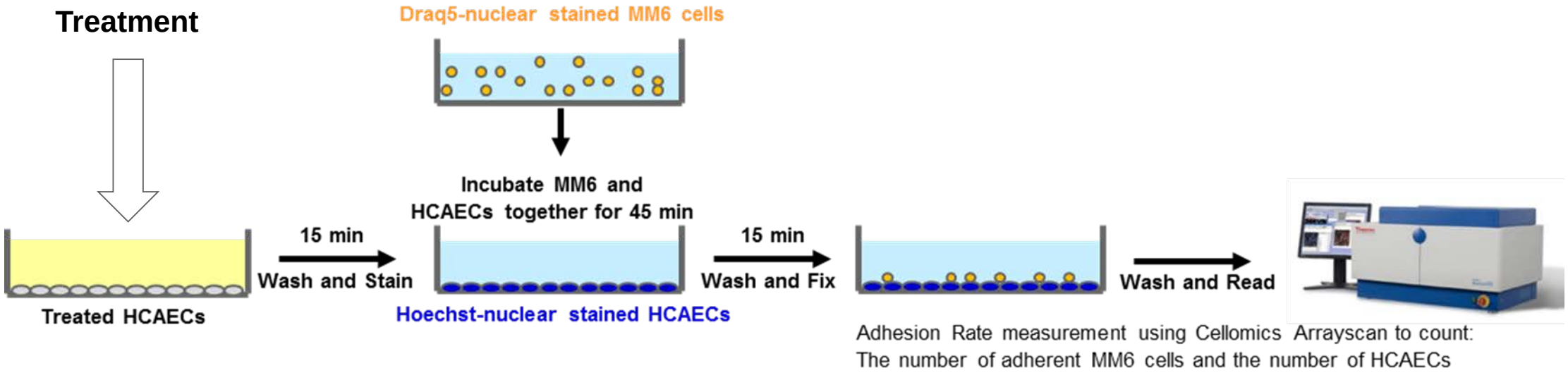
Carine Poussin*, Alexandra Laurent, Manuel C. Peitsch, Julia Hoeng, Hector De Leon

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

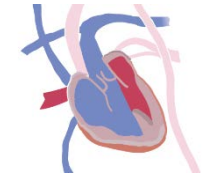
In Vitro Leukocyte-Endothelial Cell Adhesion Assay - Principles

- **Mono Mac-6 cells (MM6)**
  - Adhesion properties with characteristics of mature human monocytes (Erl et al, Atherosclerosis, 133:99-107 (1995))

- **Human coronary artery endothelial cells (HCAECs)**
  - Disease-relevant human primary cells



Concentrations of Carbonyls are reduced in THS2.2 Aqueous Extract Compared with 3R4F While Nicotine Concentrations remain Similar



Mode of Exposure: Aqueous extracts

	3R4F·sbPBS		THS2.2·abPBS		3R4F/THS2.2
Unit: ug/item	*Mean*	±*SD*	*Mean*	±*SD*	%
Formaldehyde	42.0	6.4	2.8	0.9	15.2
Acetaldehyde	1168.4	155.0	161.9	23.7	7.1
Acetone	526.2	48.3	29.4	5.3	17.8
Acrolein	128.3	15.4	7.0	1.4	18.3
Propionaldehyde	59.1	18.2	8.6	2.9	6.9
Crotonaldehyde	57.8	7.9	2.7	0.6	21.4
Methyl ethyl ketone	127.6	17.1	5.8	1.2	21.9
Butyraldehyde	21.2	3.8	8.6	0.7	2.5
Nicotine	77.6	8.4	68.4	4.9	1.1

Analytes highligthed in grey showed significant differences (*t*-test; $p<0.05$) between 3R4F and THS2.2.

s/abPBS: smoke/aerosol-bubbled phosphate buffered saline

Number of independent aqueous extracts (N):
For carbonyl analysis, N=34 and 16 for 3R4F and THS2.2, respectively.
For nicotine, N=5 for both.

Poussin et al, Toxicology 339:73-86 (2016)

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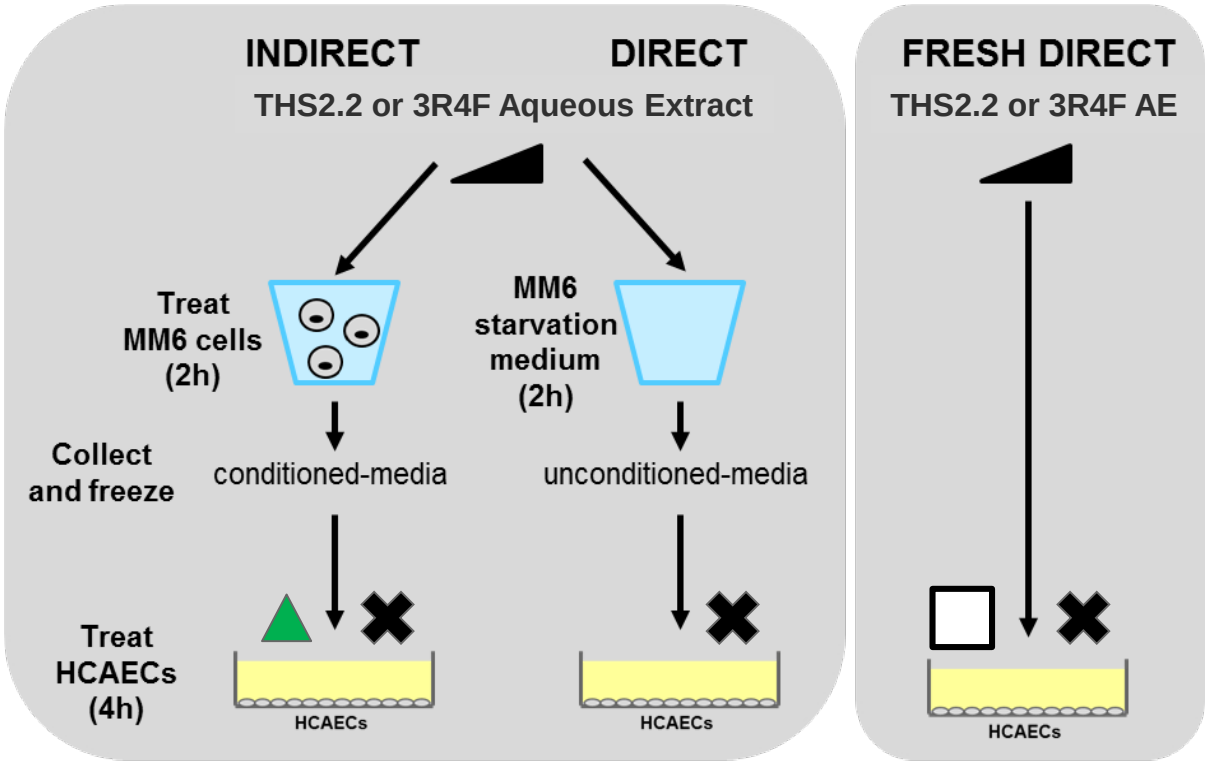
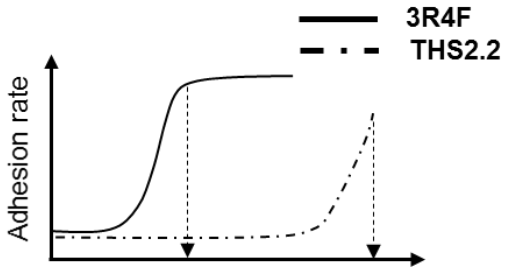
Study Design





Concentration: puffs/ml

- Adhesion assay (functional endpoint)
- Transcriptomics (molecular endpoints)

Monocyte-derived molecules
Stable aerosol chemicals
Unstable aerosol chemicals

Poussin et al, Toxicological sciences 147(2):370-85 (2015)
Poussin et al, Toxicology 339:73-86 (2016)

