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

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Reduced Risk Products ("RRPs") is the term PMI uses to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. PMI has a range of RRPs **in various stages of development, scientific assessment, and commercialization**. Because PMI's RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.

Background

- Smoking causes serious diseases (U.S. Surgeon General, 2004).
- Smoking cessation remains the most effective approach to minimize the risk of 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).
- To determine whether RRPs have the potential to reduce individual risk, we are conducting extensive and rigorous scientific studies to understand their biological impact and compare it with that of cigarettes and smoking abstinence/air exposure.

Innovation Cube, Neuchatel, Switzerland

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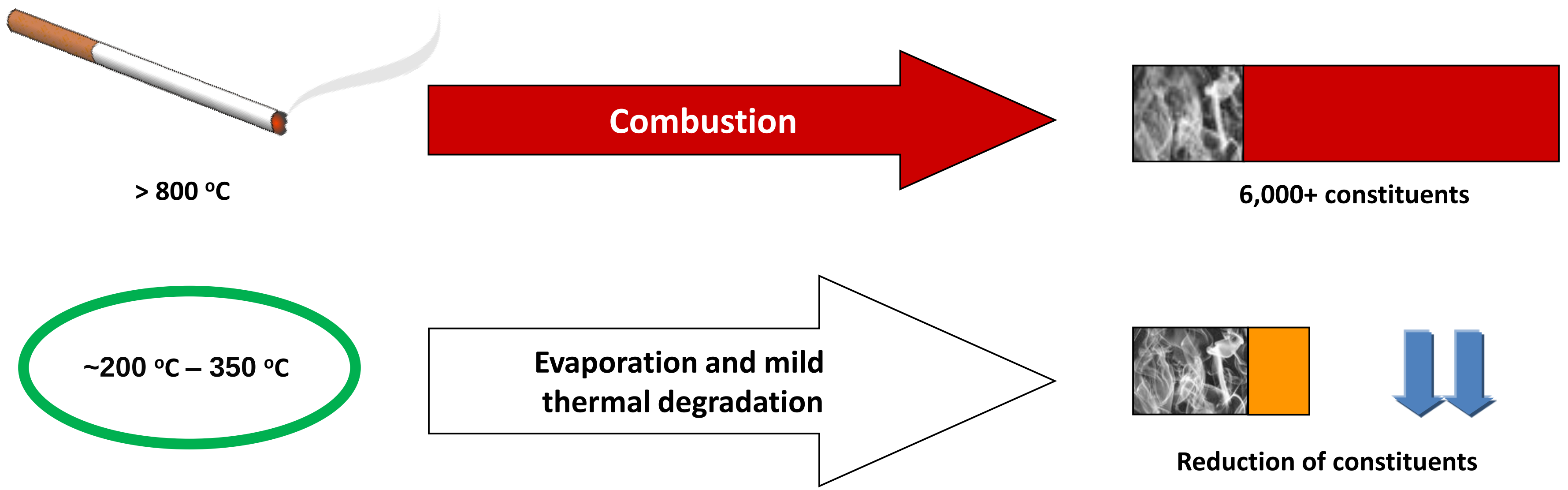
Cigarette Smoke (CS) vs. Heat-not-Burn aerosol

More than 6,000 constituents have been identified in CS. About 100 of them are considered harmful and potentially harmful constituents (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.

