# ASSESSMENT OF CANDIDATE MODIFIED RISK TOBACCO PRODUCTS ON ORAL HEALTH *IN VITRO*

***Dr. Filippo Zanetti***
***Philip Morris International R&D***
***Neuchatel, Switzerland***

September 28, 2018
*Sofia*

# Background

- Smoking causes serious diseases, such as cardiovascular diseases, lung cancer, and chronic obstructive pulmonary disease (U.S. Surgeon General, 2004).
- Smoking cessation remains the most effective approach to minimizing the risk for smoking-related diseases (Godtfredsen et al. 2008; Gepner et al., 2011).
- Providing reduced-risk alternatives to adult smokers who would otherwise continue to smoke cigarettes represents the basis of the “Tobacco Harm Reduction” strategy (IOM, 2002).
- Philip Morris International (PMI) is developing novel products with the potential to reduce individual risk and population harm in comparison with smoking cigarettes.
- To determine whether such reduced-risk products (**RRPs***) have the potential to reduce individual risk, we are conducting extensive and rigorous scientific studies comparing their biological impact with that of cigarettes.

Innovation Cube, Neuchatel, Switzerland

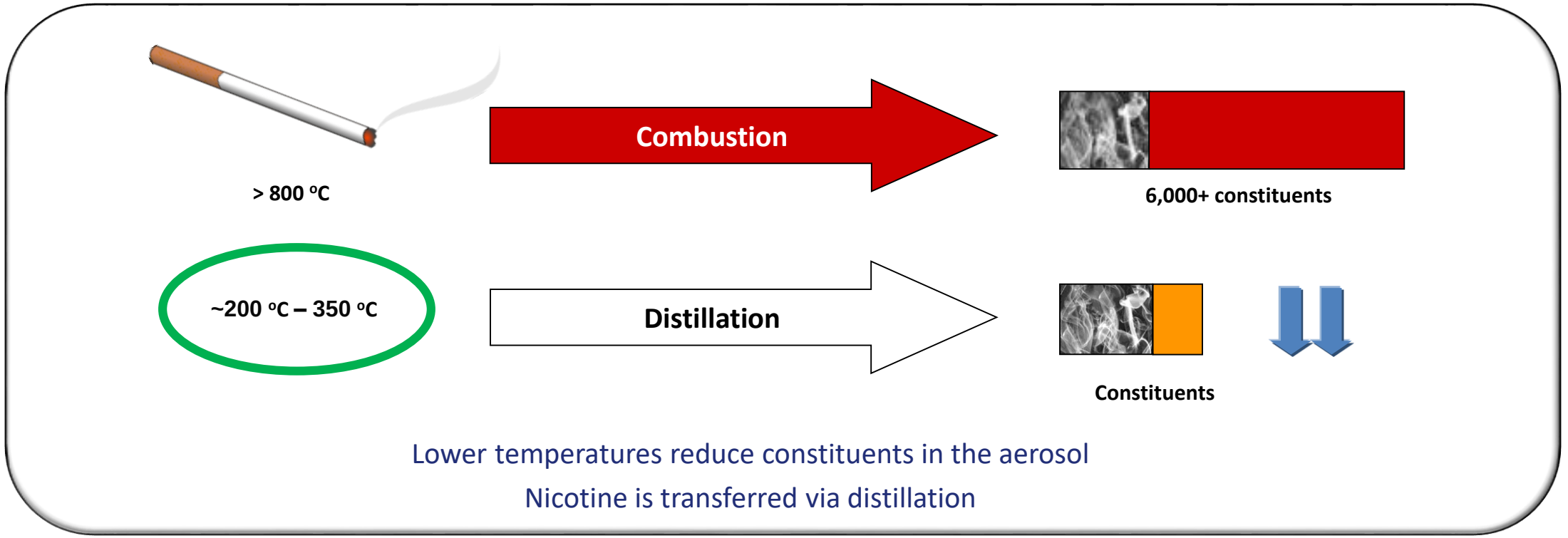
*Note: Reduced-Risk Products ("RRPs") is the term PMI uses to refer to products with the potential to reduce individual risk and population harm in comparison with smoking cigarettes.

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Cigarette Smoke vs. Heat-not-Burn

More than 6,000 constituents have been identified in cigarette smoke. Some are harmful and potentially harmful (HPHC), many of which are formed during combustion (burning) of the tobacco.

It is not known which HPHCs are responsible for tobacco-related diseases – selective reduction is not an effective approach.



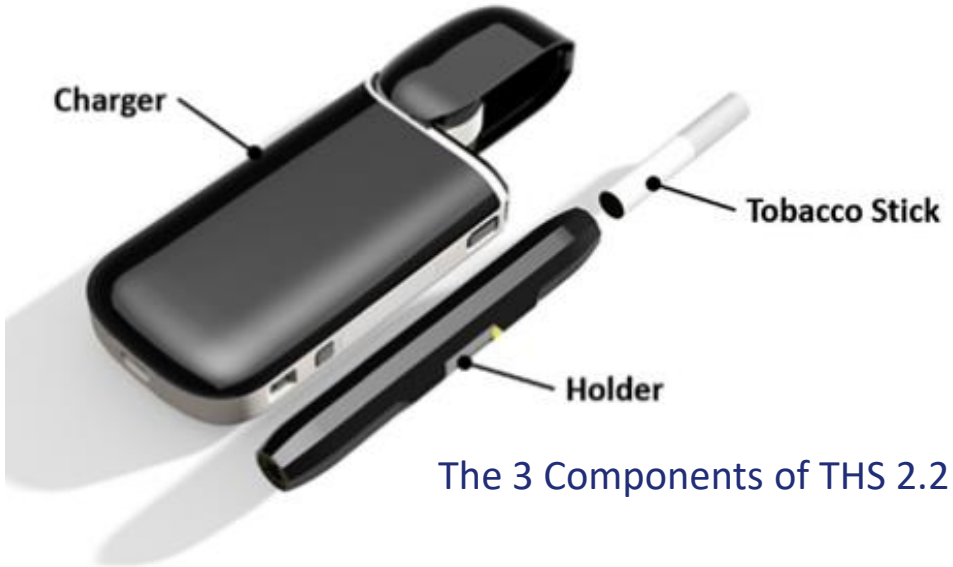

Lower temperatures reduce constituents in the aerosol

Nicotine is transferred via distillation

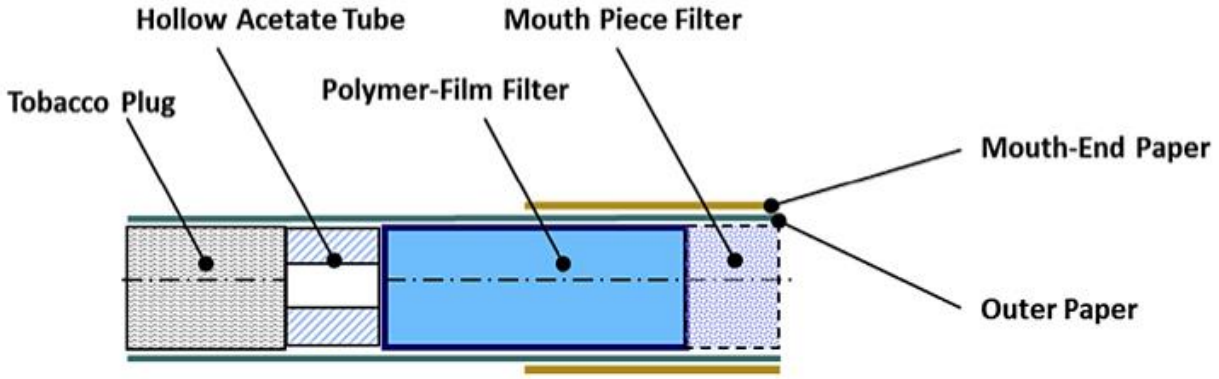
Smith, MR., et al. "Evaluation of the Tobacco Heating System 2.2. Part 1: description of the system and the scientific assessment program." Regulatory Toxicology and Pharmacology 81 (2016): S17-S26.

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# RRPs: Tobacco Heating System (THS) 2.2 Operating Principles




The 3 Components of THS 2.2



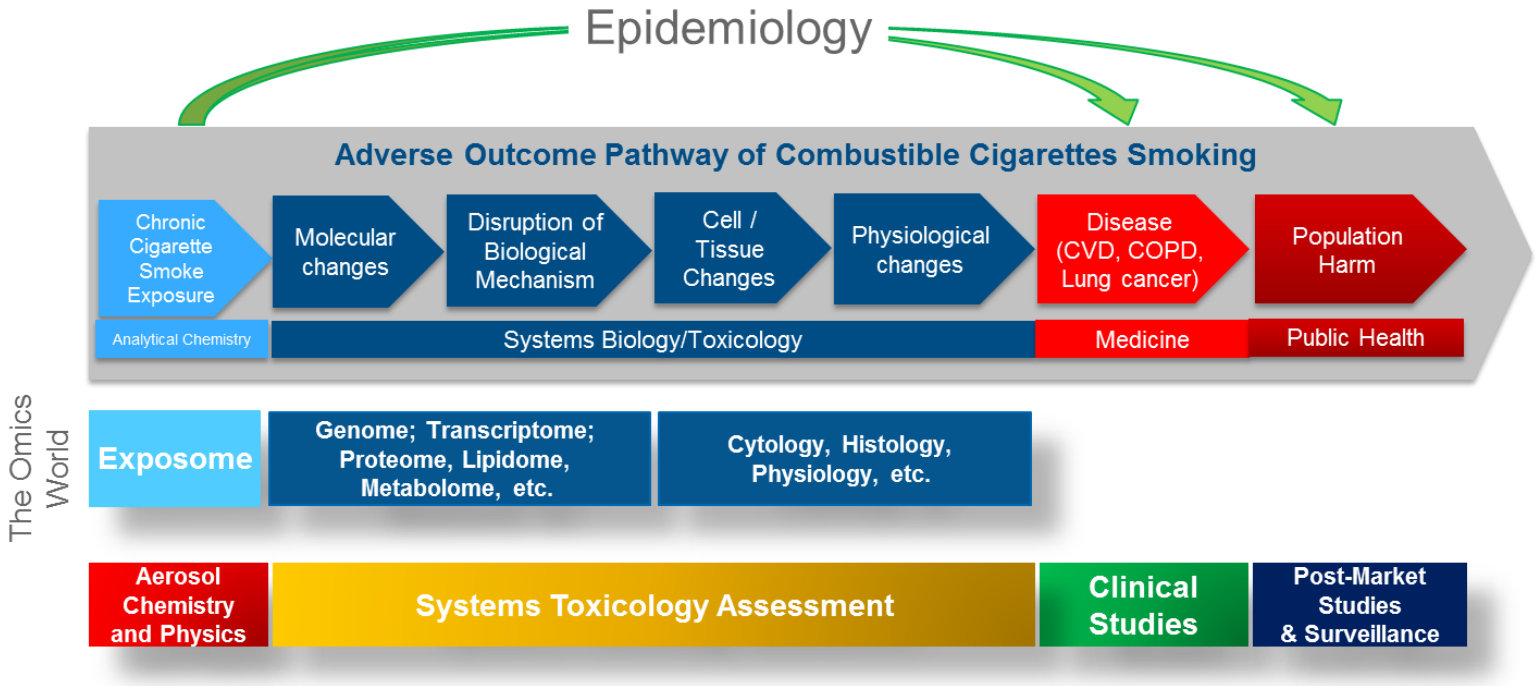

Cross-Sectional View of the Tobacco Stick

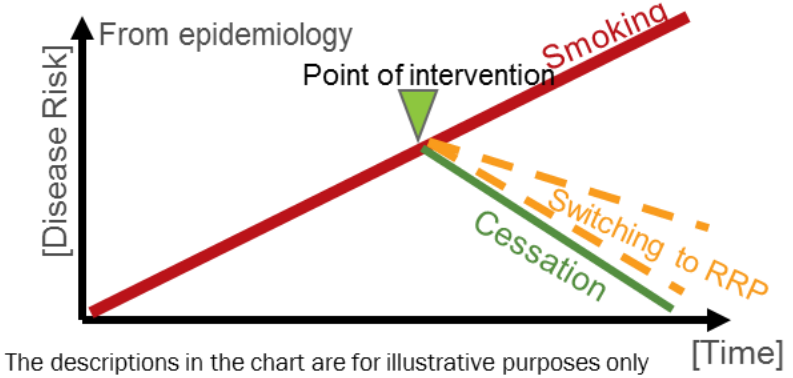
## Key Principles

- Electrically heated tobacco system version 2.2 (THS 2.2)
  - Tobacco plug
  - Tobacco blends and flavor systems developed to suit lower operating temperature (< 350 $^{o}$C)
- Heating engine precisely controlled using built-in software
  - Tobacco is heated in a controlled fashion rather than burned, which is intended to prevent generation of HPHCs through pyrogenesis and pyrosynthesis
  - The heater also acts as a temperature sensor