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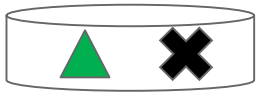
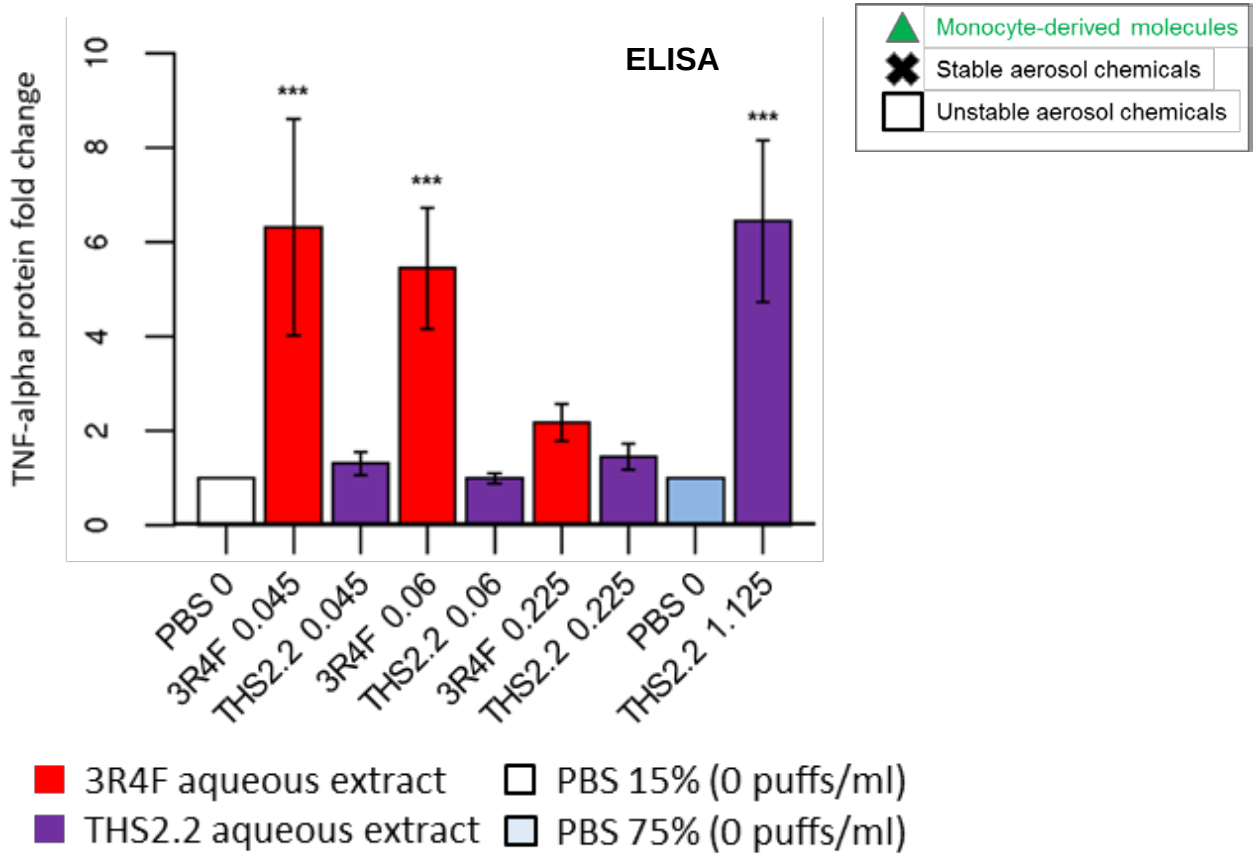
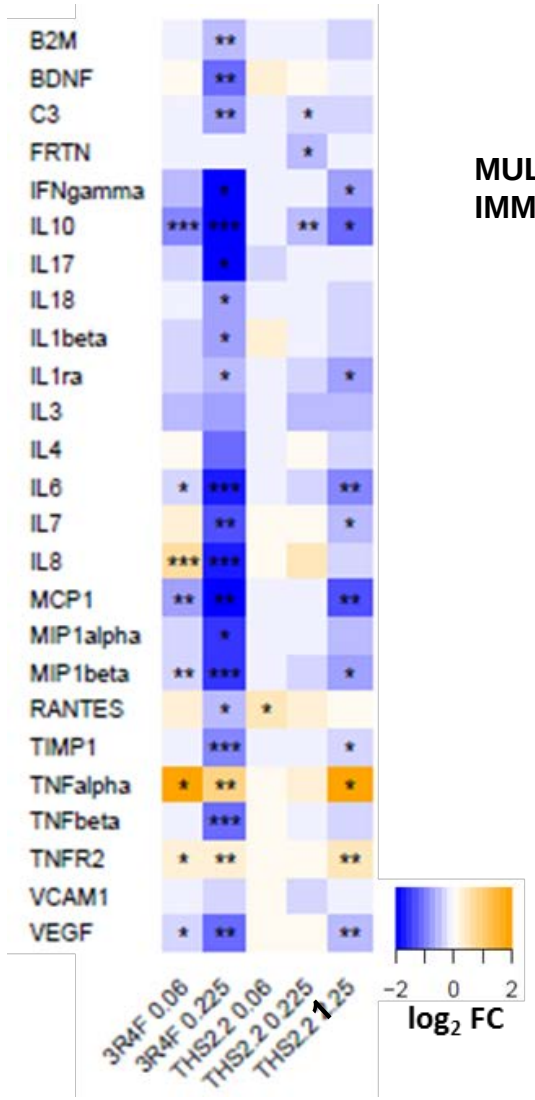
The Release of Inflammatory Mediators by Monocytic Cells is Reduced with THS2.2 Compared with 3R4F

TNF-alpha measured in MM6 conditioned-media







MULTIPLEXED IMMUNOASSAYS

Mean±SEM

***p≤0.05,**p≤0.01, ***p≤0.0001 vs. 0 puffs/ml (vehicle control)*

Poussin et al, Toxicology 339:73-86 (2016)

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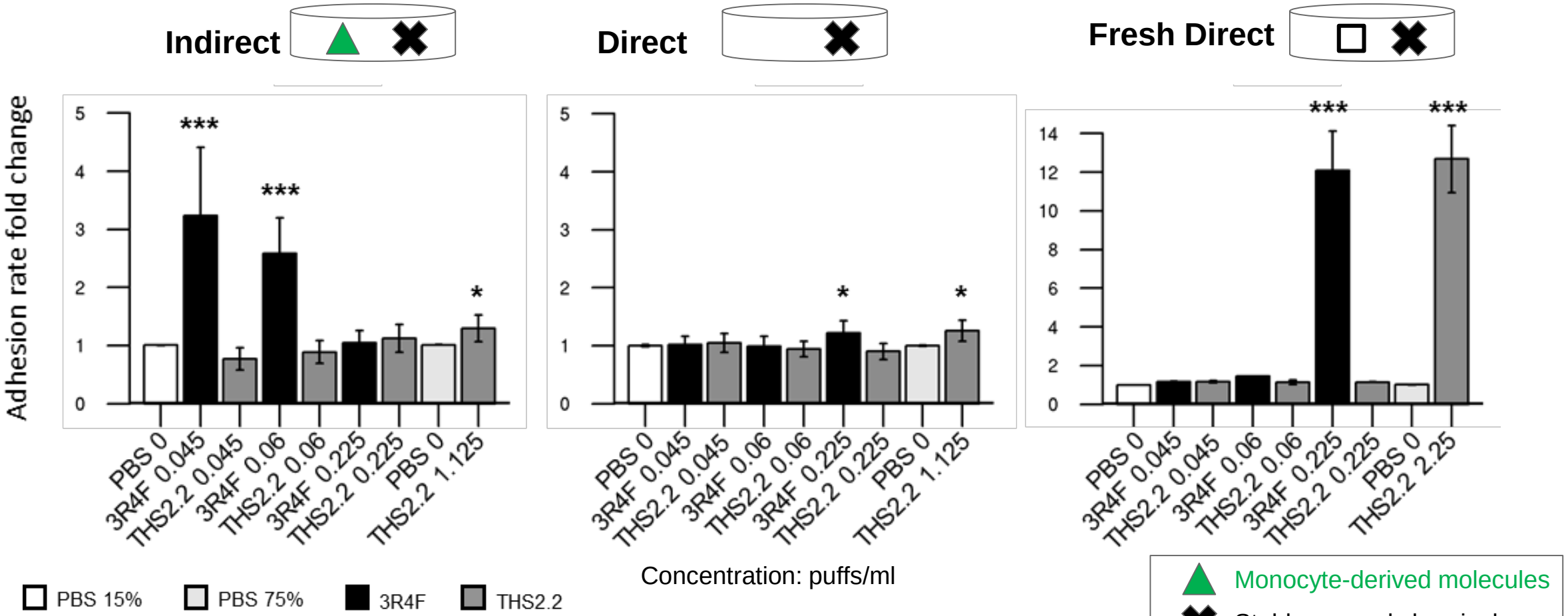
Reduced Effects of THS2.2 Compared with 3R4F on the Adhesion of Monocytic Cells to Coronary Artery Endothelial cells