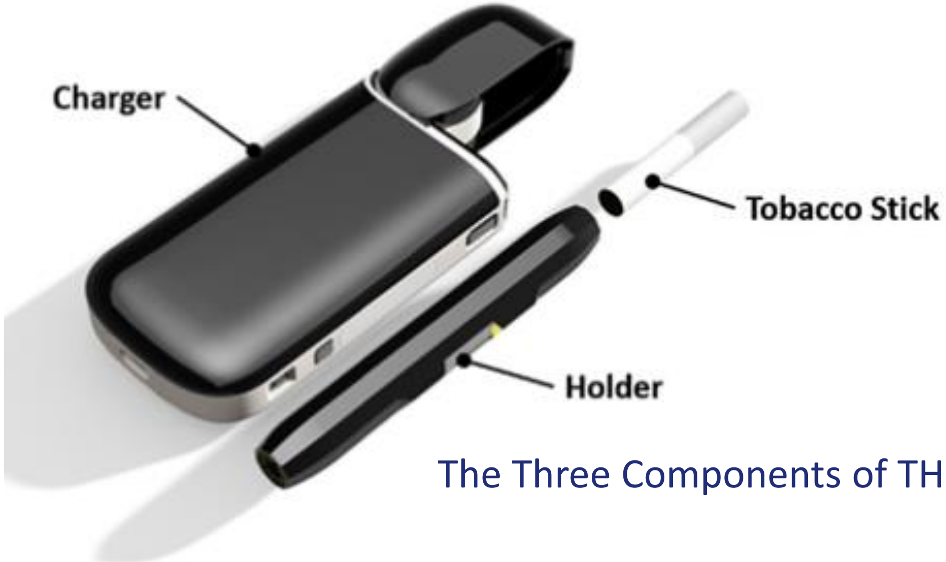


Lower temperatures reduce constituents in the aerosol

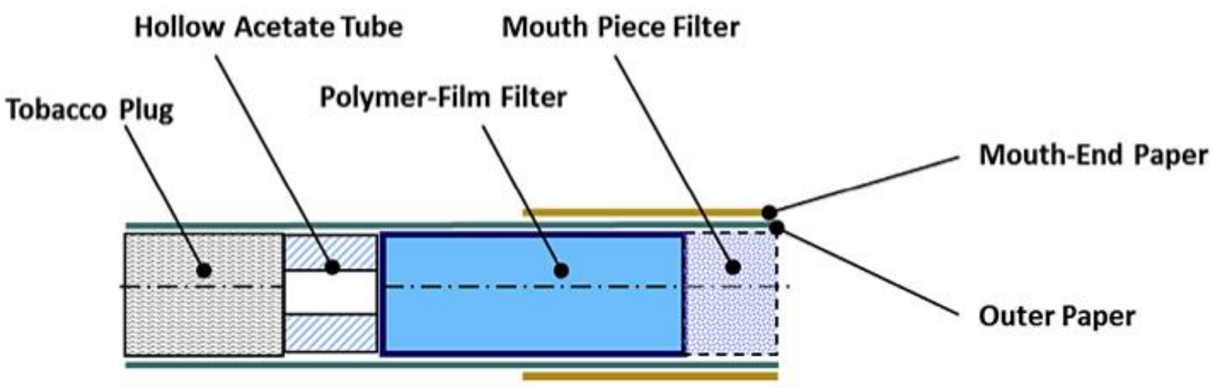
Nicotine is transferred via distillation

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.

RRPs: Tobacco Heating System (THS) 2.2 Operating Principles



The Three Components of THS 2.2



Cross-Sectional View of the Tobacco Stick

Key Principles

- Electrically heated 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

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

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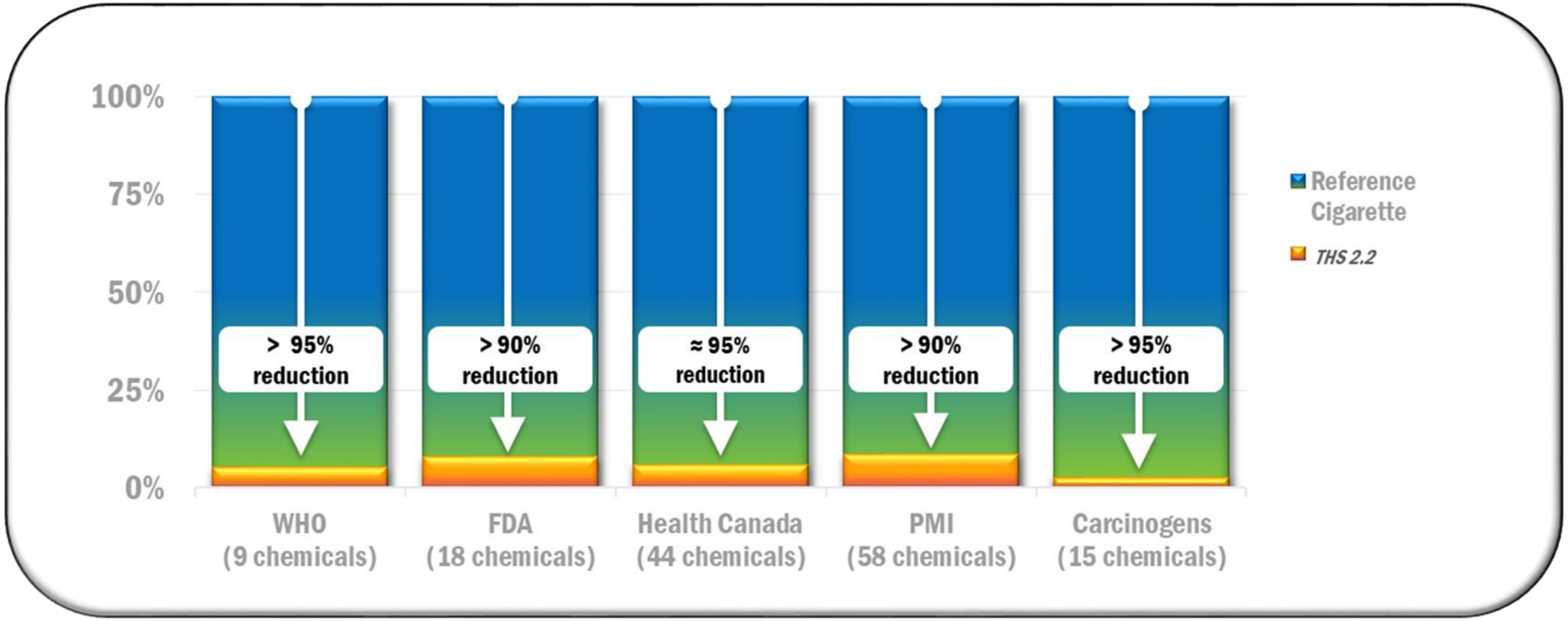
THS 2.2: Aerosol Chemistry

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



*Aerosol collection with Health Canada Intense smoking regime (55 mL puff volume, 2-second puff duration, 30-second puff interval)
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 chemicals and 15 carcinogens of IARC Groups 1.