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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



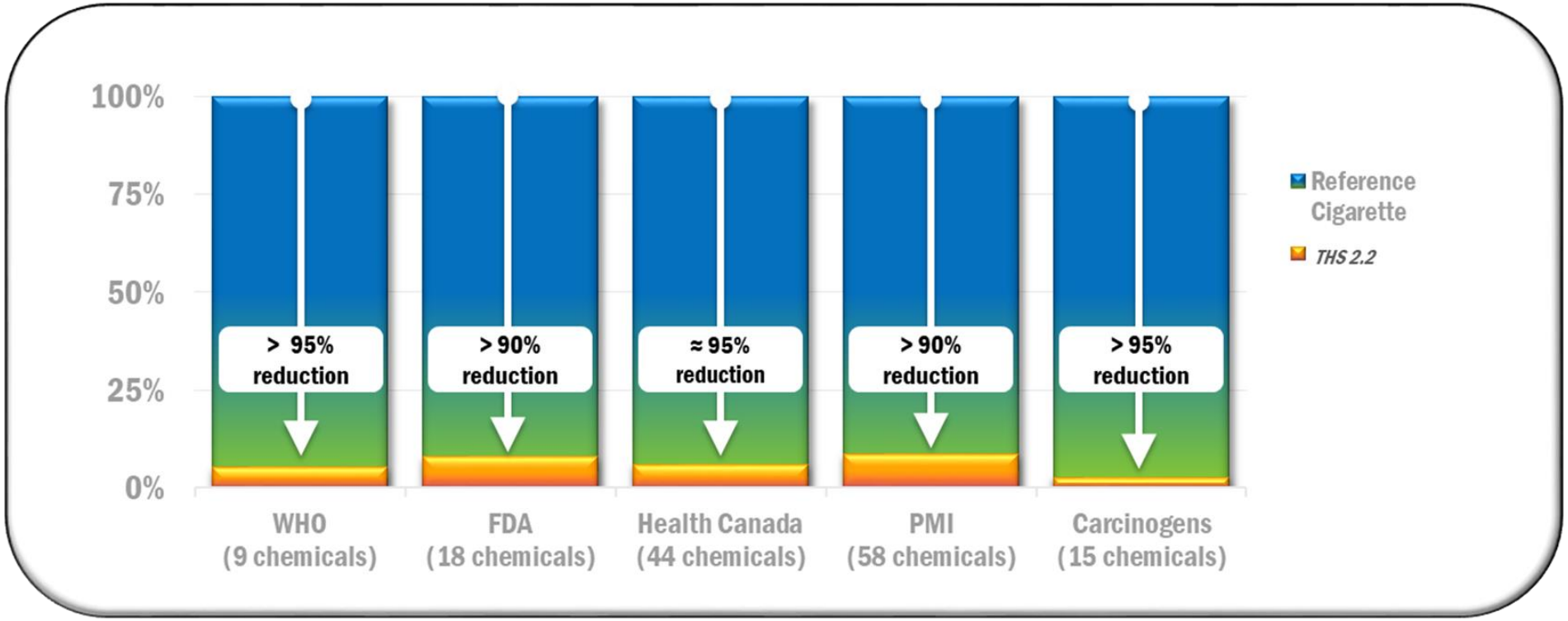

The descriptions in the chart are for illustrative purposes only

- Compare switching to RRP with continued smoking
- Assess how close switching to RRP is to smoking cessation

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# THS 2.2: Aerosol Chemistry

Average reductions in formation of HPHCs for THS 2.2 aerosol compared with levels measured in 3R4F reference cigarette smoke*




*Aerosol collection with Health Canada Intense 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 U.S. Food and Drug Administration 18 and the 15 carcinogens of the IARC Groups 1.

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

Smith, MR., et al. "Evaluation of the Tobacco Heating System 2.2. Part 1: description of the system and the scientific assessment program." Regulatory Toxicology and Pharmacology 81 (2016): S17-S26.

# Systems Toxicology: A Comprehensive Toxicity Assessment

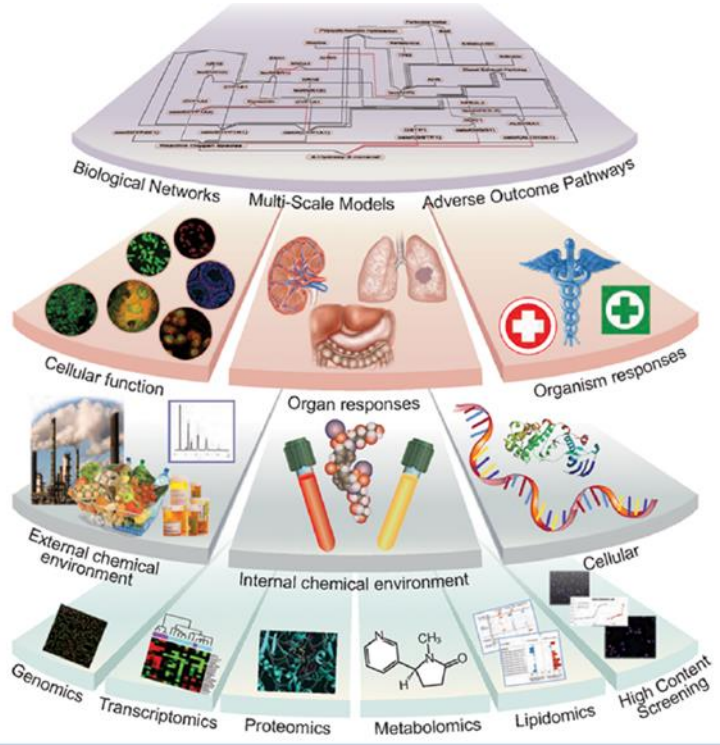
- Considers biological systems as a whole and aims at elucidating detailed **biological mechanisms** that link exposure to active substances with their adverse consequences
- Integrates classic toxicology approaches with the quantitative analysis of molecular and functional changes
- Combines high-throughout methods with advanced computational methods
- Enables the shift to a new paradigm for risk assessment (21st century toxicology) (*Product Assessment*)

Computational models

Apical measurements

Molecular measurements

Enabling technologies



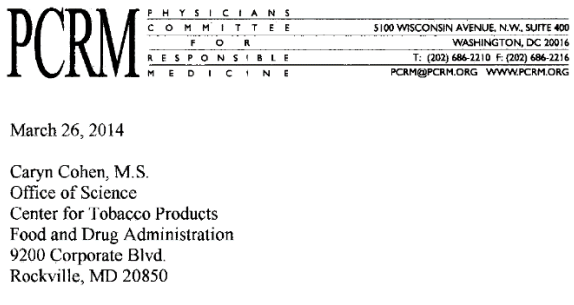

Sturla et al., 2014

System toxicology enables the shift from regulatory toxicological assessment toward a detailed mechanistic understanding of biological pathways perturbed by exposure to toxicants

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Background: Replacing, Reducing, and Refining

- 2009: European Commission published a report on Alternative Testing Strategies for Replacing, Reducing, and Refining (3R) the use of animals in research.
- Human organotypic tissues reconsituted using primary cells cultured in three dimensions, with proper cell-cell contact, recapitulating biological functions (e.g., mucus secretion, mucociliary clearance) and allowing whole cigarette smoke exposure at the air-liquid interface.
- "The Food, Drug, and Cosmetic Act directs the FDA to consult with the Institute of Medicine (IOM) on the design and conduct of studies for the assessment of MRTPs [modified risk tobacco products]. In its 2011 report, *Scientific Standards for Studies on Modified Risk Tobacco Products*, the IOM identified, as a standard step in this assessment, *in vitro* tests for cytotoxicity, genotoxicity, proliferation, apoptosis, oxidative stress, inflammation, mucus production, and endothelial cell activation."
- PETA issued a letter in March 2014 whereby PETA stated the *in vitro* field has progressed significantly, with many varied technological advances, and the *in vitro* testing of tobacco products is no longer "limited to a small number of cytotoxicity and genotoxicity assays."



PCRM PHYSICIANS COMMITTEE FOR RESPONSIBLE MEDICINE
5100 WISCONSIN AVENUE, N.W., SUITE 400
WASHINGTON, DC 20016
T: (202) 686-2210 F: (202) 686-2216
PCRM@PCRM.ORG WWW.PCRM.ORG

March 26, 2014

Caryn Cohen, M.S.
Office of Science
Center for Tobacco Products
Food and Drug Administration
9200 Corporate Blvd.
Rockville, MD 20850

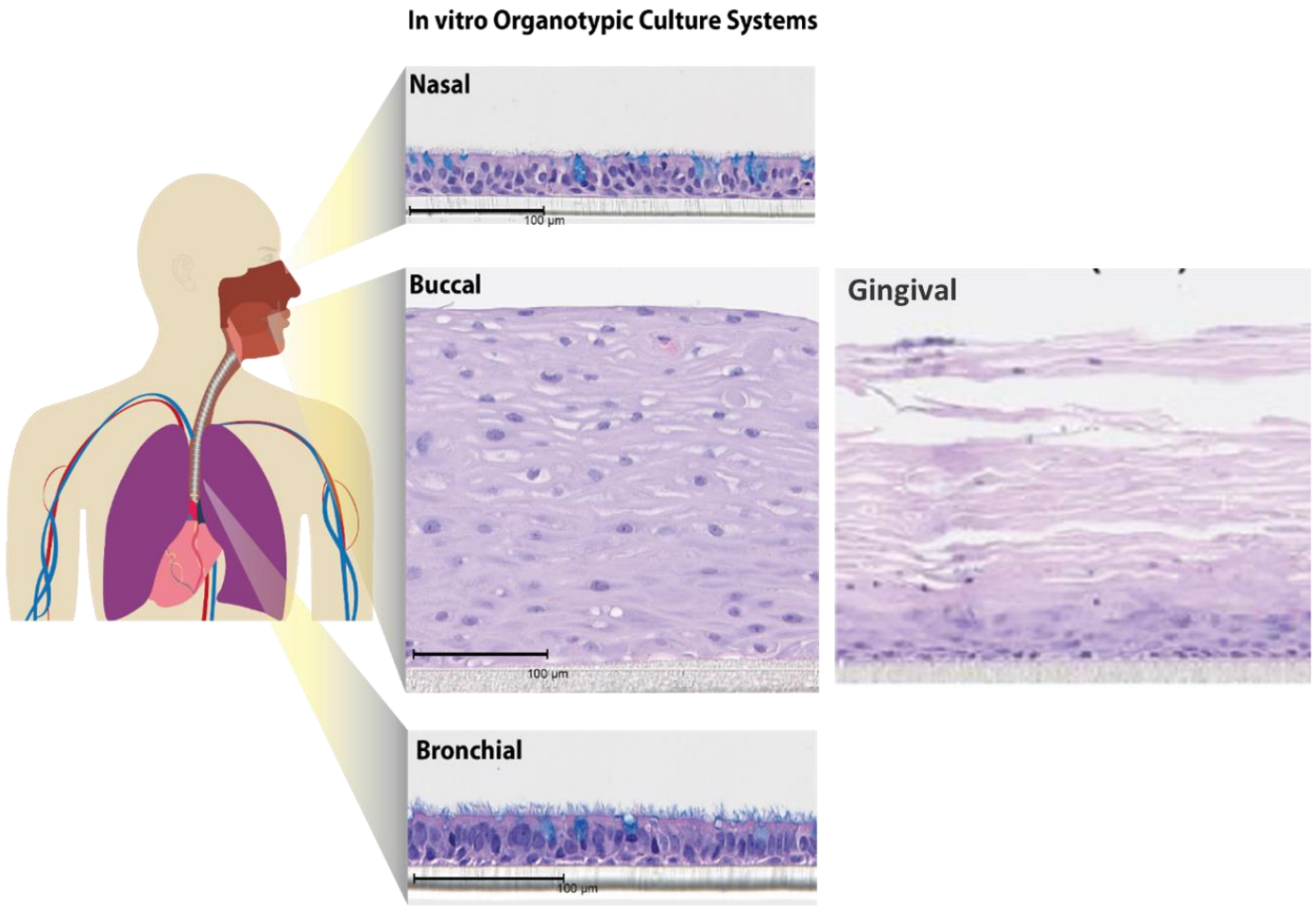
PETA
PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