Mean±SEM

***p≤0.05, ***p≤0.0001 vs. 0 puffs/ml (vehicle control)*

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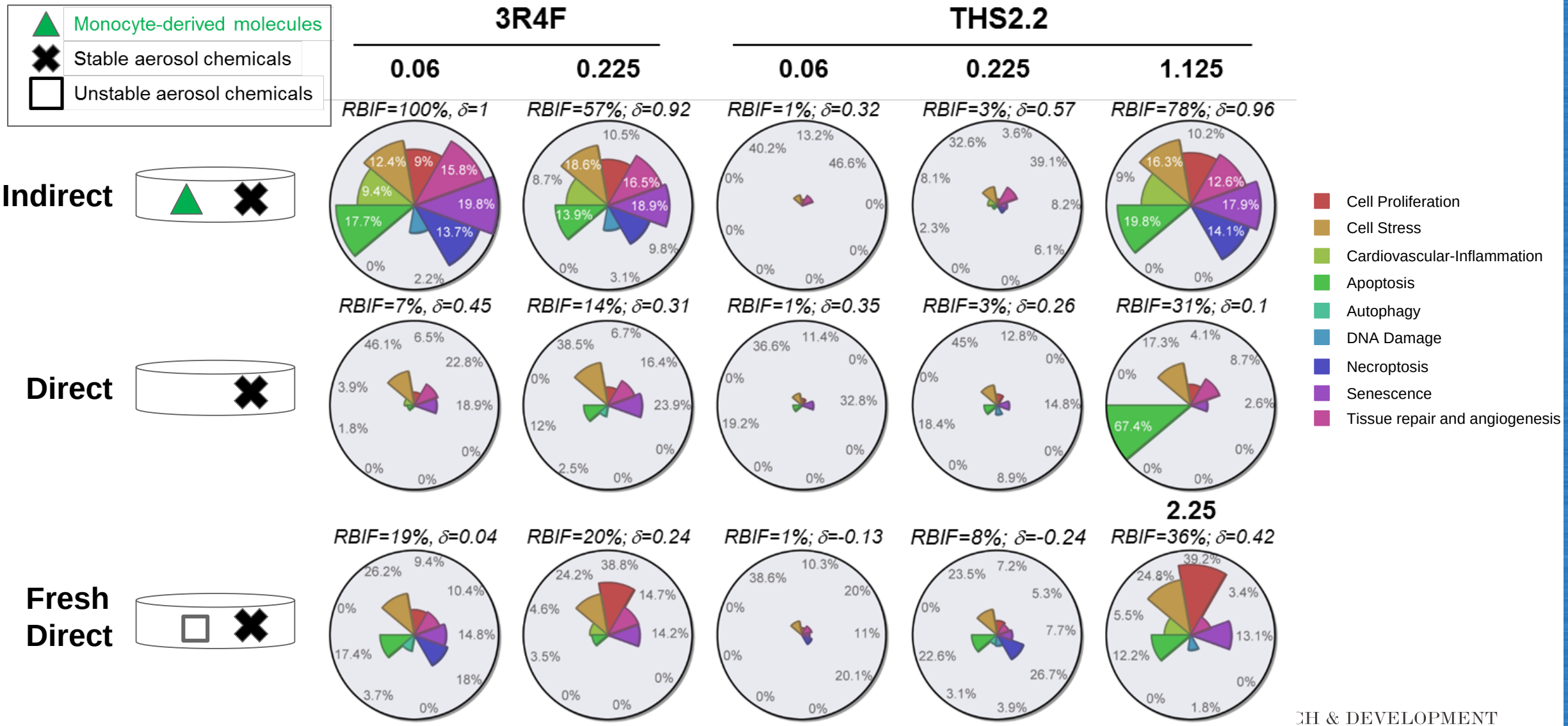
Poussin et al, Toxicology 339:73-86 (2016)

Reduced Biological Impact of THS2.2 Compared with 3R4F on Human Coronary Artery Endothelial Cells



Poussin et al, Toxicology 339:73-86 (2016)

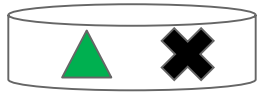
Underlying Mechanisms

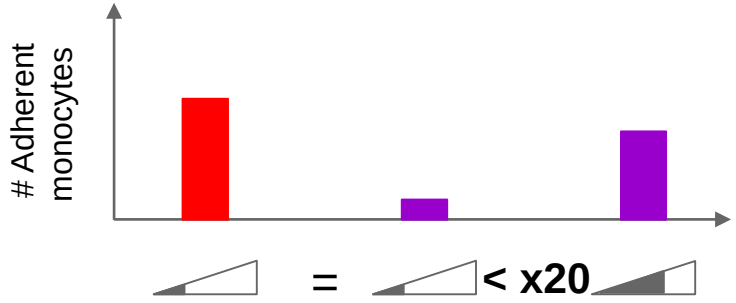
Monocyte-derived molecules
Stable aerosol chemicals
Unstable aerosol chemicals

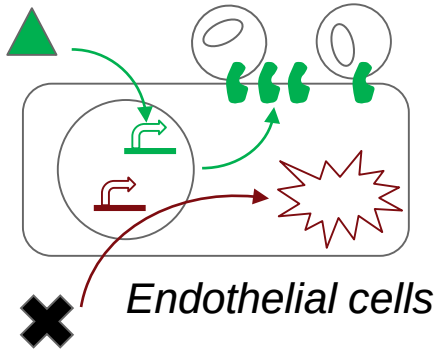
How many adherent monocytes?

What mechanism(s)?

Indirect

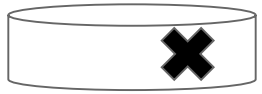


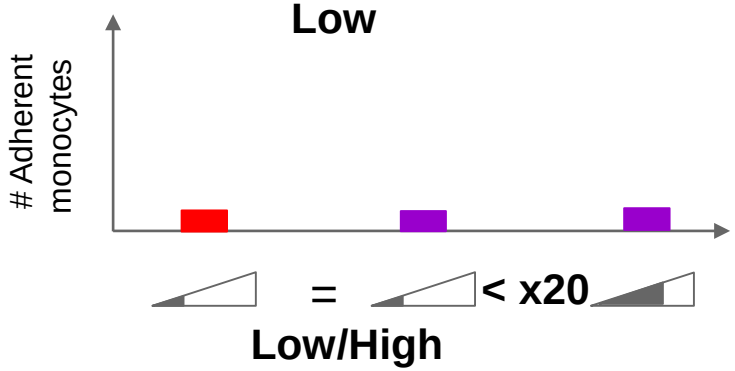


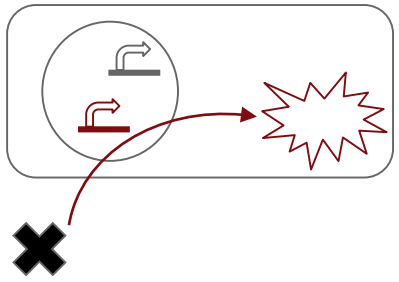


Inflammatory-driven adhesion

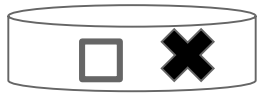
Direct

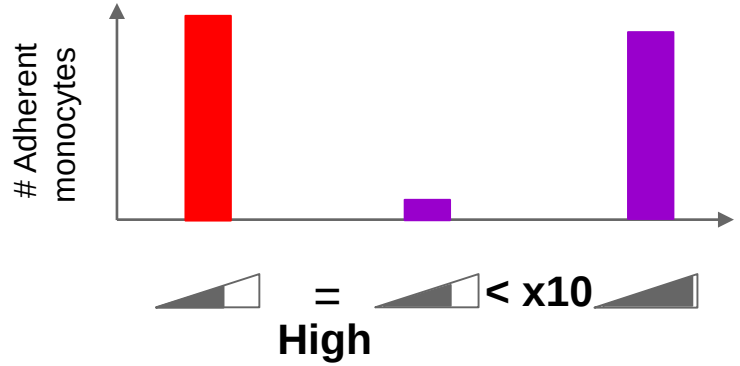


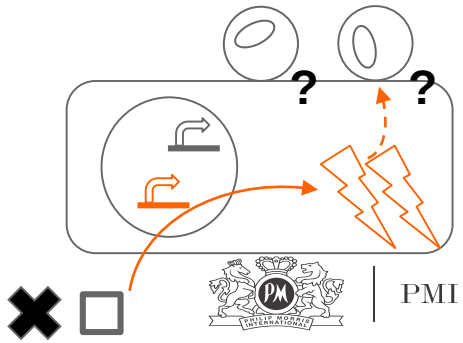




Fresh Direct







Cell Stress (cytotoxicity)-driven adhesion

PMI RESEARCH & DEVELOPMENT

Case Study 2: Assessment of THS2.2 compared with 3R4F on the Migratory Behavior of Monocytic cells (THP-1)

Food and Chemical Toxicology 86 (2015) 81–87

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short communication

Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes

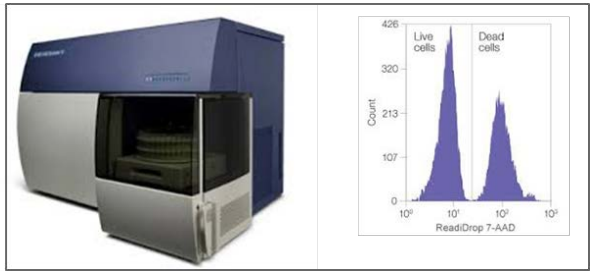
Marco van der Toorn[1,*], Stefan Frentzel[1], Hector De Leon, Didier Goedertier, Manuel C. Peitsch, Julia Hoeng

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

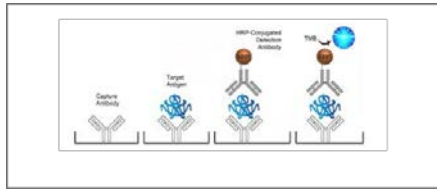
Reduced Effects of THS2.2 Compared with 3R4F on Monocytic Cell Cytotoxicity and Inflammatory Mediators Production