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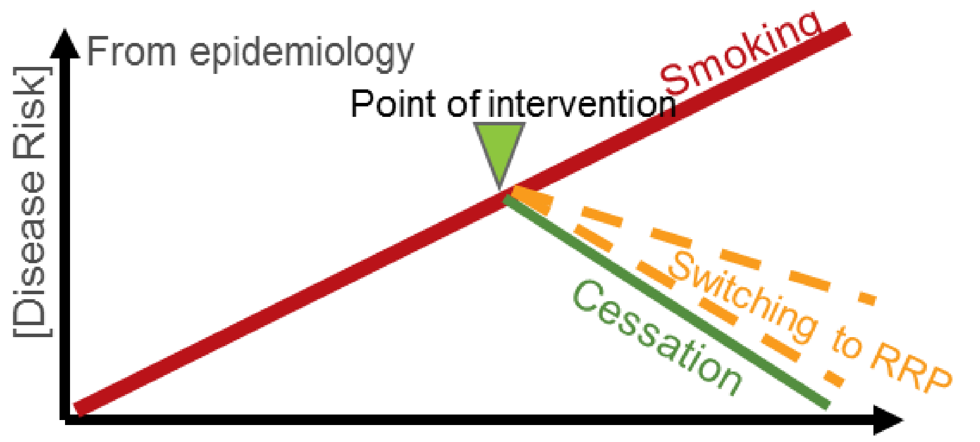
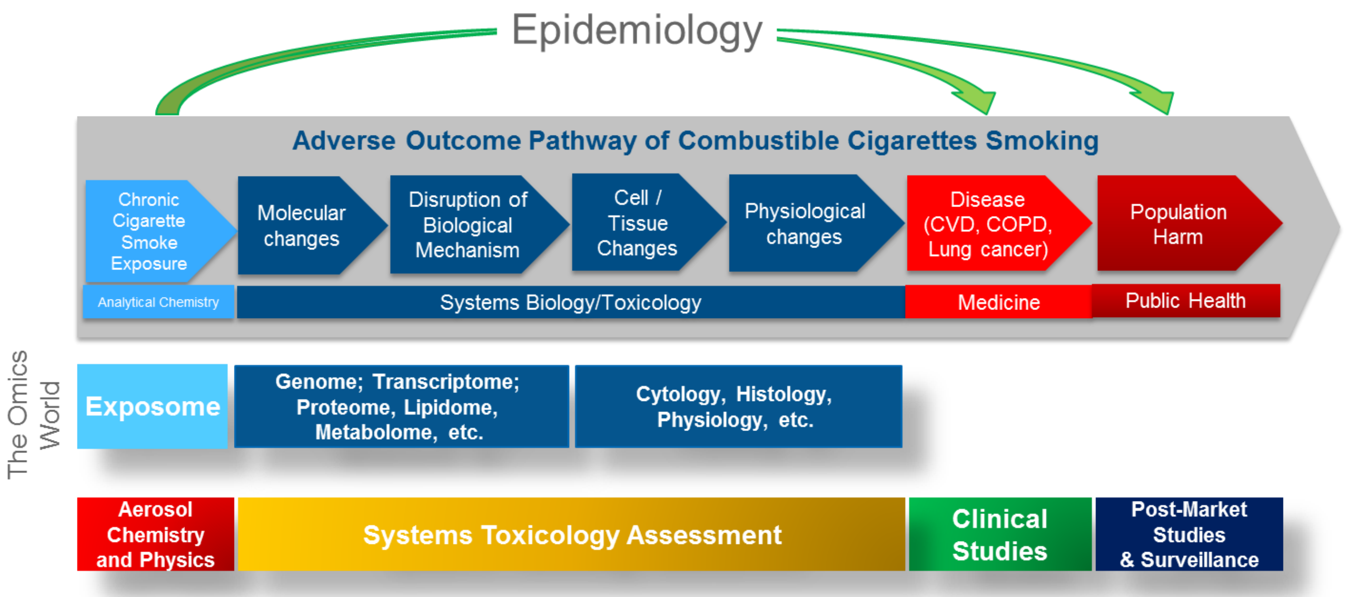
Smith, MR., et al. "Evaluation of the Tobacco Heating System 2.2. Part 1: description of the system and the scientific assessment program." Regulatory Toxicology and Pharmacology 81 (2016): S17-S26.

Conceptual Framework for Product Assessment



The descriptions in the chart are for illustrative purposes only



[1]Source: IOM (Institute of Medicine), 2012, Scientific Standards on Modified Risk Tobacco Products. Washington, DC: The National Academy Press
Source: Smith, M.R., 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 (2016). http://dx.doi.org/10.1016/j.yrtph.2016.07.006

Dental Color Stability Study

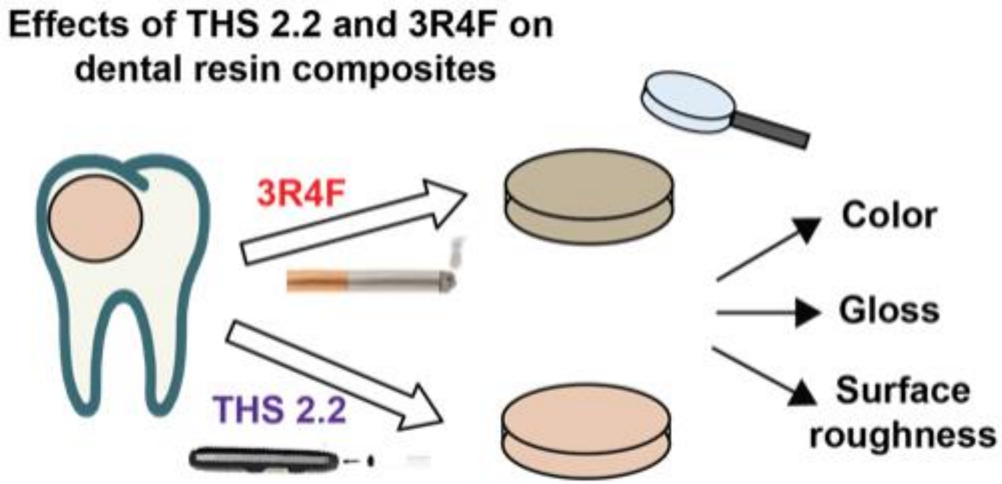
Collaboration with the University of Rochester – Prof. Yanfang Ren