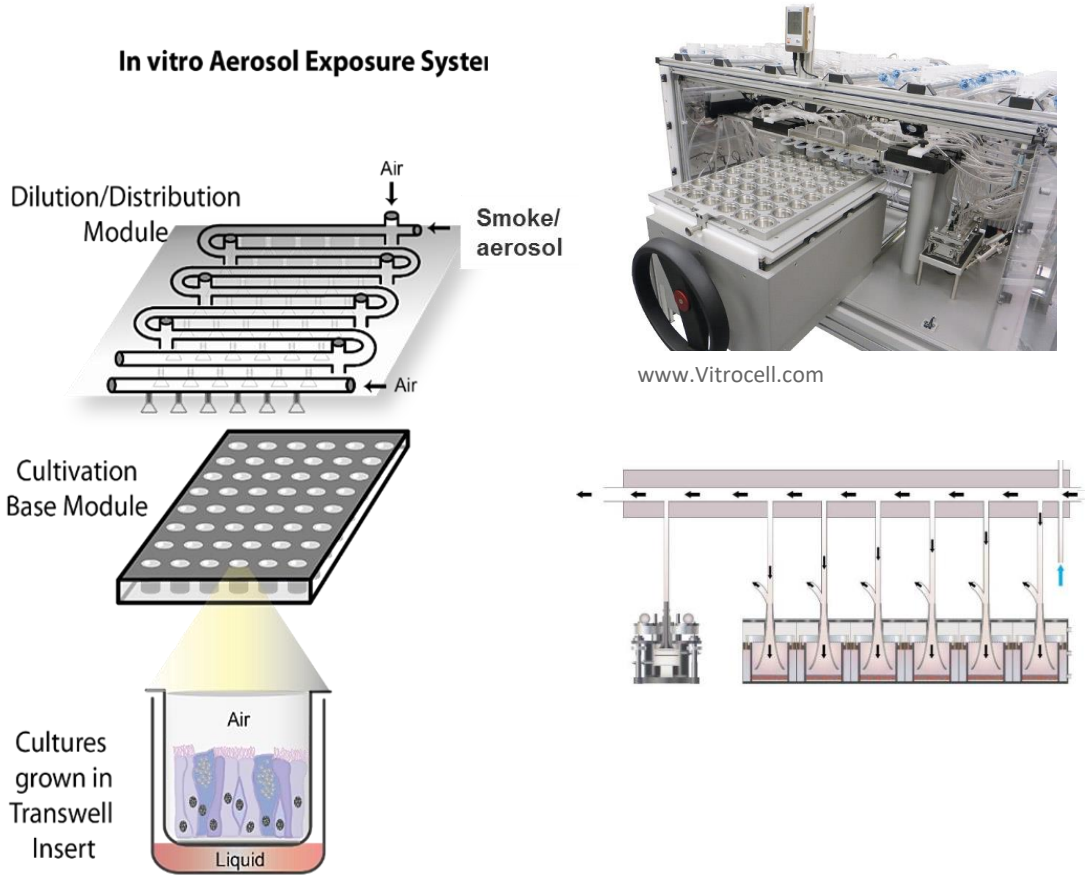
HEADQUARTERS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

Belgium, Estonia, Germany, UK, and Slovakia have banned animal testing for tobacco research

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Human Organotypic Culture Models of the Aerodigestive Tract and Exposure System

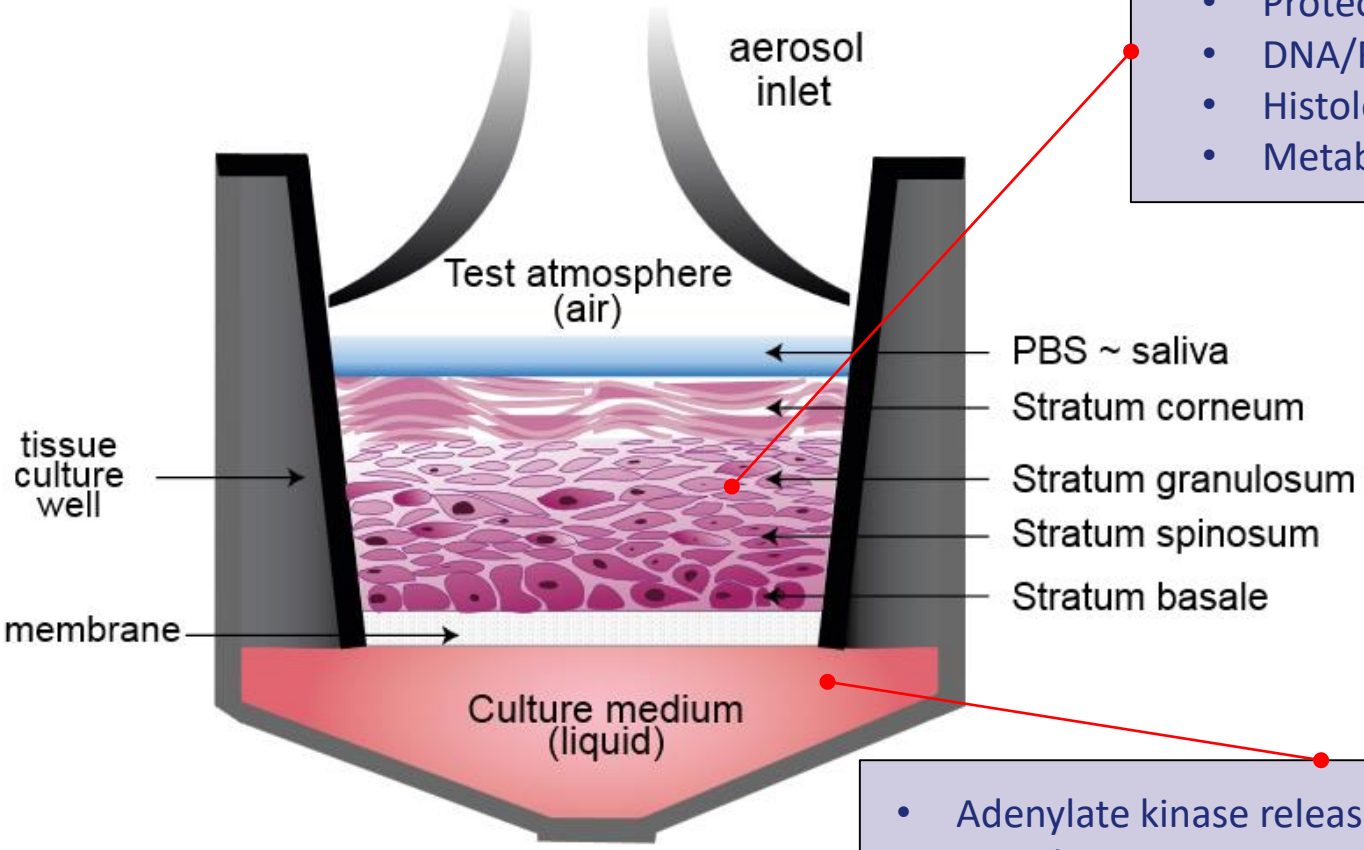



www.Vitrocell.com

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Systems Toxicology: A Comprehensive Toxicity Assessment




- Transcriptomics (mRNA + miRNA microarray)
- Proteomics (targeted/non-targeted approach)
- DNA/RNA Sequencing
- Histological analysis
- Metabolomics

- Adenylate kinase released assay (cytotoxicity)
- Cytochrome P450 activity
- Profiling of inflammatory mediators

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Outline

## Results from series of studies assessing heated tobacco products

Toxicol Mech Methods. 2014 Oct;24(7):470-87. doi: 10.3109/15376516.2014.943441. Epub 2014 Sep 11.

**In vitro systems toxicology approach to investigate the effects of repeated cigarette smoke exposure on human buccal and gingival organotypic epithelial tissue cultures.**

Schlage WK[1], Iskandar AR, Kostadinova R, Xiang Y, Sewer A, Majeed S, Kuehn D, Frentzel S, Talikka M, Geertz M, Mathis C, Ivanov N, Hoeng J, Peitsch MC.

Chem Res Toxicol. 2016 Aug 15;29(8):1252-69. doi: 10.1021/acs.chemrestox.6b00174. Epub 2016 Aug 5.

**Systems Toxicology Assessment of the Biological Impact of a Candidate Modified Risk Tobacco Product on Human Organotypic Oral Epithelial Cultures.**

Zanetti F[1], Sewer A[1], Mathis C[1], Iskandar AR[1], Kostadinova R[1], Schlage WK[2], Leroy P[1], Majeed S[1], Guedj E[1], Trivedi K[1], Martin F[1], Elamin A[1], Merg C[1], Ivanov NV[1], Frentzel S[1], Peitsch MC[1], Hoeng J[1].

Food Chem Toxicol. 2017 Mar;101:15-35. doi: 10.1016/j.fct.2016.12.027. Epub 2016 Dec 23.

**Comparative systems toxicology analysis of cigarette smoke and aerosol from a candidate modified risk tobacco product in organotypic human gingival epithelial cultures: A 3-day repeated exposure study.**

Zanetti F[1], Titz B[2], Sewer A[2], Lo Sasso G[2], Scotti E[2], Schlage WK[3], Mathis C[2], Leroy P[2], Majeed S[2], Torres LO[2], Keppler BR[4], Elamin A[2], Trivedi K[2], Guedj E[2], Martin F[2], Frentzel S[2], Ivanov NV[2], Peitsch MC[2], Hoeng J[2].

Food Chem Toxicol. 2018 May;115:148-169. doi: 10.1016/j.fct.2018.02.062. Epub 2018 Mar 2.

**Assessment of the impact of aerosol from a potential modified risk tobacco product compared with cigarette smoke on human organotypic oral epithelial cultures under different exposure regimens.**

Zanetti F[1], Sewer A[2], Scotti E[2], Titz B[2], Schlage WK[3], Leroy P[2], Kondylis A[2], Vuillaume G[2], Iskandar AR[2], Guedj E[2], Trivedi K[2], Schneider T[2], Elamin A[2], Martin F[2], Frentzel S[2], Ivanov NV[2], Peitsch MC[2], Hoeng J[2].

## Results from dental color stability study

Am J Dent. 2017 Dec;30(6):316-322.

**Effects of cigarette smoking on color stability of dental resin composites.**

Zhao X[1,2], Zanetti F[3], Majeed S[3], Pan J[2], Malmstrom H[1], Peitsch MC[3], Hoeng J[3], Ren Y[1].