THP1 cells

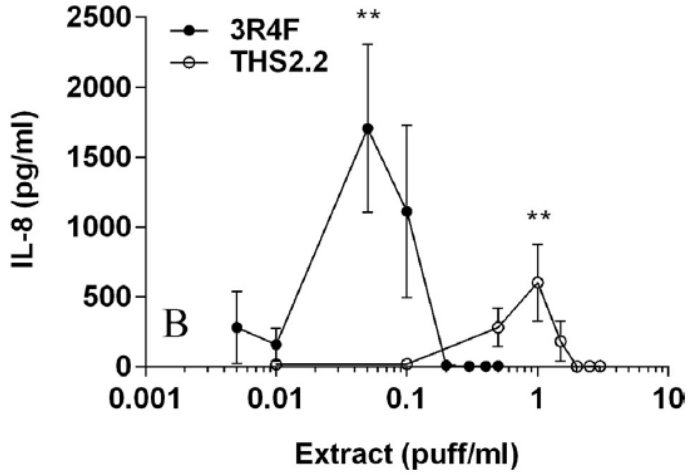
Cytototoxicity



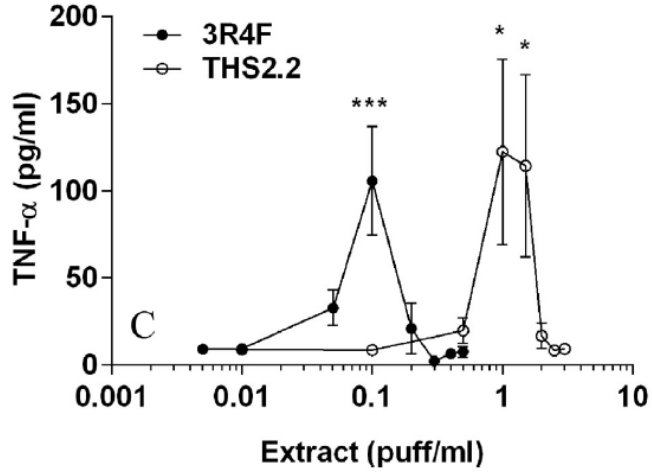
Inflammatory mediators

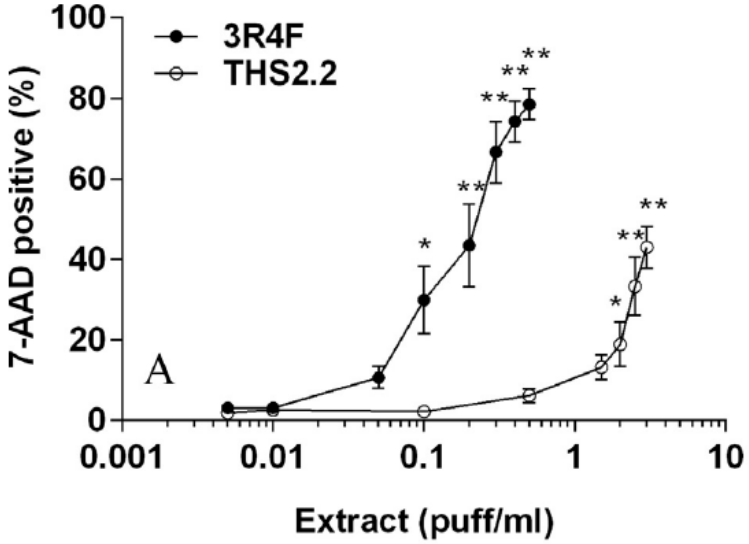


IL-8



TNF-Alpha

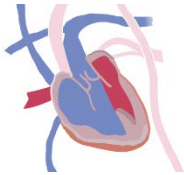


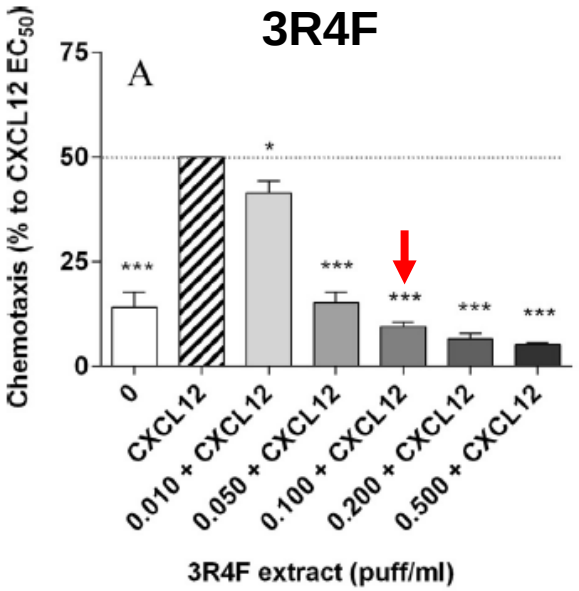


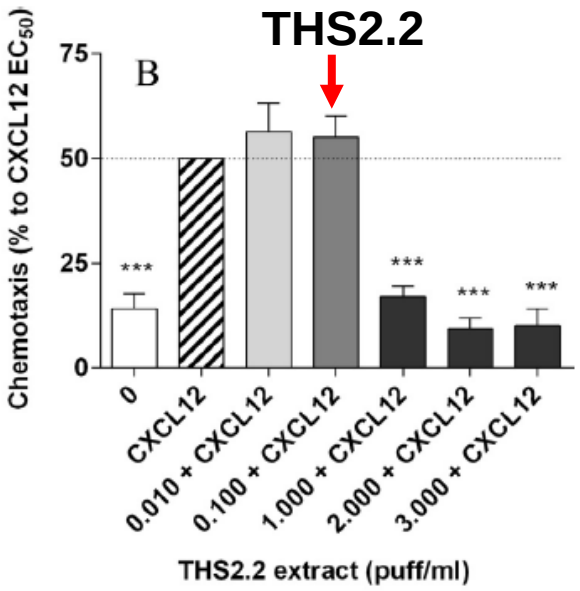
Van der Toorn et al, Food and Chemical Toxicology 86:81-87 (2015)

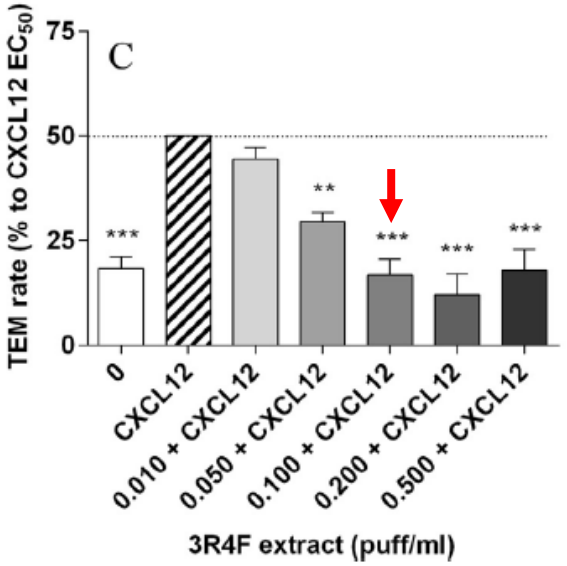
PMI RESEARCH & DEVELOPMENT

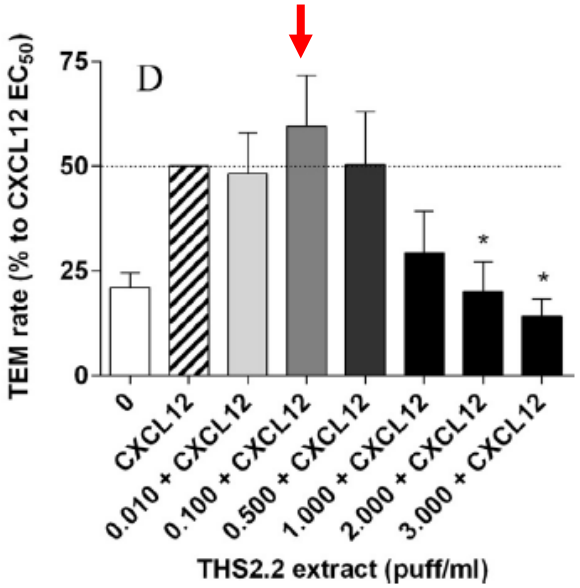
Reduced Effects of THS2.2 Compared with 3R4F on Transmigration of Monocytic Cells



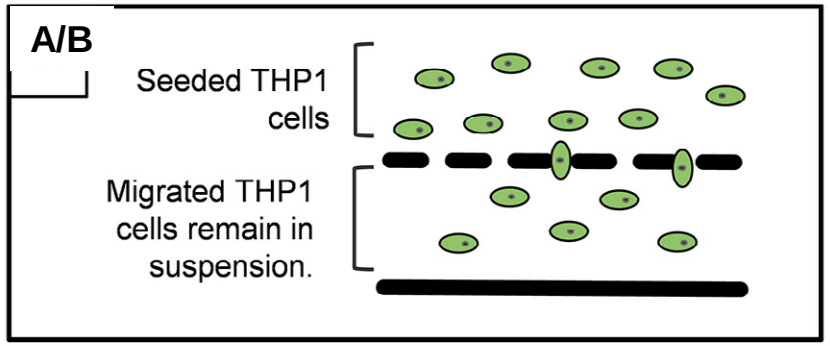




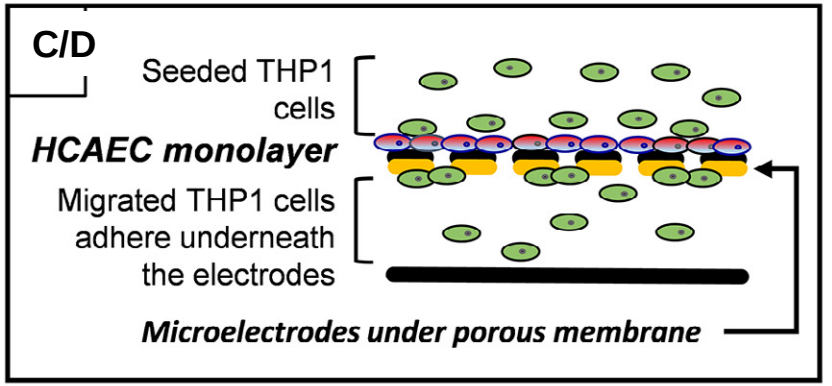




Transwell chemotaxis (Cell counting)