CS and Tooth Color Stability

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

Nicotine and other CS constituents (e.g., carbon-based nanoparticles) are responsible for:

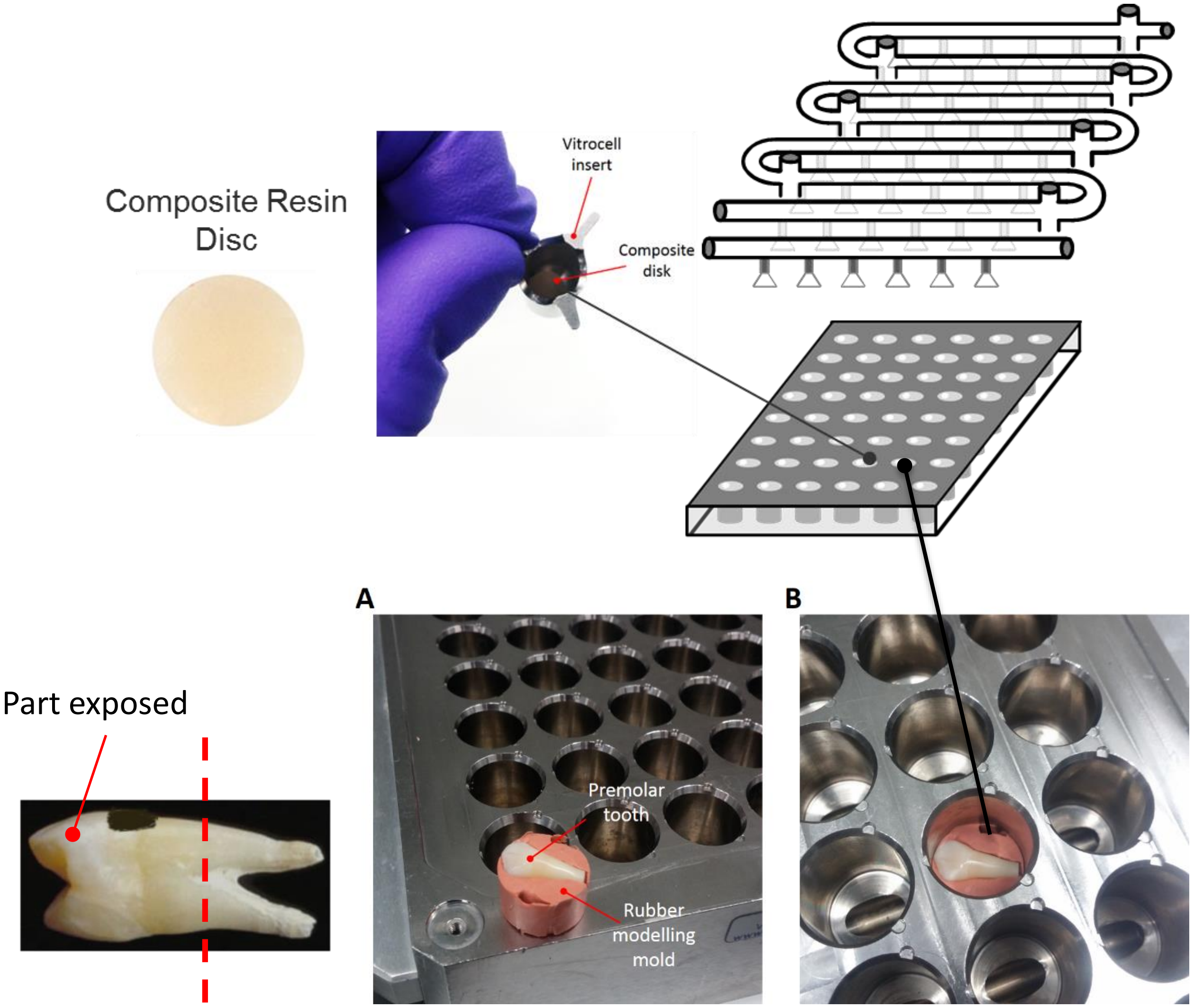
- Tooth color alterations
- Increased build-up of plaque and tartar on teeth
- Gloss alteration
- Surface roughness alterations



Measure *in vitro* the impact of CS and THS 2.2 on color stability of composite resins and human premolar teeth

https://twitter.com/hashtag/discoverdental

Experimental Setup



Composite resins and premolar teeth were allocated in the Vitrocell® 24/48 exposure plate

Source: Zhao X et al., American Journal of Dentistry, Vol. 30, No. 6, December, 2017; Zanetti et al., in preparation