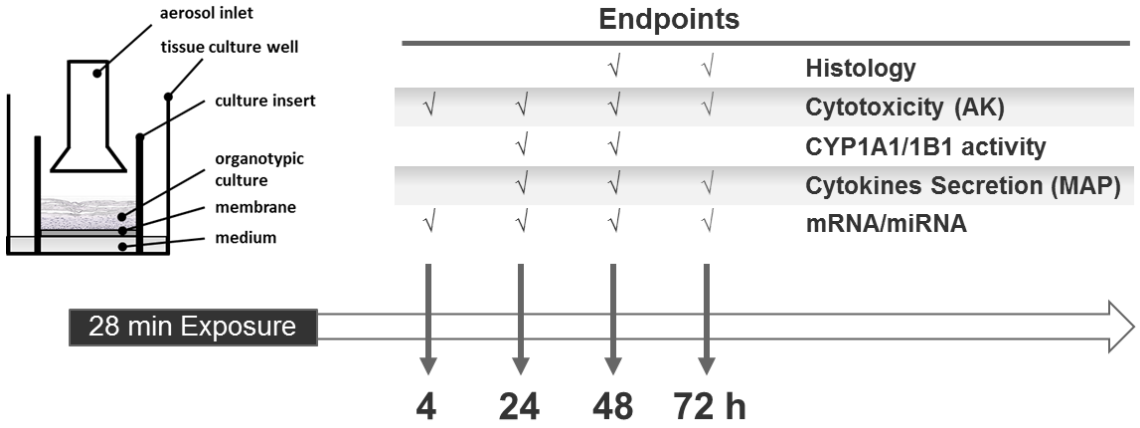
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

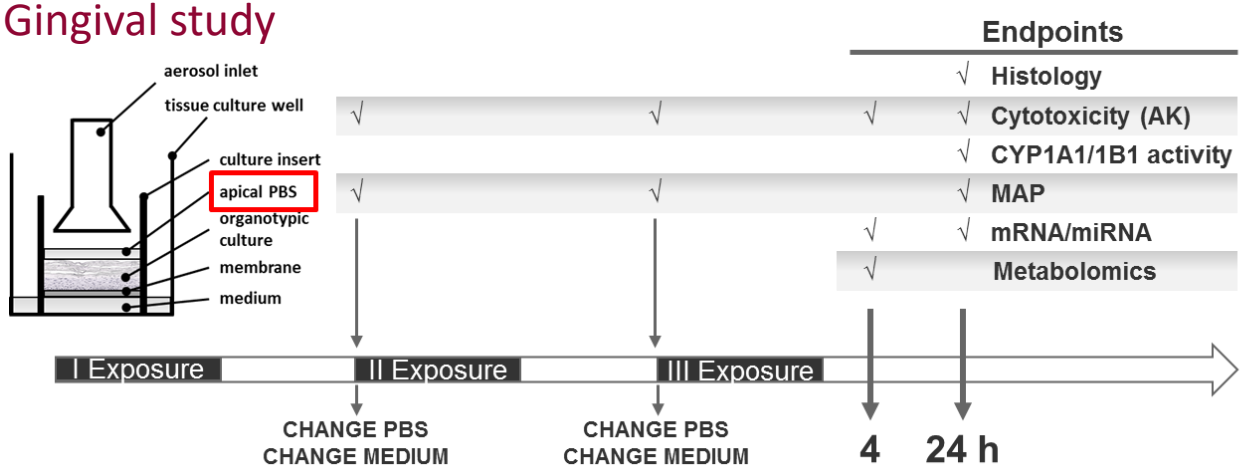
# Assessment Studies of Heated Tobacco Aerosols Using Organotypic Oral Cultures (Buccal and Gingival)

# Study Design: To Assess the Reduced Impact of an RRP compared with Cigarette Smoke

## Buccal study




## Gingival study




- 3 experimental repetitions

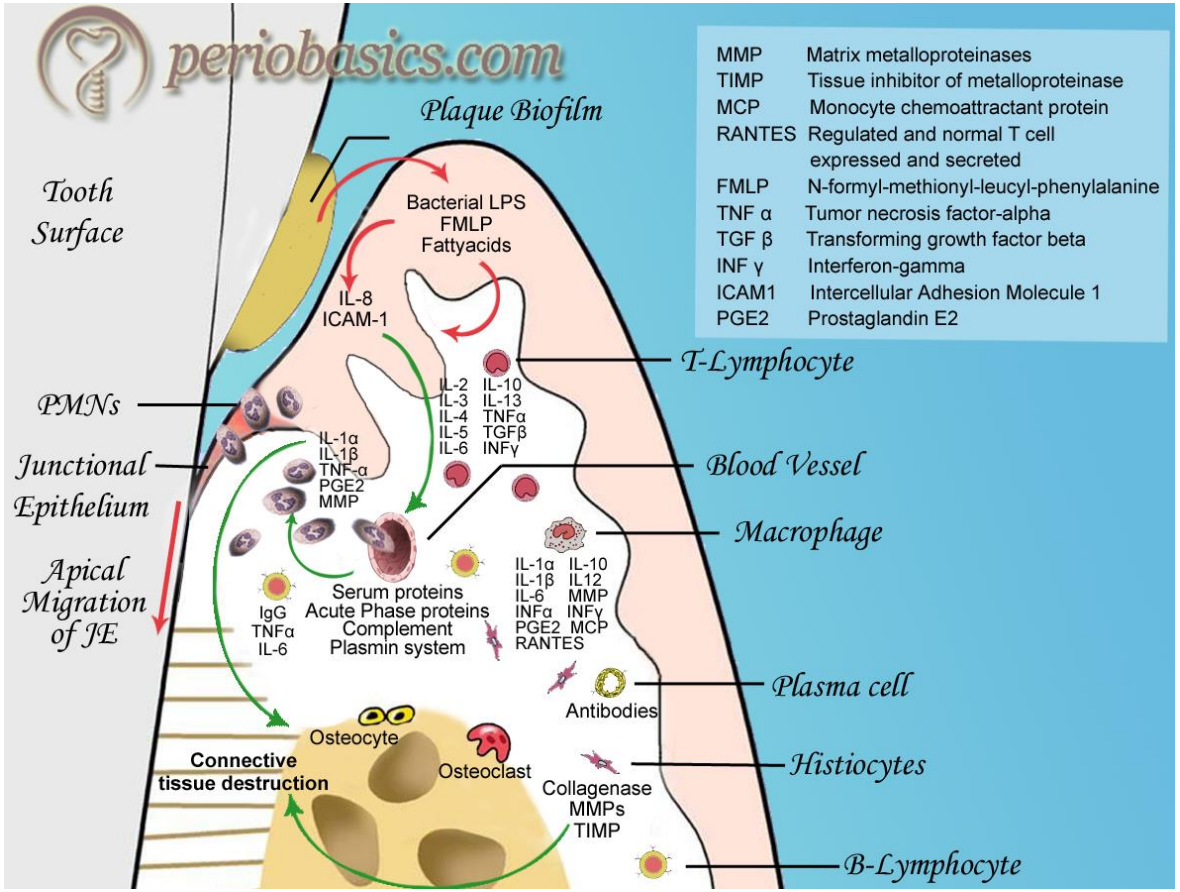
| Tissue | Experimental Phase | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Buccal | ●●● | ●●● | ●●● |
| Gingival | ●●● | ●●● | ●●● |

● independent smoke/aerosol generation

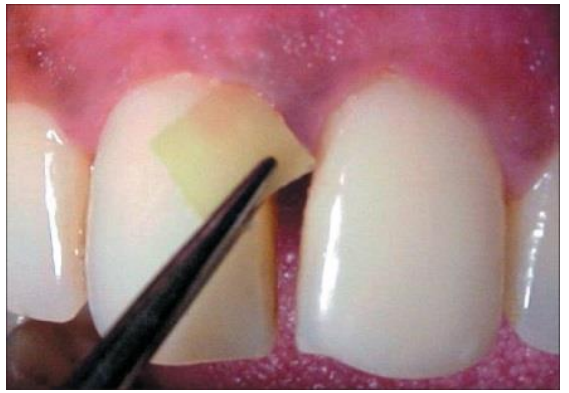
- Exposure at comparable nicotine concentrations
- 3R4F reference cigarettes were used as the control to assess the effects of cigarette smoke exposure

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

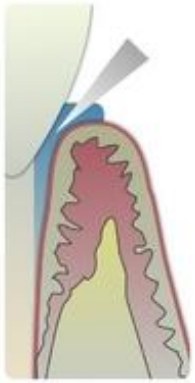
# Periodontal Diseases and Inflammation




- Cytokines involved in inflammation and repair of periodontal tissues
- Cytokine profiles can potentially be used for diagnosis and prognostic markers



https://www.researchgate.net/figure/257839870_fig2_Gingival-crevicular-fluid-collection-by-micro-capillary-pippettes



http://www.hindawi.com/journals/tswj/2013/105873/

- Cigarette smoke constituents directly affect immune cells present in gingival tissue

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Inflammatory Mediators in Gingival Crevicular Fluid

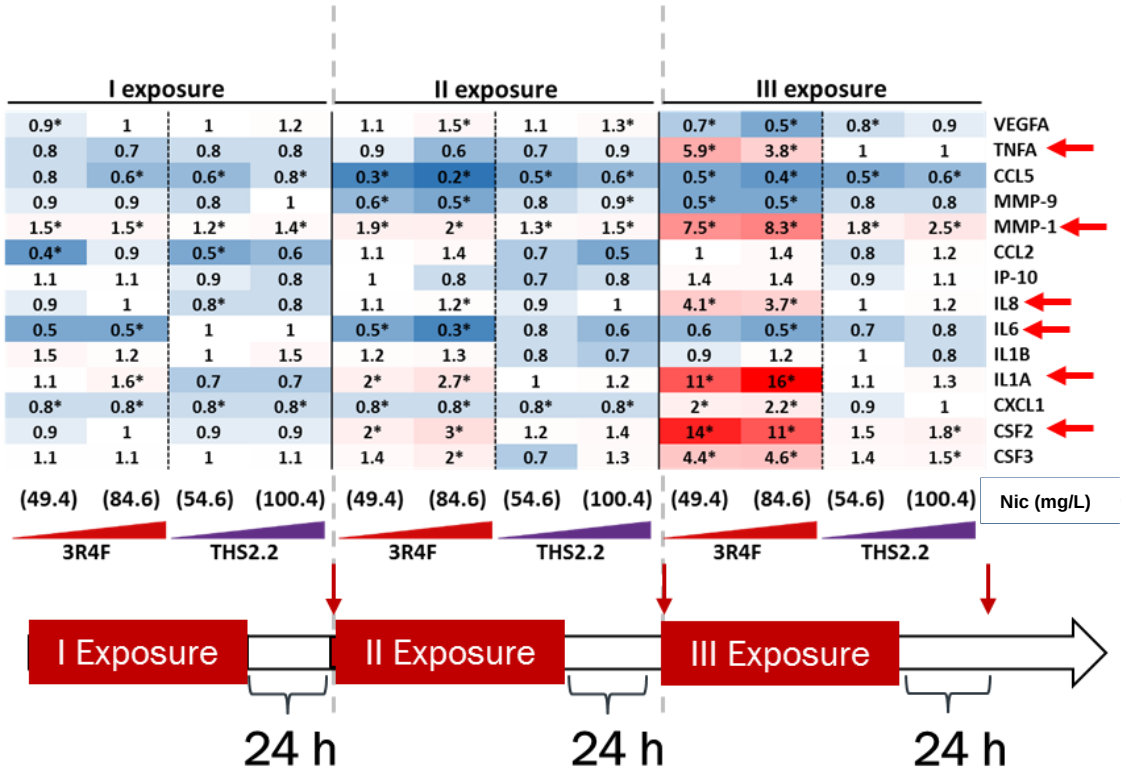
| Component | Source | Function |
|---|---|---|
| Bacteria | Oral biofilm plaque | Initiate the host immune response |
| Epithelial cells | Oral sulcular and junctional epithelium | Represent the high cell turnover of the gingival sulcus |
| Leukocytes | Gingival blood vessel plexus | Polymorphonuclear neutrophils are involved in innate immunity Monocytes/macrophages and lymphocytes are involved in cell-mediated immunity |
| Erythrocytes | Gingival blood vessels | Result from damage to small blood vessels and capillaries |
| Alkaline phosphatase | Fibroblasts, osteoblasts, osteoclasts, neutrophils | Plays a role in superoxide generation and in the first line of defense |
| Cathepsin B | Macrophages | Active enzyme in proteolysis |
| Collagenase-2 (matrix metalloproteinase-8) | Neutrophils | Active enzyme associated with collagenatic activity |
| Gelatinase (matrix metalloproteinase-9) | Neutrophils | Hydrolysis of intercellular matrix |
| Neutrophil elastase | Neutrophils | Cleavage of elastin, collagen and proteoglycans |
| Macrophage elastase (matrix metalloproteinase-12) | Macrophages | Cleavage of elastin, collagen and proteoglycans |
| Carboxyterminal telopeptide of type I collagen | Fragment of bone type I collagen | Highly correlated with bone turnover |
| Interleukin-1beta | Macrophages | Regulates immune and inflammatory reactions Stimulates bone resorption |
| Interleukin-4 | Basophils | Anti-inflammatory Macrophage inhibition T-helper 2 cell differentiation |
| Transforming growth factor-beta | Macrophages | Modulates proinflammatory cytokine production |
| Tissue inhibitors of metalloproteinases | Neutrophils, macrophages, fibroblasts, keratinocytes | Inhibits matrix metalloproteinases |
| Tumor necrosis factor-alpha | Neutrophils, macrophages, lymphocytes | Delays neutrophil apoptosis |
| Interleukin-6 | T-cells, macrophages, osteoblasts | Regulator of T- and B-cell growth Stimulates osteoclast formation |
| Interleukin-8 | Macrophages, epithelial cells | Recruitment and activation of neutrophils |
| Interferon gamma | Leukocytes, lymphocytes | Macrophage activation Suppression of T-helper 2 cells |
| IgA | Plasma cells | Antigen neutralization |
| IgG | Plasma cells | Antigen neutralization |
| IgM | Plasma cells | Antigen neutralization |
| Lactoferrin | Neutrophils, acinar cells | Antibacterial Creates iron-limiting environment |
| Lysozyme | Neutrophils, macrophages | Hydrolysis of peptidoglycans of bacterial cell walls |
| Osteoprotegerin | Osteoblasts | Decoy receptor for RANKL Inhibits osteoclast formation |
| Osteocalcin | Osteoblasts | Calcium binding |
| Prostaglandin $E_2$ | All cell types | Proinflammatory and immunomodulatory effects |
| Transforming growth factor-alpha | Macrophages, keratinocytes | Regulation of tissue repair, cell proliferation, chemotaxis, differentiation and matrix synthesis |