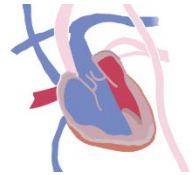
Transendothelial migration (Impedance-based)



PMI RESEARCH & DEVELOPMENT

Van der Toorn et al, Food and Chemical Toxicology 86:81-87 (2015)

Conclusions – *In vitro* Adhesion and Transmigration Studies



- Reduced effects *in vitro* of THS2.2 compared with 3R4F (at matching nicotine concentrations) on **cellular mechanisms relevant for the development of atherosclerosis**

- The concentrations of **THS2.2 had to be increased by 10 to 20 times** depending on the context to observe similar effects as those induced by 3R4F

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In vivo Systems Toxicology

This research was funded by Philip Morris International

Rodent Models for Assessment

- Respond to cigarette mainstream smoke
- Develop pathologies with characteristics of smoking-induced human diseases such as:
  - *Lung inflammation*, altered *pulmonary function*, *emphysema* in Chronic obstructive pulmonary disease (COPD) → **C57Bl6** mouse strain
  - A*therosclerosis* in cardiovascular diseases (CVD)→ **Apoe**$^{-/-}$ mouse strain (C57Bl6 background)
  - *Tumor development in* Lung Cancer → **A/J** mouse strain

Lo Sasso G, Schlage WK, Boué S, Veljkovic E, Peitsch MC, Hoeng J. (2016) The Apoe(-/-) mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. J Transl Med. 2016 14(1):146.Epub. Review. **PMID 27207171**.

Stinn W, Buettner A, Weiler H, Friedrichs B, Luetjen S, van Overveld F, Meurrens K, Janssens K, Gebel S, Stabbert R, Haussmann HJ. (2013) Lung inflammatory effects, tumorigenesis, and emphysema development in a long-term inhalation study with cigarette mainstream smoke in mice. Toxicol Sci. 131(2):596-611. **PMID: 23104432**

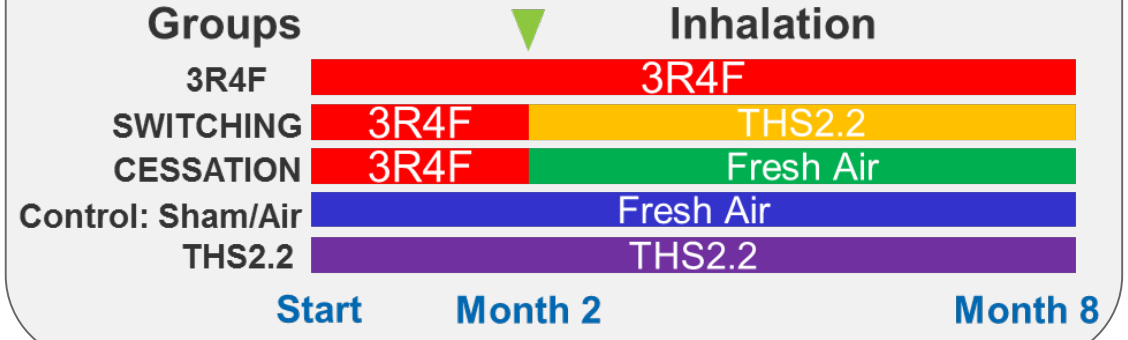
Systems Toxicology Mouse Inhalation Studies (IS) for MRTP Assessment

STUDY OBJECTIVES

- **To compare the effects of THS2.2 and 3R4F**
- To use a Switching design **upon initiation of disease**:
  - to assess **reversibility** (switch to fresh air, i.e. cessation)
  - to **quantify how similar switching to THS2.2** is to **cessation**

STUDY DESIGN: 5 exposure groups

(e.g. Apoe-/-)



MEASUREMENTS

- **In-life observations, body weight**
- **Markers of exposure**: in plasma (e.g. carboxyhemoglobin, nicotine and cotinine) and urine e.g. (nicotine metabolites)
- **Hematology**
- **Clinical chemistry** (e.g. cholesterol, HDL, LDL, Glucose)
- **Respiratory endpoints** (BALF cell count, lung histopathology, function, volume)
- **Cardiovascular endpoints** (Aortic plaque volume, area, occlusion)
- **Molecular analysis**: Omics (Transcriptomics, Proteomics, Lipidomics) (various tissues and biofluids)

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Case Study 3: ApoE$^{-/-}$ Mouse Inhalation and Switching Study

OXFORD
SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

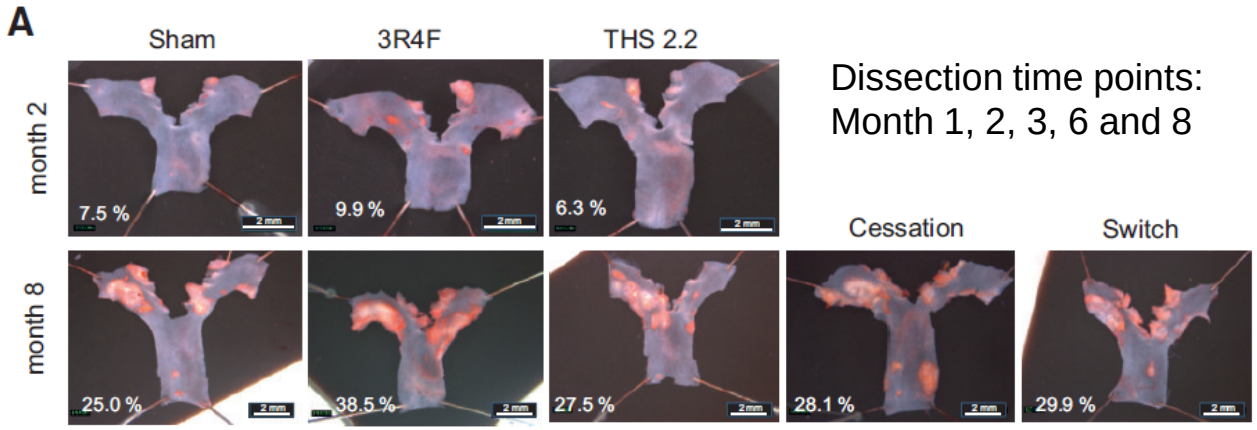
TOXICOLOGICAL SCIENCES, 149(2), 2016, 411–432

doi: 10.1093/toxsci/kfv243
Advance Access Publication Date: November 25, 2015
Research Article

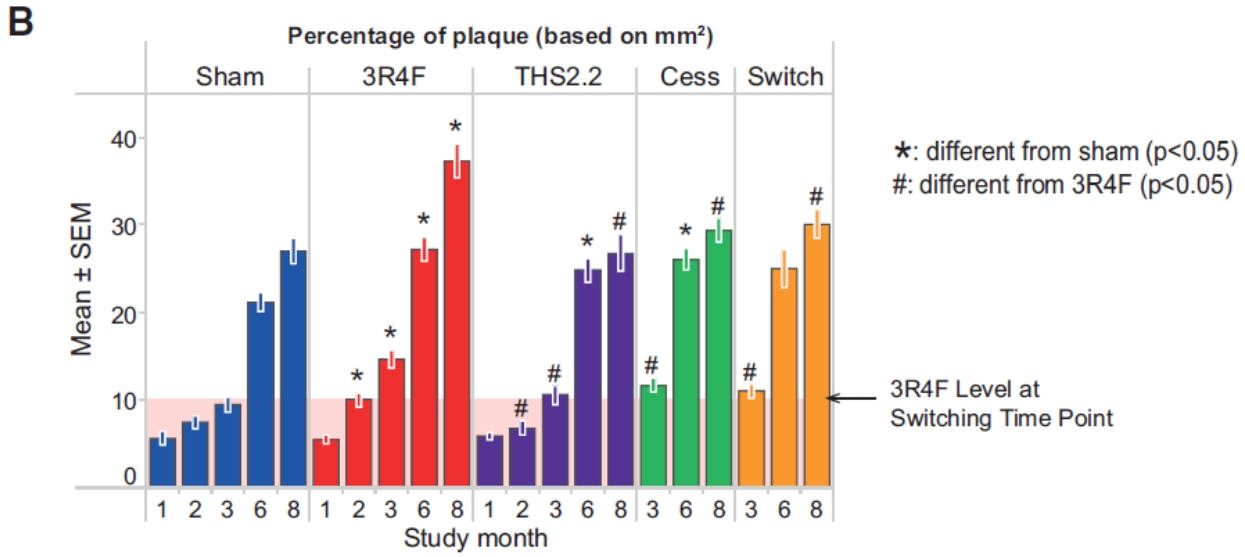
An 8-Month Systems Toxicology Inhalation/Cessation Study in Apoe$^{-/-}$ Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared With Conventional Cigarettes