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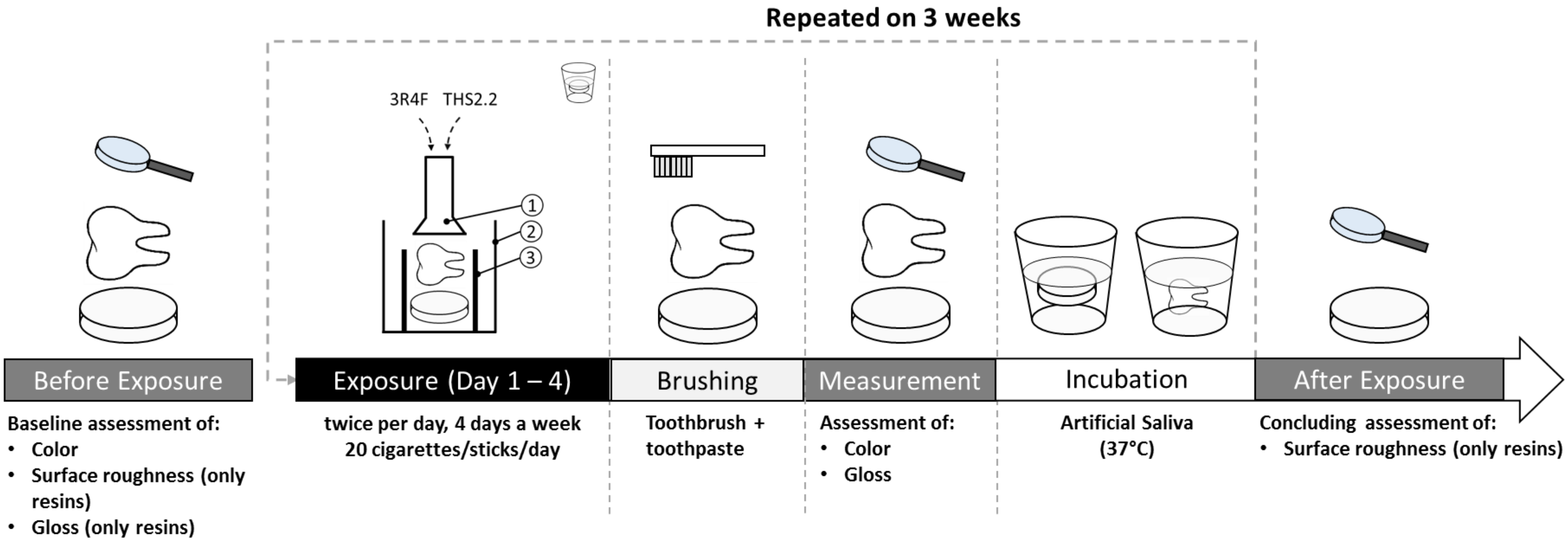
Study Design



1. Aerosol inlet
2. Vitrocell® plate well
3. Insert

Source: Zhao X et al., American Journal of Dentistry, Vol. 30, No. 6, December, 2017; Zanetti et al., in preparation

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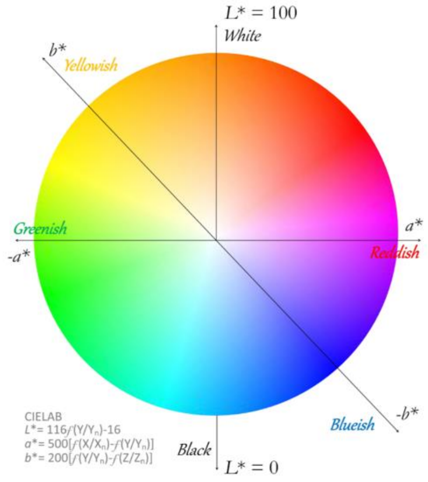
CS Exposure Causes a Higher Impact on Color Stability of Composite Resins than THS 2.2 Aerosol

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



$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

ΔE values (mean and standard deviation) of composite resins after three weeks of CS exposure

	DVS		TEC		FSU	
	THS 2.2	3R4F	THS 2.2	3R4F	THS 2.2	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

DVS (Durafill VS), TEC (Tetric EvoCeram BulkFill), FSU (Filtek Supreme Ultra);
Source: Zhao X et al., American Journal of Dentistry, Vol. 30, No. 6, December, 2017

CS Exposure Causes a Higher Impact on Color Stability of Human Premolar Teeth than THS 2.2 Aerosol

ΔE									
	Enamel			**Dentin**			**Composite resin**		
	Week1	Week2	Week3	Week1	Week2	Week3	Week1	Week2	Week3
3R4F	5.2 ±2.2	7.1 ±2.3	8.8 ±2.6	13.9 ±3.0	18.2 ±3.9	21.3 ±4.4	12.5 ±2.6	19.0 ±4.3	25.6 ±3.8
THS 2.2	1.2 ±0.8	2.0 ±1.0	2.8 ±1.2	1.2 ±0.6	2.4 ±0.7	3.1 ±0.8	1.1 ±0.9	1.9 ±0.8	3.0 ±1.0
p*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

FSU- Filtek Supreme Ultra composite resin

Source: Zanetti F et al., in preparation

Conclusions Part I

- CS causes discoloration of dental hard tissues and color mismatch of esthetic composite resin restorations.
- THS 2.2 induces much less discoloration in comparison with CS.
- The effects of CS and THS 2.2 aerosol on the gloss of composite resins was largely resin type-dependent. The exposure to CS and THS 2.2 aerosol did not affect the surface roughness in any of the three composite resins tested.
- These results are consistent with the available evidence that THS 2.2 generates an aerosol with a different chemical composition from CS and with none of the combustion-related solid particles typically found in CS.