Barros et al., 2016

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Greater Changes in the Concentrations of Secreted Inflammatory Mediators Were Detected Following Cigarette Smoke than THS 2.2 Aerosol Exposure

## Assessment of THS 2.2 using human organotypic gingival cultures



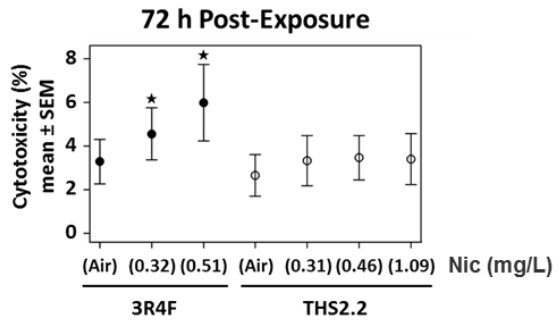

- IL8: released in gingival crevicular fluid of smokers and periodontitis patients[1]
- MMP-1: potential marker of tissue repair in periodontitis[2], increased in oral inflammatory models[3]
- IL1A: shown to increase bone resorption and collagen turnover and stimulate other inflammatory cytokines[4]
- TNFA: upregulated in gingival tissues of smokers[5]
- IL6: decreased in smokers' saliva[6]

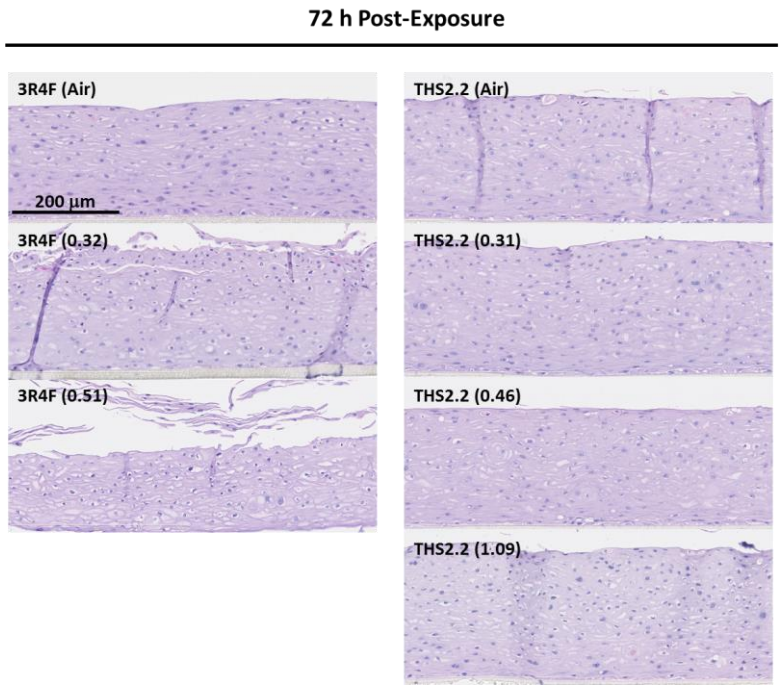
[1]Giannopoulou et al., 2003
[2]Romanelli et al., 1999
[3]Kim et al., 2006
[4]De Nardin et al., 2001
[5]Bostrom et al., 1998; Bostrom et al., 1999; Ojima and Hanioka, 2010,
[6]Tymkiw et al., 2011

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

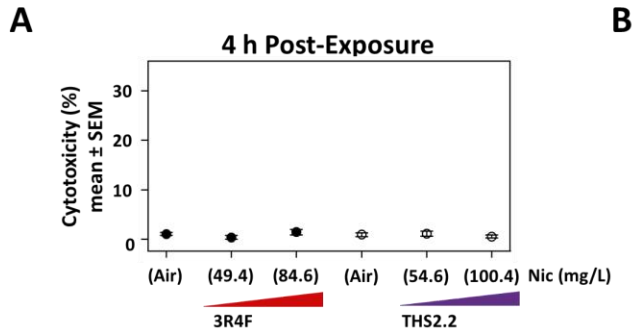
# Tissue Damage Was Not Observed in Buccal and Gingival Cultures Following CHTP 1.2 Aerosol Exposure