Blaine Phillips,* Emilija Veljkovic,† Stéphanie Boué,† Walter K. Schlage,‡ Gregory Vuillaume,† Florian Martin,† Bjoern Titz,† Patrice Leroy,† Ansgar Buettner,§ Ashraf Elamin,† Alberto Oviedo,* Maciej Cabanski,†,1 Héctor De León,† Emmanuel Guedj,† Thomas Schneider,† Marja Talikka,† Nikolai V. Ivanov,† Patrick Vanscheeuwijck,† Manuel C. Peitsch,† and Julia Hoeng,†,2

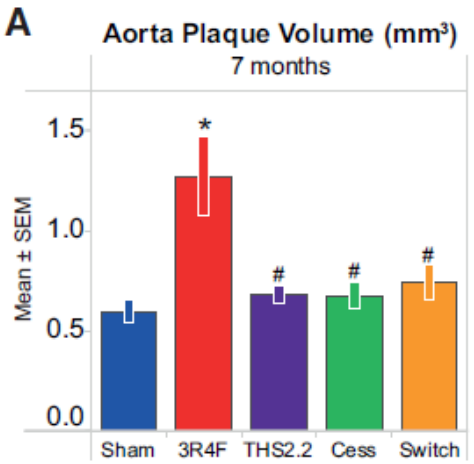
Reduced Effects of THS2.2 Compared with 3R4F on the Growth of Aortic Arch Plaques

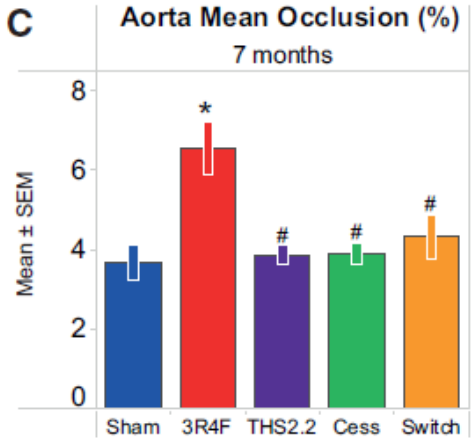


Dissection time points:
Month 1, 2, 3, 6 and 8



Aortic arches were dissected, longitudinally opened, pinned down, and stained with OilRedO for planimetry





Aortic arch (in situ) plaque measurements: 3D reconstruction from high resolution micro computed tomography (micro-CT)

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Phillips et al, Toxicological Sciences 149(2):411-432 (2016)

Systems Toxicology Mouse Inhalation Studies (IS) for MRTP Assessment

MRTP= modified-risk tobacco product

Strain	Study	Reference	Focus
C57BL/6	7-month IS: MRTP= SMAR (prototype)	Philips et al, 2015, PMID 25843363	Study description / Respiratory disorders
		Ansari et al, 2016, PMID 26731301	Data description and availability
		Elamin et al, 2016, PMID 27268958	Lung proteome
Apoe-/-	8-month IS: MRTP= THS2.2	Phillips et al, 2016, PMID 26609137	Cardiovascular and respiratory disorders
		Szostak et al, 2017, PMID 28111298	Heart transcriptomics
		Lasso et al, 2016, PMID 27027324	Liver transcriptomics
Both models	Mentioned above	Titz et al, 2016, PMID 26582801	Integrative systems toxicology: lung lipidomics, proteomics and transcriptomics

Systems Toxicology Animal Inhalation Studies (IS) for MRTP Assessment

- **Rat inhalation studies according to regulatory guidelines (*TG 413) from the Organization for Economic Cooperation and Development (OECD) – Executed according to GLP**
  - Goal: to assess the degree of *reduced exposure in laboratory models*
  - Measurements: Standard and systems toxicology endpoints
  - The results show *reduced exposure and effects* of THS2.2 compared with 3R4F in laboratory models
  - Publications:
    - *Wong et al, Regul Toxicol Pharmacol, 81(S2): S59-S81 (2016)*
    - *Kogel et al, Regul Toxicol Pharmacol, 81(S2): S123-S138 (2016)*
    - *Oviedo et al, Regul Toxicol Pharmacol, 81(S2): S93-S122 (2016)*
    - *Sewer et al, Regul Toxicol Pharmacol, 81(S2): S82-S92 (2016)*

***OECD Guidelines for the Testing of Chemicals, Section 4.**
Test No. 413: Subchronic Inhalation Toxicity: 90-day Study, OECD 2009

MRTP= modified-risk tobacco product

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Systems Toxicology - Conclusions

This research was funded by Philip Morris International

Systems Toxicology-based Assessment Studies - Conclusions

In comparison with the 3R4F reference cigarette, the data shows that:

- THS2.2 yield **significantly lower levels of harmful and potentially harmful constituents** (HPHCs) → on average of about 90-95%
- This leads, in laboratory models (*in vitro* and *in vivo*), to:
  - **Reduced exposure** to HPHCs and **reduced toxicity** in laboratory models
  - **Reduced biological impact on key cellular mechanisms** (i.e. endothelial inflammation)
  - **Reduced impact on disease-associated mechanisms** (reduced severity of disease endpoints) (i.e. aortic plaque growth)
- The **effects of Switching from 3R4F to THS2.2 approach those observed in Cessation in these models**

HPHCs: harmful and potentially harmful constituents

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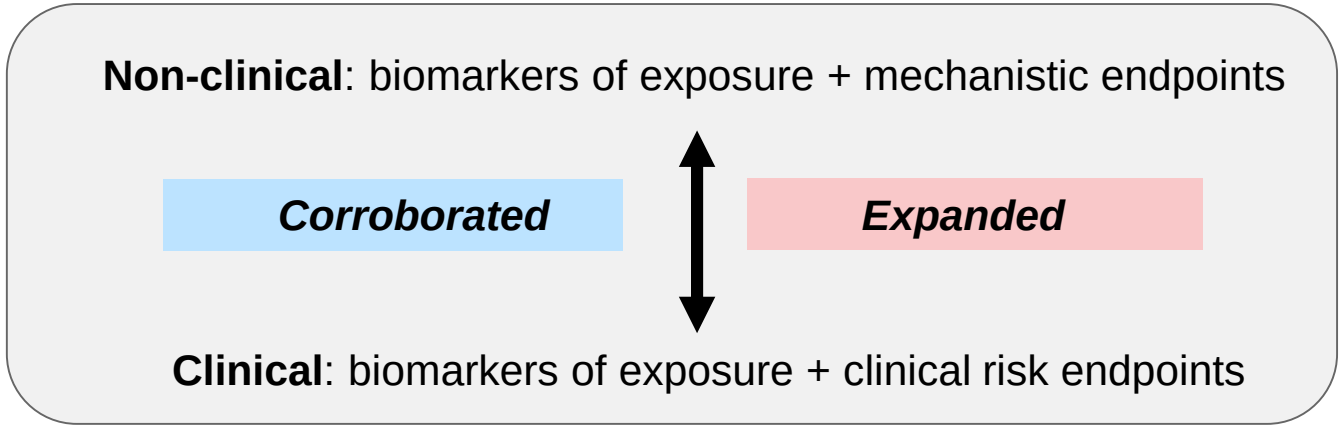
The Relationship between PMI's Non-Clinical and Clinical MRTP Assessment Programs

This research was funded by Philip Morris International

The Relationship between PMI's Non-Clinical and Clinical MRTP Assessment Programs

- **Developed** using the body of scientific literature on:
  - Epidemiological evidence linking smoking and disease
  - Mechanistic understanding of disease onset and progression associated with smoking
- **Aligned**, so that evidence generated in:

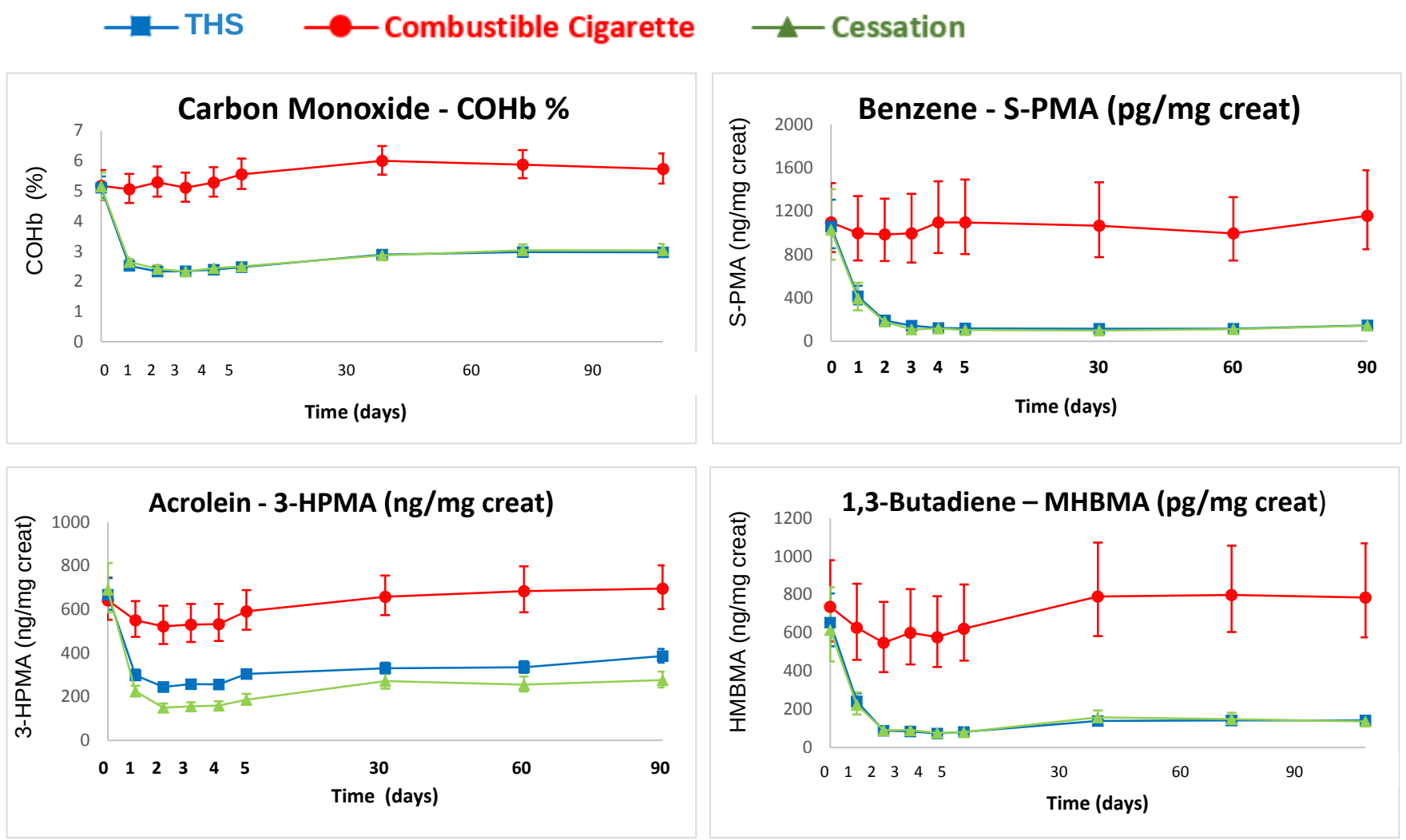


Case Study 4 : Reduced Exposure Clinical Study in Japan

Adult smokers used the products ad libitum

Adult smokers randomized to cigarettes or THS2.2 were free to use the product as often as they wished, in confinement (5 days) and then ambulatory (85 days)

Levels of reduced exposure approached those observed in people who stopped smoking for the duration of the studies



Note: These data alone do not represent a claim of reduced risk
Source: PMI Research and Development
Registered on clinicaltrials.gov: NCT01970995

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Transparency and Independent Verification

This research was funded by Philip Morris International

Transparency and Independent Verification of PMI's Results

Independent Studies	Launched an **investigator-initiated studies program that supports external scientists** who can conduct independent research related to our MRTPs through the provision of products, equipment, and financial or technical support
Independent Verification	Crowd-sourced verification of data and methods using double blind assessment → **sbvimprover.com**
Independent Expert Review	Conducting in-depth analysis of study reports by independent experts
Publish Results	> 200 peer-reviewed articles since 2008 → **pmiscience.com**

sbv IMPROVER
SYSTEMS BIOLOGY VERIFICATION

PMI SCIENCE
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PMI RESEARCH & DEVELOPMENT

sbv IMPROVER

Project initiated 6 years ago and funded by Philip Morris International

Aims to provide a measure of quality control in R&D by identifying the building blocks that need verification in a complex industrial research pipeline

Aims to verify methods & data in systems biology / toxicology using double blind performance assessment

Complements the classical peer review system

sbv IMPROVER
SYSTEMS BIOLOGY VERIFICATION

sbvimprover.com

_computational BIOLOGY

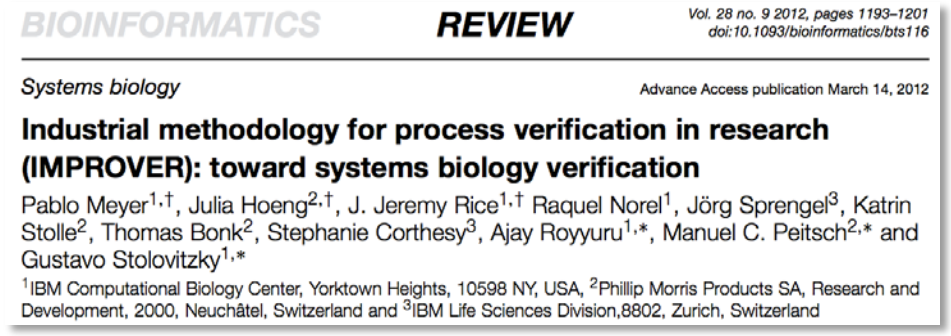
COMMENTARY

Verification of systems biology research in the age of collaborative competition

Pablo Meyer[1], Leonidas G Alexopoulos[2], Thomas Bonk[3], Andrea Califano[4], Carolyn R Cho[5], Alberto de la Fuente[6], David de Graaf[7], Alexander J Hartemink[8], Julia Hoeng[3], Nikolai V Ivanov[3], Heinz Koeppl[9], Rune Linding[10], Daniel Marbach[11], Raquel Norel[1], Manuel C Peitsch[3], J Jeremy Rice[1], Ajay Royyuru[1], Frank Schacherer[12], Joerg Sprengel[13], Katrin Stolle[3], Dennis Vitkup[4] & Gustavo Stolovitzky[1]

Collaborative competitions in which communities of researchers compete to solve challenges may facilitate more rigorous scrutiny of scientific results.

Nature Biotechnology 2011 Sep 8;29(9):811-5



BIOINFORMATICS REVIEW

Vol. 28 no. 9 2012, pages 1193–1201
doi:10.1093/bioinformatics/bts116

Systems biology

Advance Access publication March 14, 2012

Industrial methodology for process verification in research (IMPROVER): toward systems biology verification

Pablo Meyer[1,†], Julia Hoeng[2,†], J. Jeremy Rice[1,†] Raquel Norel[1], Jörg Sprengel[3], Katrin Stolle[2], Thomas Bonk[2], Stephanie Corthesy[3], Ajay Royyuru[1,*], Manuel C. Peitsch[2,*] and Gustavo Stolovitzky[1,*]

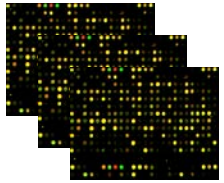
[1]IBM Computational Biology Center, Yorktown Heights, 10598 NY, USA, [2]Phillip Morris Products SA, Research and Development, 2000, Neuchâtel, Switzerland and [3]IBM Life Sciences Division,8802, Zurich, Switzerland

Bioinformatics 2012 28(9):1193-1201

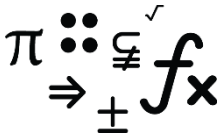
PMI RESEARCH & DEVELOPMENT

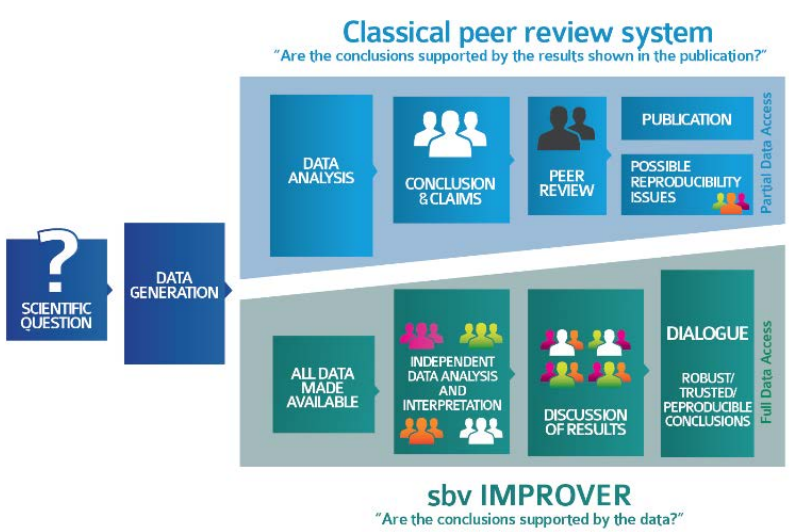
sbv IMPROVER leverages the crowd to complement the classical peer review system

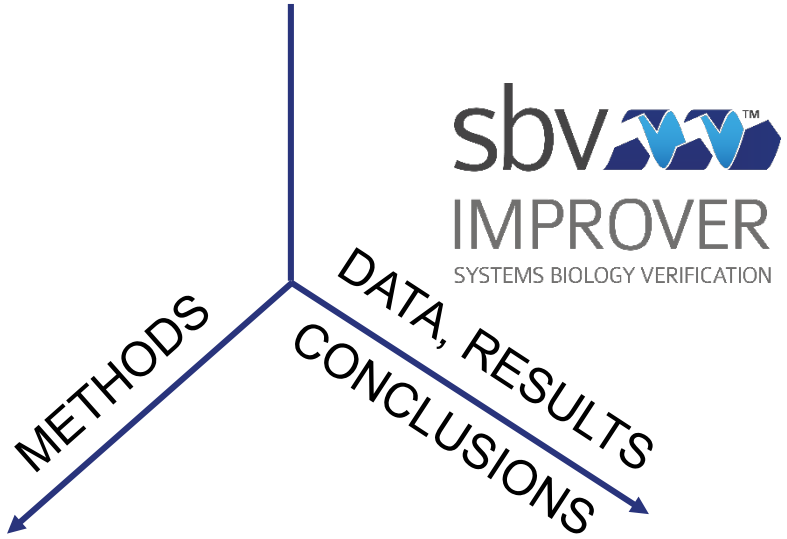
Systems Toxicology data



Systems Toxicology methods







sbv IMPROVER
SYSTEMS BIOLOGY VERIFICATION

COMPUTATIONAL CHALLENGES

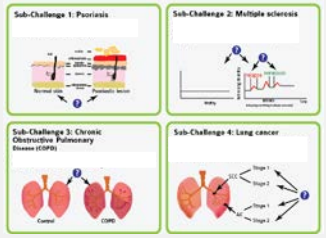
VERIFICATION PROJECT

PMI RESEARCH & DEVELOPMENT

Past sbv IMPROVER computational challenges

Diagnostic signature challenge (2012)