Assessment of THS 2.2 Using Organotypic Oral Cultures (Buccal and Gingival)

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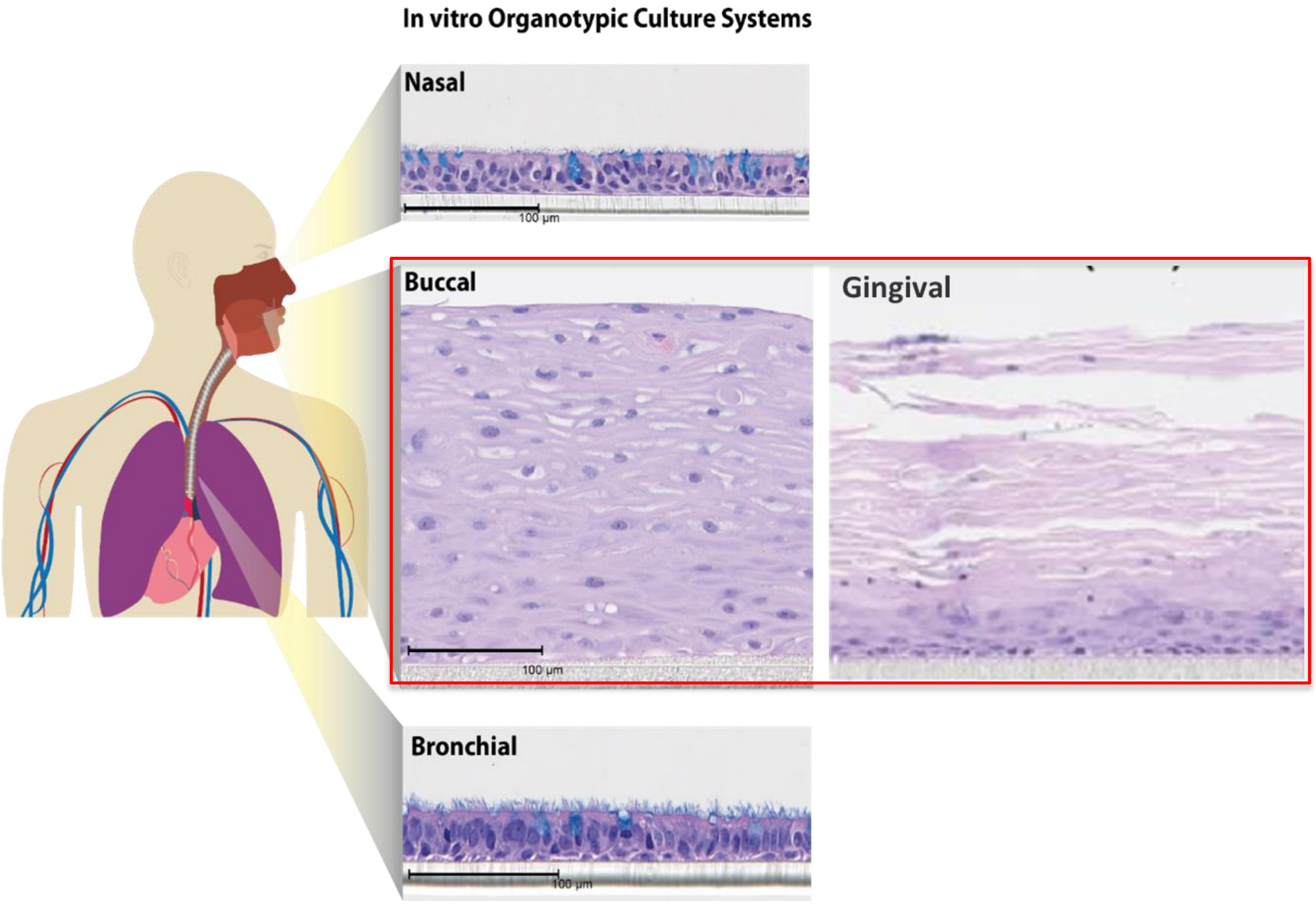
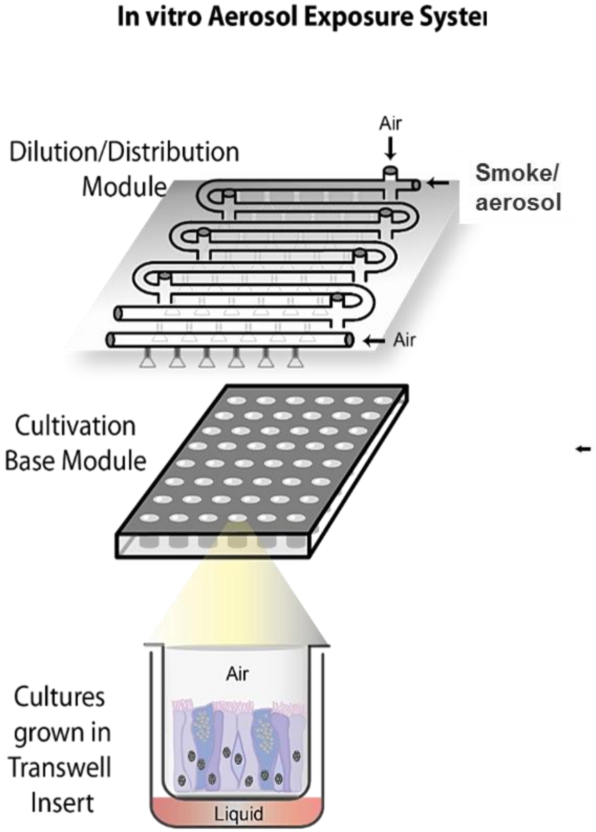
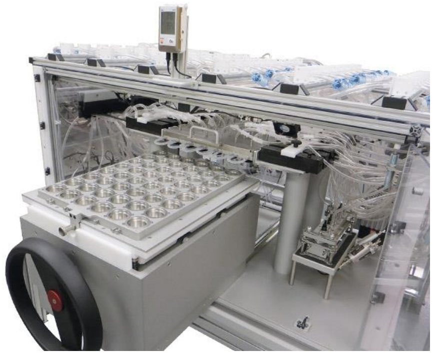
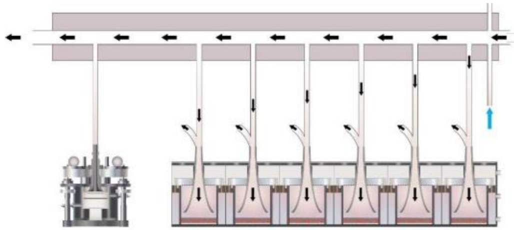
Human Organotypic Culture Models of the Aerodigestive Tract and Exposure System