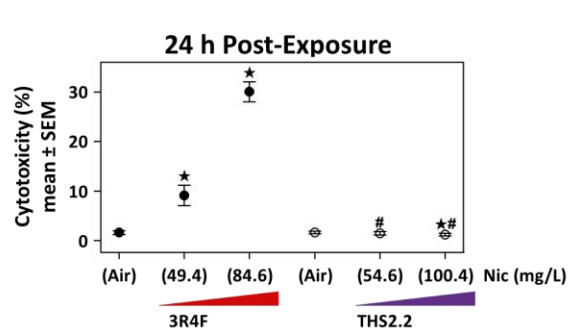
## Buccal

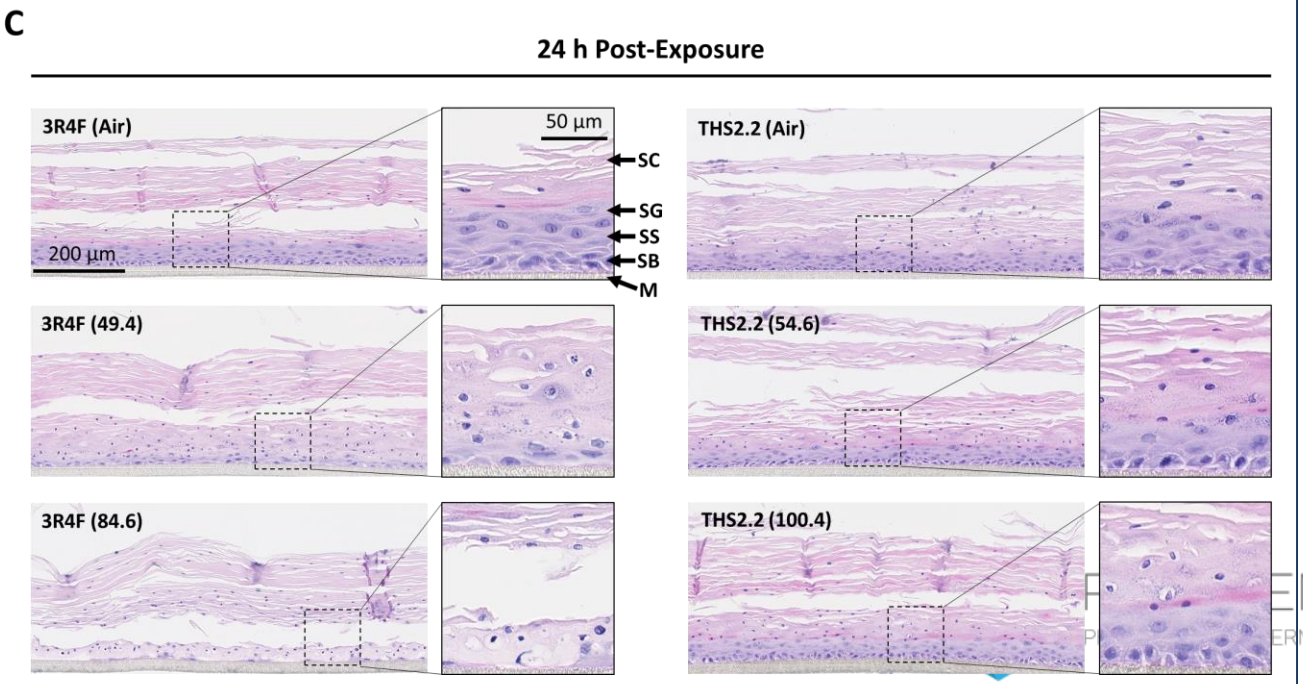



## Gingival

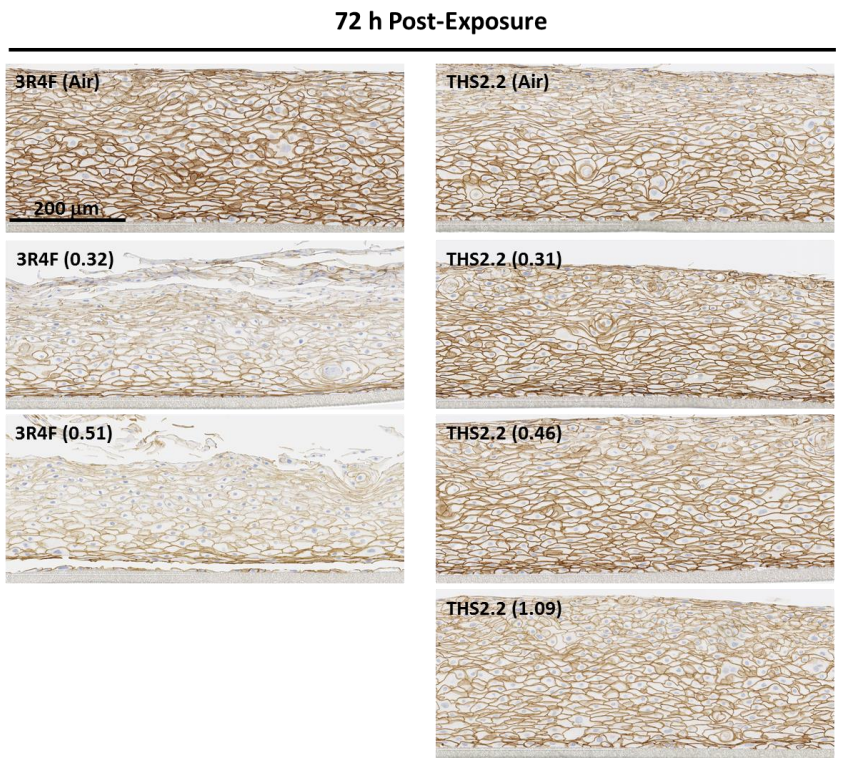


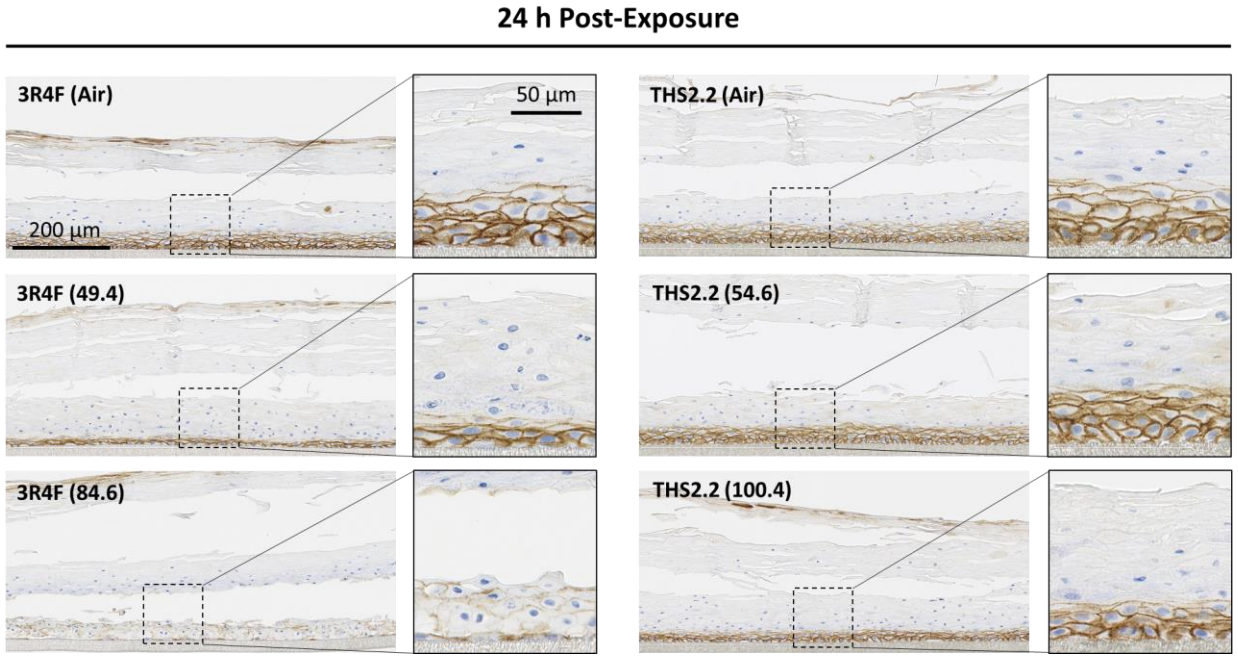



# Decreased Expression of E-cadherin Was Detected Following Cigarette Smoke but Not CHTP 1.2 Aerosol Exposure

## Buccal




## Gingival



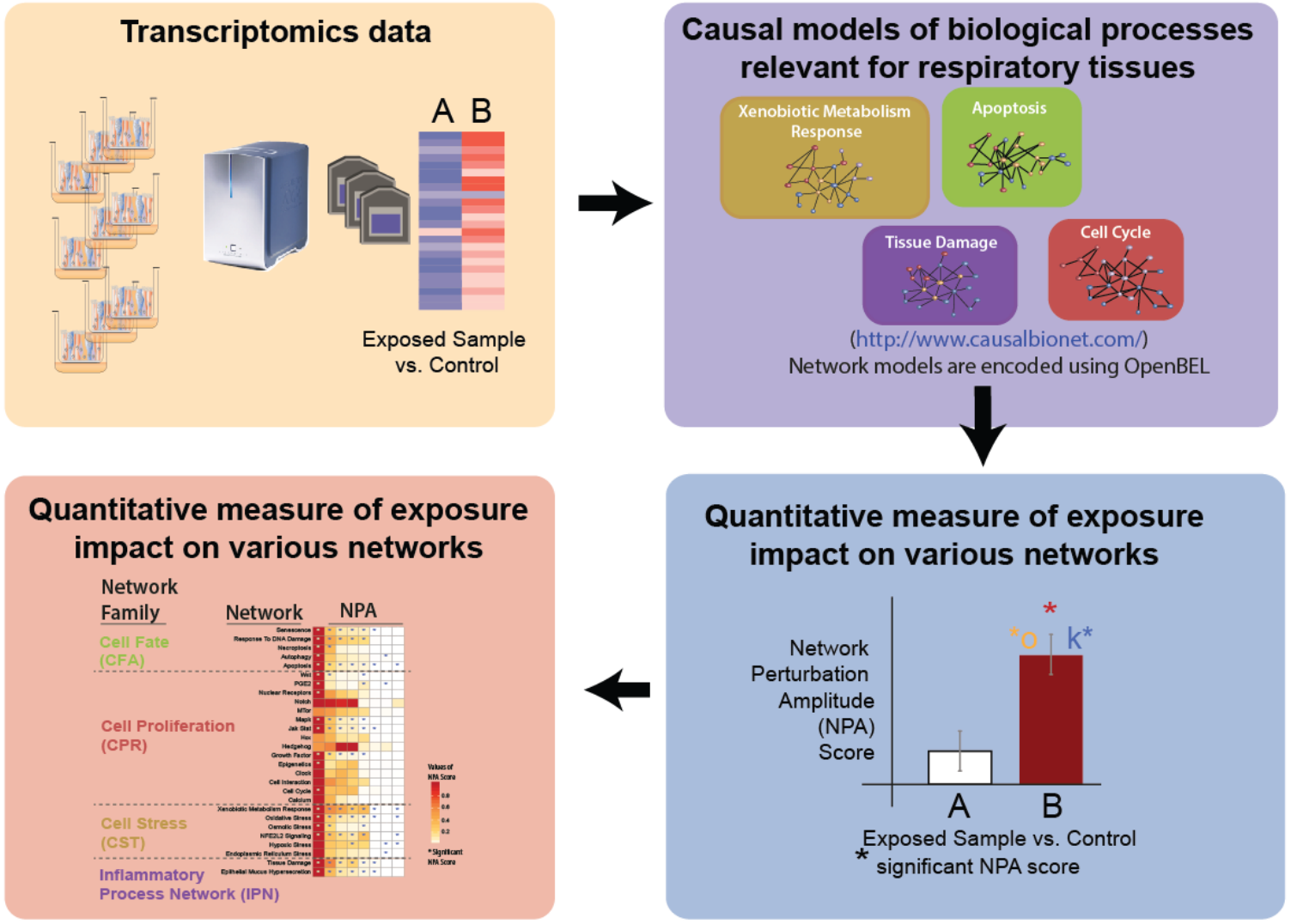

A destabilization of E-cadherin expression by CS was also demonstrated in oral mucosa cells[1,2]

A significant reduction in E-cadherin levels was reported in periodontal disease compared with healthy conditions[3]

[1]Hasegawa et al., 2002. [2]Coppe et al., 2008. [3]Arun R et al., 2010.

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

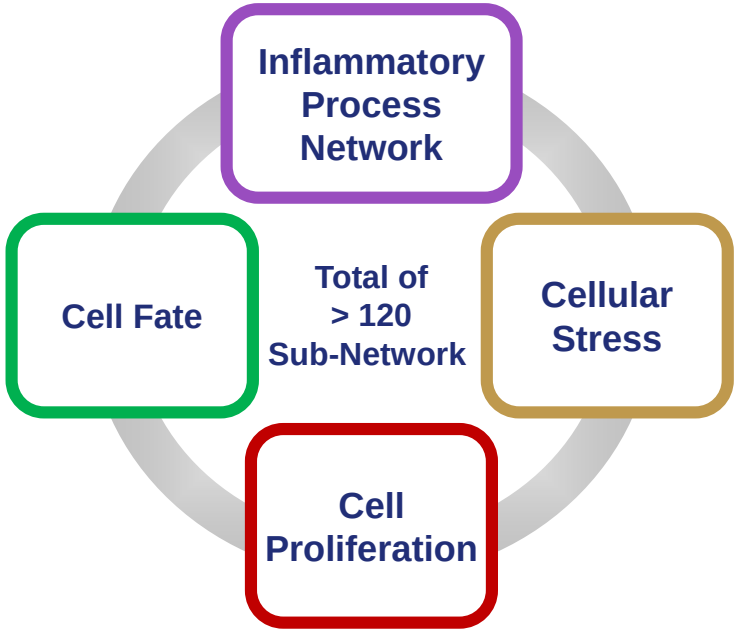
# Quantitative Mechanism-Based System Impact Assessment

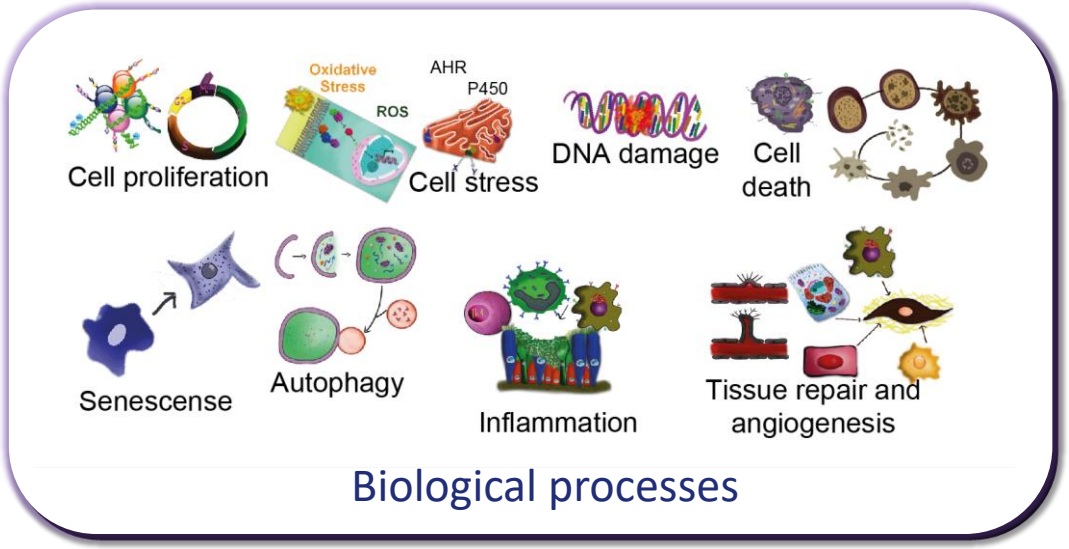



Hoeng et al., 2012

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

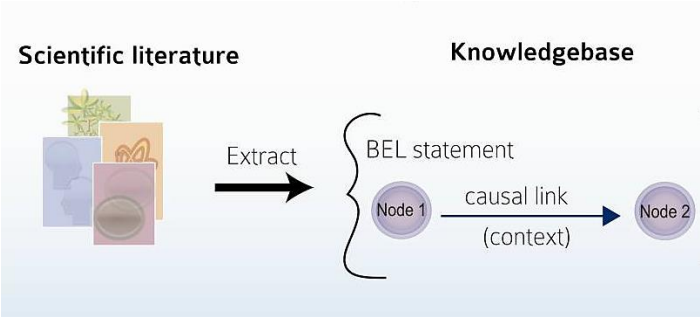
# Causal Network Models




Boué, S., et al. (2015). Causal biological network database: a comprehensive platform of causal biological network models focused on the pulmonary and vascular systems. Database, 2015, bav030.

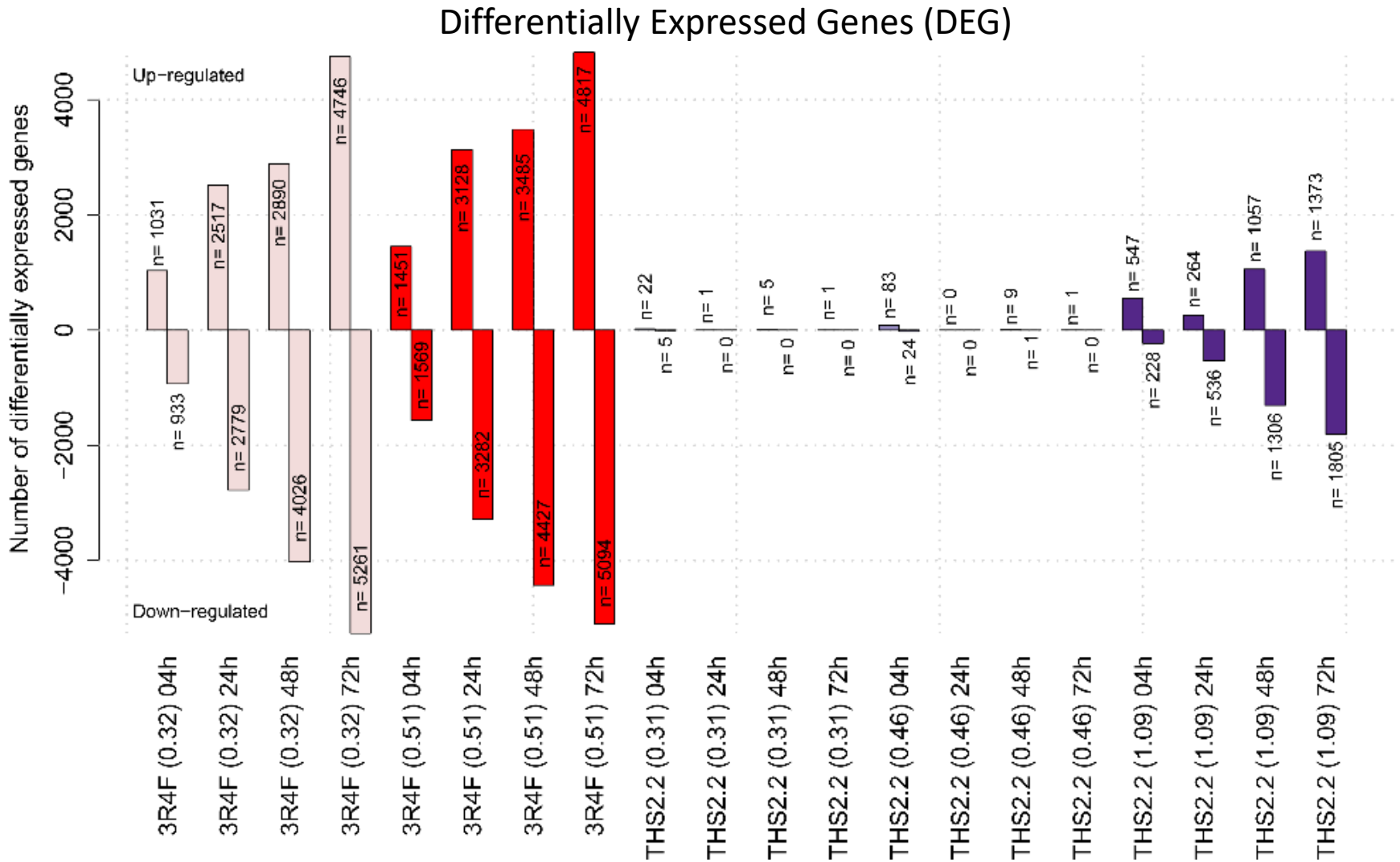
The Causal Biological Networks are composed of more than 120 manually curated and well-annotated biological network models that can be accessed at http://causalbionet.com.