To identify gene signatures for diagnostic classification in four disease area

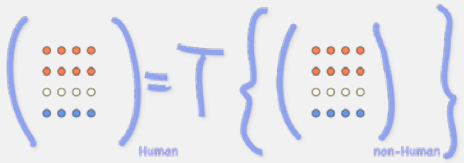


PUBLICATIONS

- *Tarca et al, Bioinformatics 29 (22) (2013)*
- *Special issue in Systems Biomedicine 1 (4) (2013) including 11 articles*

Species translation challenge (2013)

To identify and quantify a function of translatability of biological perturbations across human and rodent species



PUBLICATIONS

- *Special Issue in Bioinformatics 31 (4) (2014) including 6 articles*
- *Poussin et al, Scientific Data 1:140009 (2014)*

Network verification challenge (2014-2015)

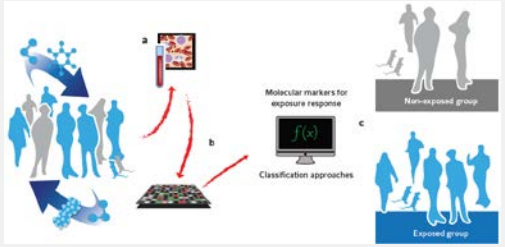
To review biological network models that are suitable for drug discovery, toxicological and mechanistic research in respiratory disease

PUBLICATIONS

- *sbv IMPROVER projet team, Gene Regul Syst Bio (2016)*
- *sbv IMPROVER projet team, F1000Res (2015)*
- *sbv IMPROVER project team, Pac Symp Biocomput (2015)*

Systems Toxicology challenge (2015-2016)

To identify robust blood-based gene signatures as predictors for smoking and cessation status



PUBLICATIONS

- *Poussin et al, Chem Res Toxicol (accepted) (2017)*
- *3 other manuscripts in preparation*

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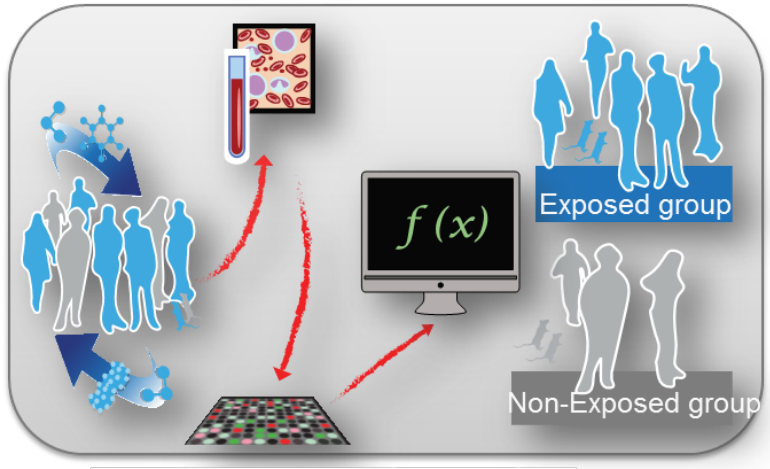
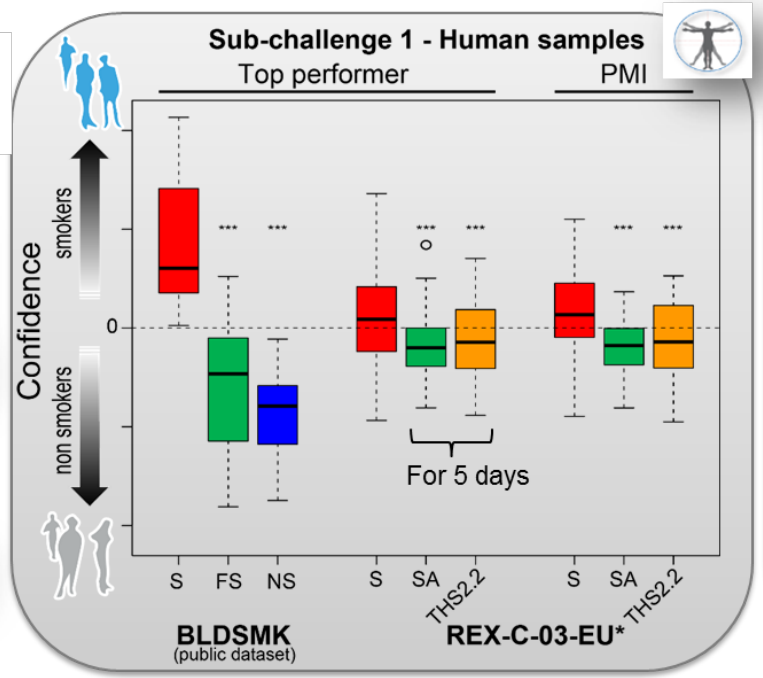
PMI RESEARCH & DEVELOPMENT

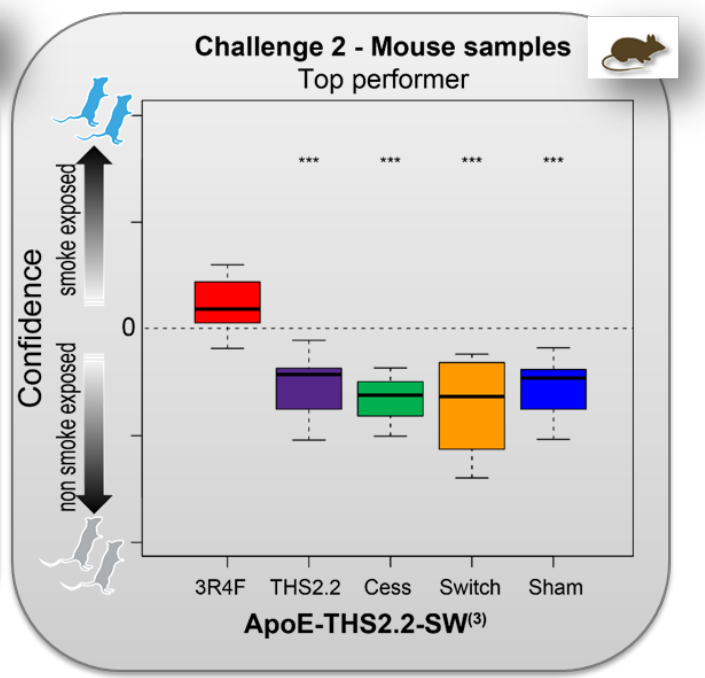
Case Study 5: Whole Blood Exposure Response Marker Identification: Crowdsourcing validation of PMI's gene expression signature

sbv IMPROVER Systems Toxicology Computational Challenge[1]



- 135 registered participants
- 61 international teams
- 23 valid submissions





Gene Signature[2]

LRRN3, SASH1, PALLD, RGL1, TNFRSF17, CDKN1C, IGJ, RRM2, ID3, SERPING1, FUCA1

*In black genes common to the crowdsourcing signature***

S / 3R4F: Smoker / 3R4F exposed
FS / Cess: Former Smoker / Cessation
NS / Sham: Never Smoker / exposed to fresh air

SA: Smoking Abstinence
THS2.2: THS2.2

Switch: Switch to THS2.2

(1) *Poussin et al. Chemical research in toxicology. 2017*
(2) *Martin et al. Human & Experimental Toxicology. 2015*
(3) *Phillips et al. Toxicol Sci. 2016*

** similar results were obtained for REX-C-04-JP (not shown)*
*** selected by at least 2 teams*
**** significantly different from smoke exposed group*

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Summary and Conclusions

This research was funded by Philip Morris International

Conclusions

Our scientific assessment program enabled us to determine already that **THS2.2**:

- **Does not generate combustion** through normal operation
- Generates an aerosol with, **on average, 90-95% lower levels of HPHCs** compared with reference cigarette smoke
- **Is on average 90-95% less toxic** in laboratory-based tests compared with reference cigarette smoke
- **Reduces the risk of smoking-related diseases** in sophisticated **laboratory-based models**
- Adults smokers **switching to THS2.2:**
  - **have reduced biomarkers of exposure (HPHCs)** compared with adult smokers who continued smoking
  - their **levels approached those observed in smokers who quit smoking** for the duration of the study

December 6th, 2016: submission of a MRTP application for THS2.2 with the U.S. Food and Drug Administration's Center for Tobacco Products

HPHC: harmful and potentially harmful constituents
MRTP: modified-risk tobacco product

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Thank you for your attention

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