www.Vitrocell.com



Zanetti F. et al., Food and Chemical Toxicology, 2017; Zanetti F. et al., Chemical Research in Toxicology, 2016

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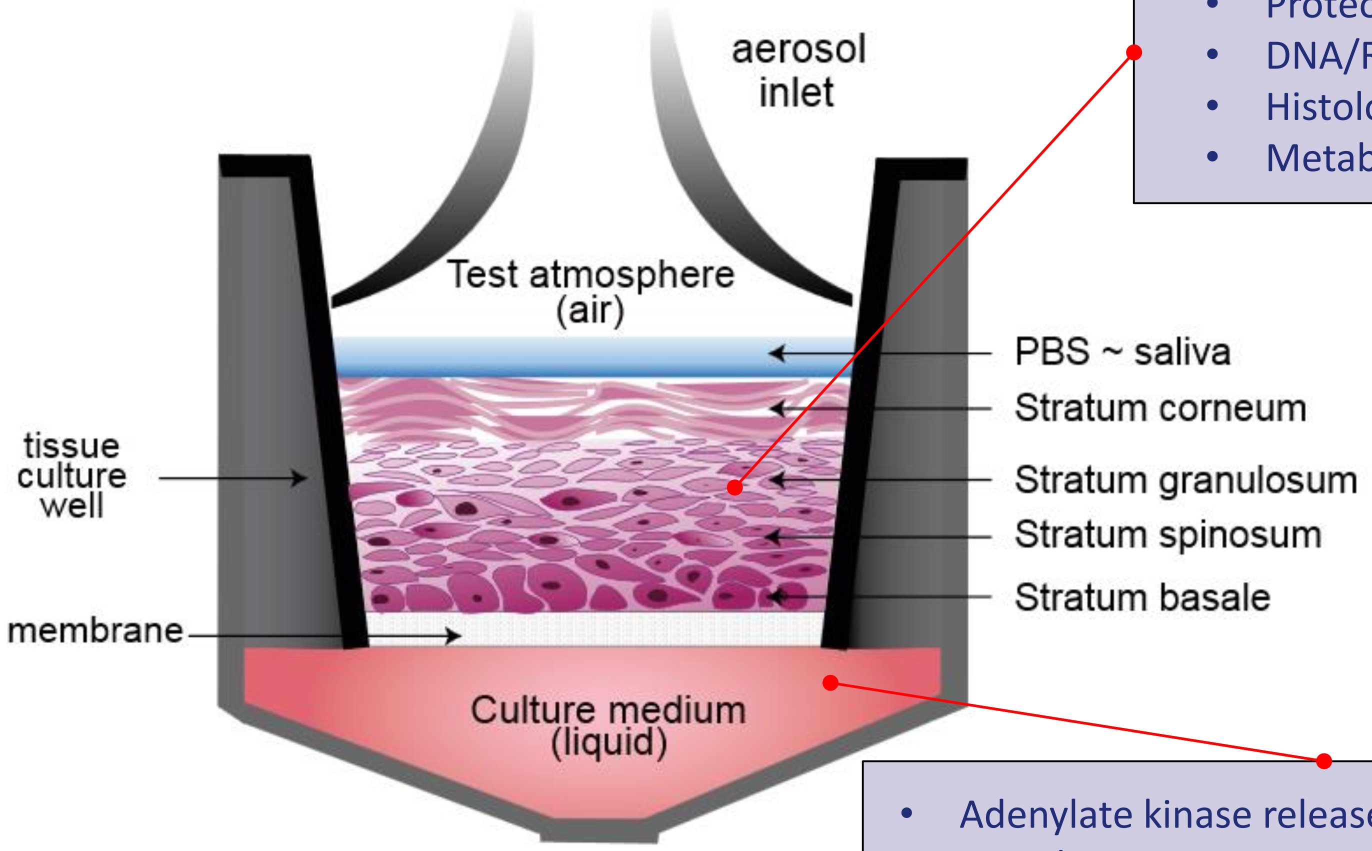
Systems Toxicology: A Comprehensive Toxicity Assessment