BEL-scripted causal network models that describe signaling pathways relevant in diseased and non-diseased pulmonary and vascular tissues

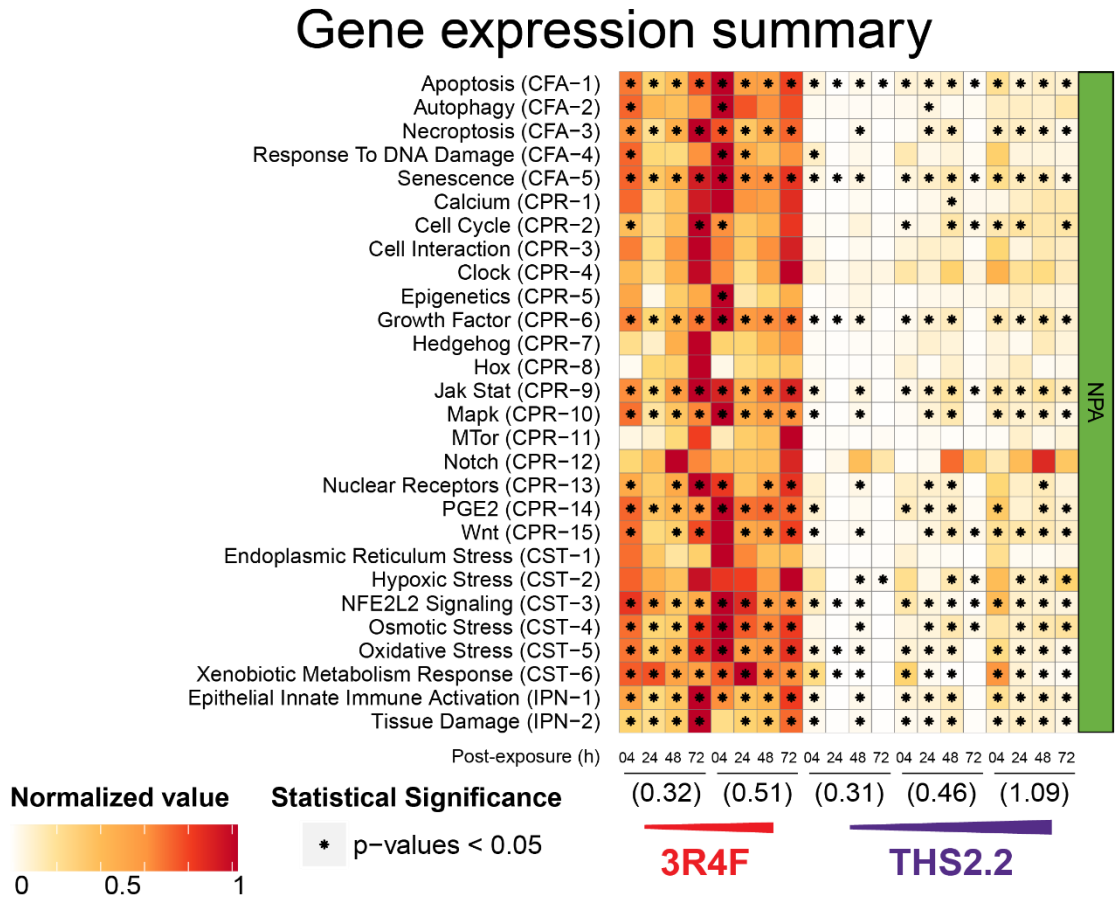
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

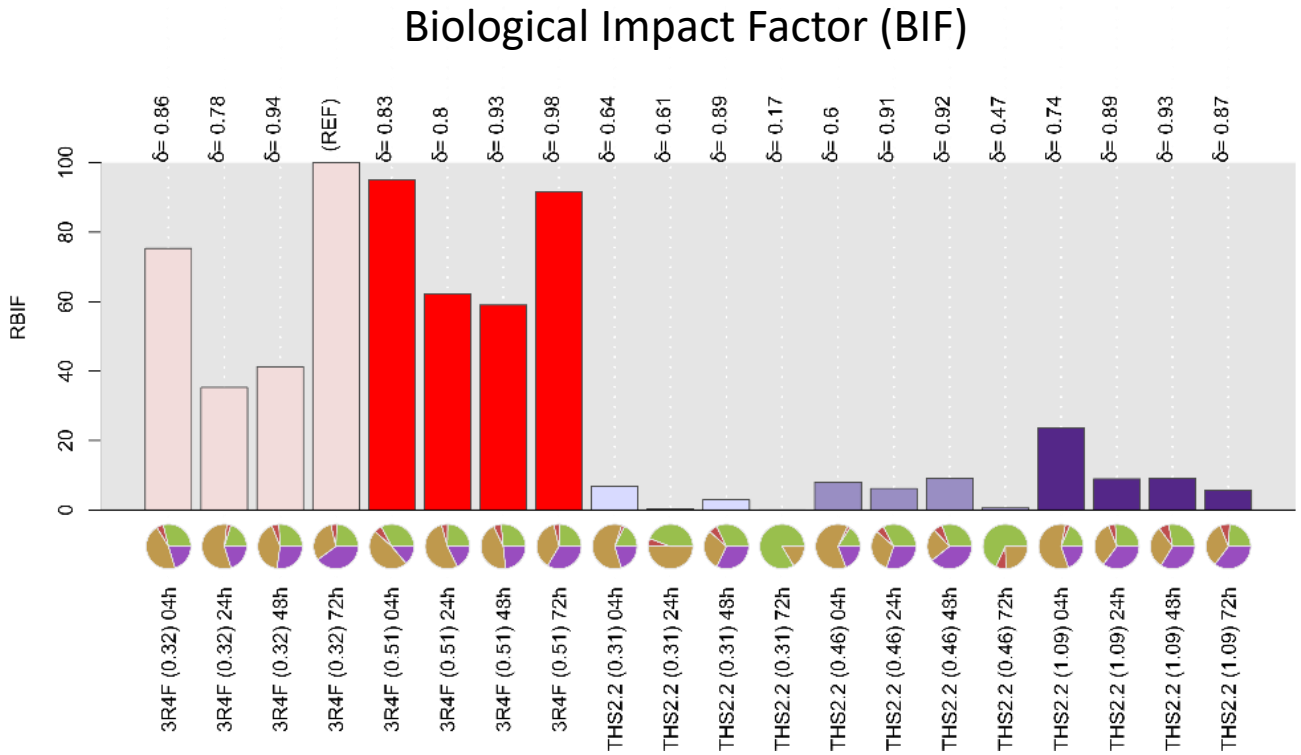
# A Network-Based Enrichment Analysis on the Transcriptome Data Demonstrated Reduced Impact on Biological Processes Following Exposure to THS 2.2 Aerosol in Buccal Cultures Compared with Cigarette Smoke Exposure




PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# A Network-Based Enrichment Analysis on the Transcriptome Data Demonstrated Reduced Impact on Biological Processes Following Exposure to THS 2.2 Aerosol in Buccal Cultures Compared with Cigarette Smoke Exposure



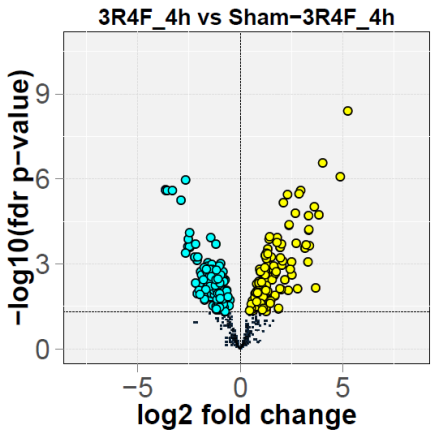



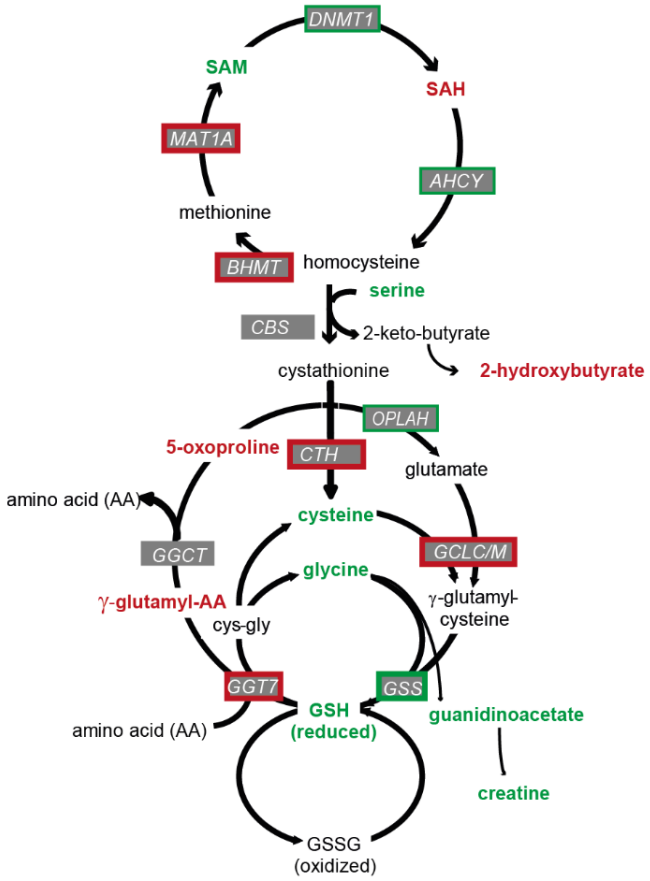

- Inflammation, oxidative stress, and xenobiotic metabolism are known to be impaired by cigarette smoke[1]
- Important network models related to periodontal diseases[2]

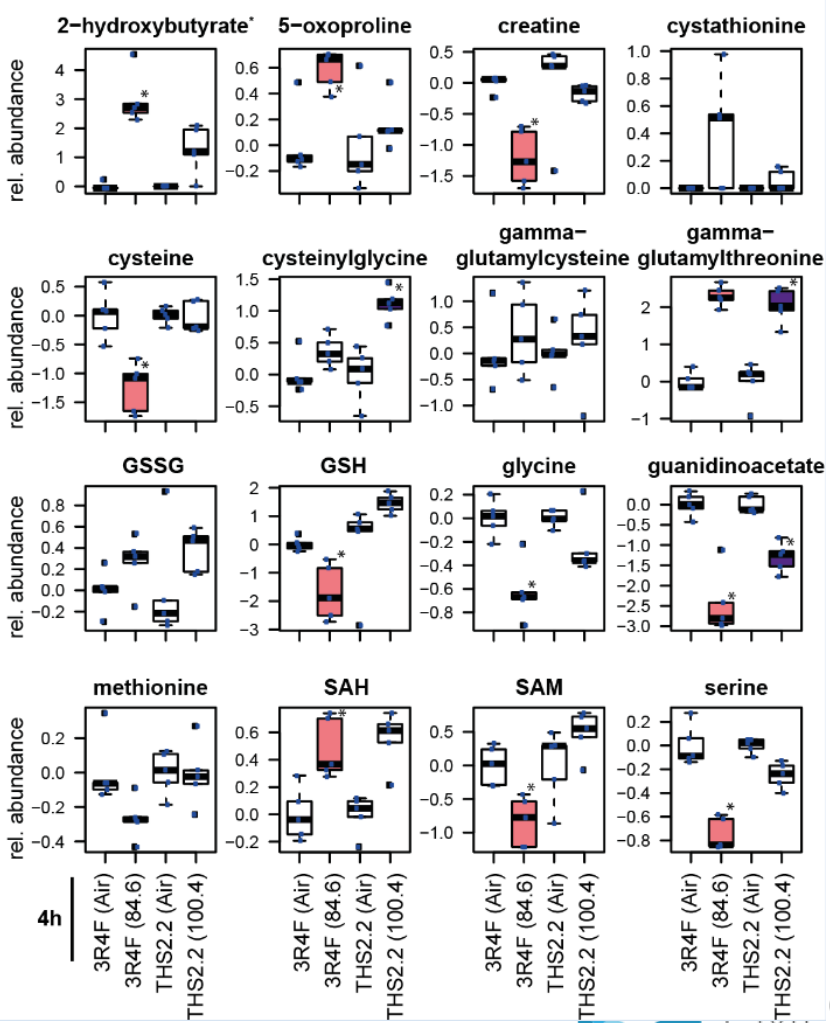
PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Results: Metabolomics Investigations Allowed Detection of a Lower Number of Metabolites Impacted by THS 2.2 Aerosol than by Cigarette Smoke

## Gingival THS 2.2 Study



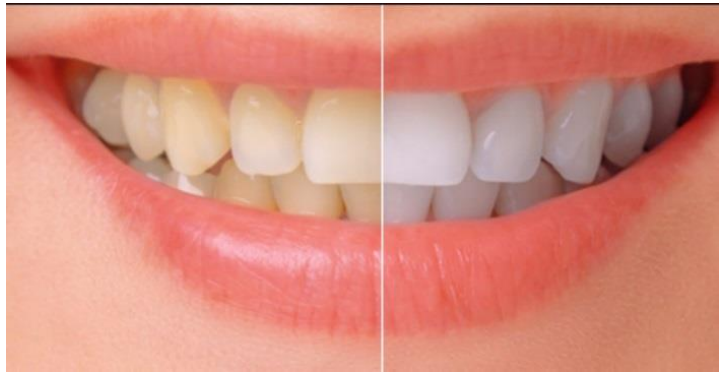

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Dental Color Stability Study

## Collaboration with the University of Rochester - Prof. Yanfang Ren

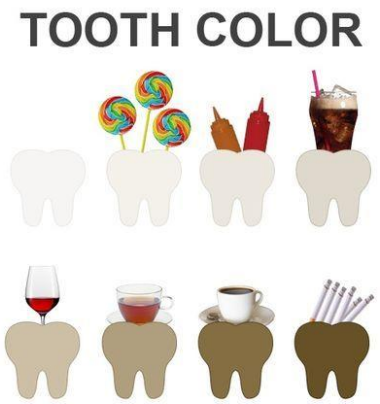
# Cigarette Smoke and Tooth Coloration



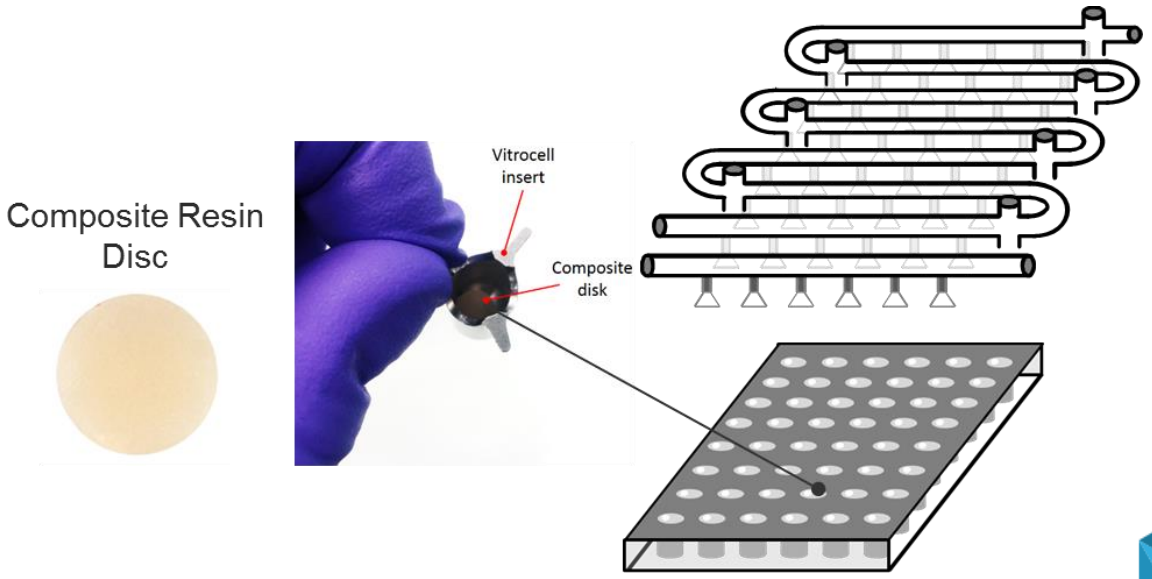
http://www.kenzdental.com/blog/tips-to-prevent-tooth-discoloration-after-teeth-whitening

Nicotine and TAR are responsible for the following smoke-related teeth alterations:

- Tooth discoloration
- Increased build up of plaque and tartar on the teeth
- Gloss alteration
- Surface roughness alterations

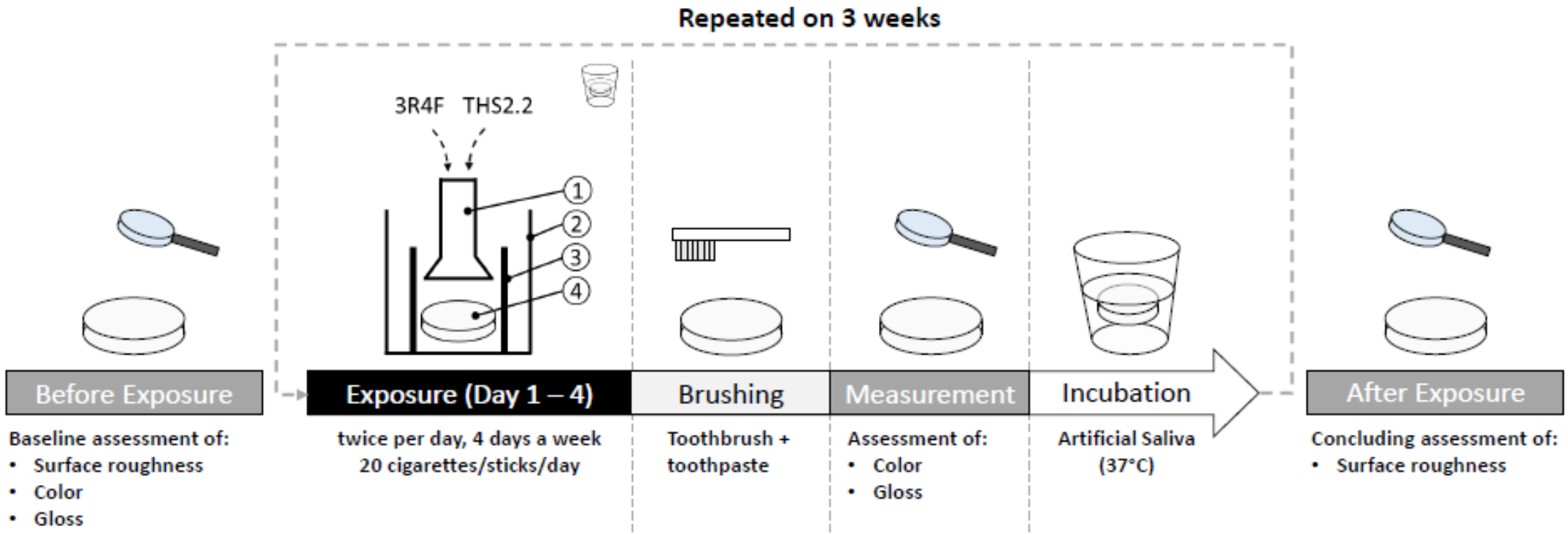



https://twitter.com/hashtag/discoverdental




PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Study Design




① Aerosol inlet

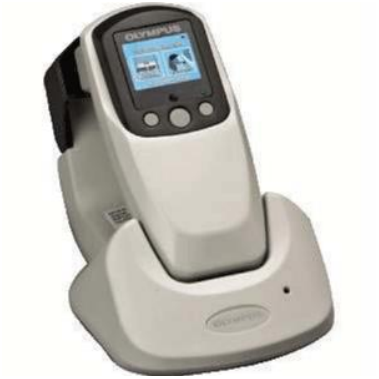
② Vitrocell plate well

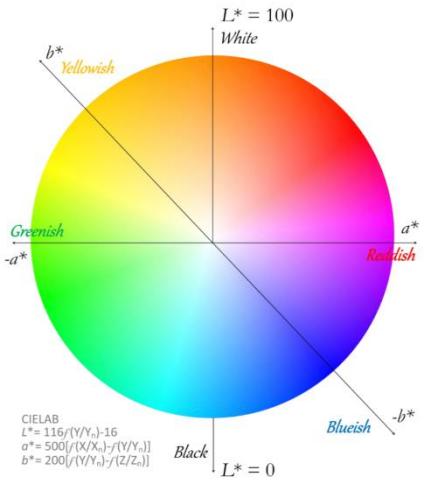
③ Insert

④ Composite resin disc

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Cigarette Smoke Exposure Causes a Higher Discoloration of Composite Resins than THS 2.2 Aerosol






http://www.fsw.cc/color-spaces/


**Δ**E values (mean and standard deviation) of composite resins after three weeks of smoking challenges

| | DVS | | TEC | | FSU | |
|---|---|---|---|---|---|---|
| | THS2.2 | 3R4F | THS2.2 | 3R4F | THS2.2 | 3R4F |
| **Week 3** | 4.0 ± 0.6 | 23.0 ± 1.2 | 5.3 ± 1.5 | 30.4 ± 1.4 | 2.6± 0.5 | 28.0± 2.5 |

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Conclusions

- Acute and repeated cigarette smoke exposure impacted organotypic buccal and gingival culture biology.
- THS 2.2 aerosol exposures minimally affected organotypic buccal and gingival cultures compared with cigarette smoke.
- A series of experimental repetitions ensured statistical robustness and reproducibility of results.
- Systems toxicology comprises several endpoints and allows a better understanding of biological processes with a mechanistic-multi-omics approach.
- Composite resin color alterations exerted by THS 2.2 aerosol are minimal and below the clinical threshold ($\Delta E < 3.3$) for the FSU product.

PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

# Acknowledgments


## Philip Morris International

Anita Iskandar
Elena Scotti
Bjorn Titz
Carole Mathis
Karsta Luettich
Walter Schlage
Julia Hoeng
Manuel C. Peitsch

Patrice Leroy
Alain Sewer
Florian Martin

Shoaib Majeed
Laura Ortega Torres
Stefan Frentzel

Celine Merg
Maica Corciulo
Keyur Trivedi
Ashraf Elamin

Karine Baumer
Sam Ansari
Remi Dulize
Dariusz Peric
Emmanuel Guedj
Nikolai V. Ivanov

## University of Rochester

Yanfang Ren
Xiaoyi Zhao


Thank you