- 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

Zanetti F. et al., Food and Chemical Toxicology, 2017;
Zanetti F. et al., Chemical Research in Toxicology, 2016

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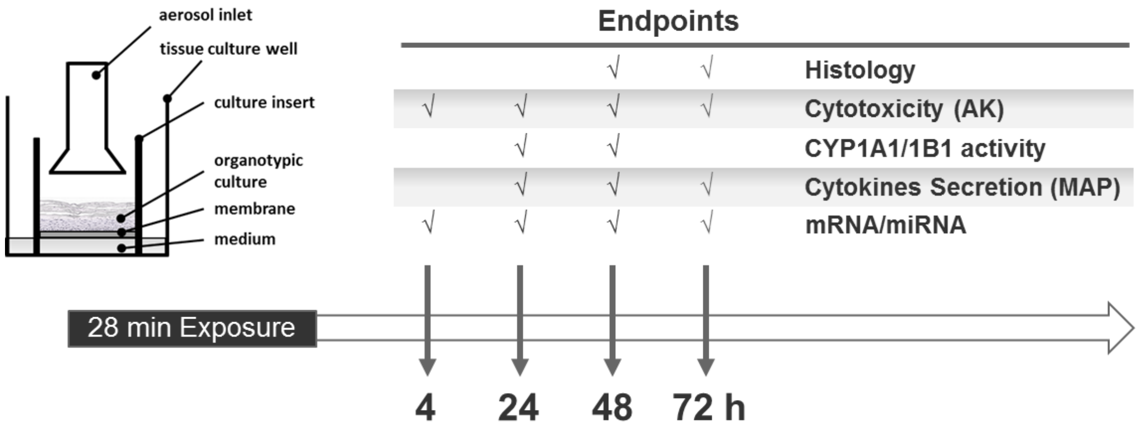
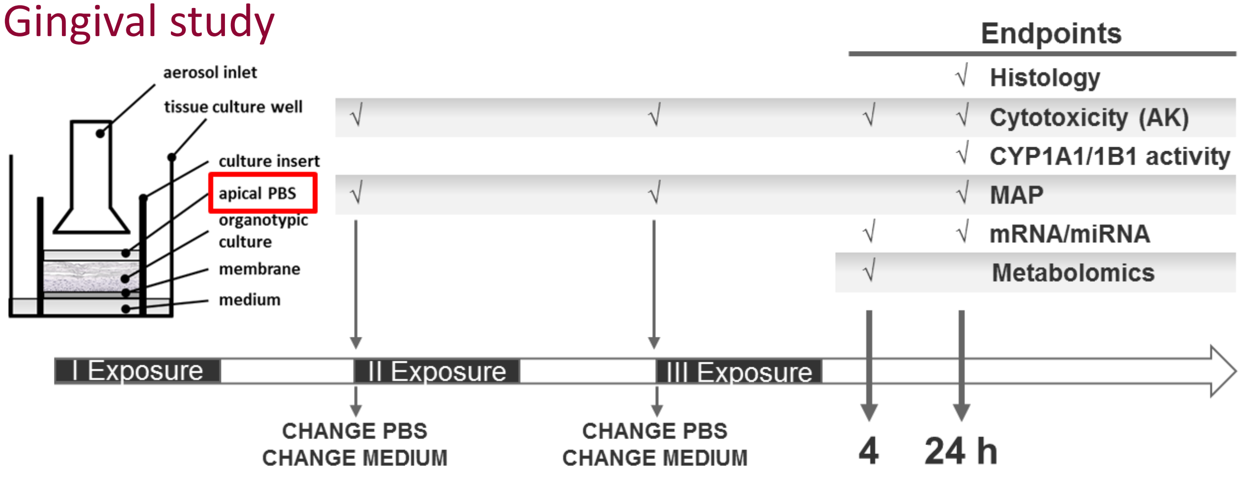
Study Design: To Assess the Reduced Impact of an RRP Compared with CS

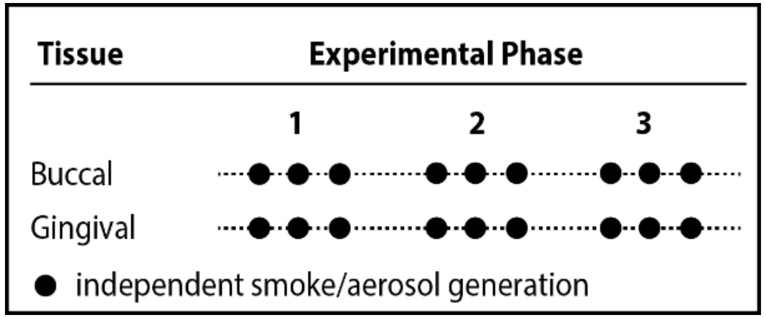
Buccal study



Gingival study



- Three experimental repetitions



- Exposure at comparable nicotine concentrations
- Use of 3R4F reference cigarettes

Zanetti F. et al., Food and Chemical Toxicology, 2017; Zanetti F. et al., Chemical Research in Toxicology, 2016

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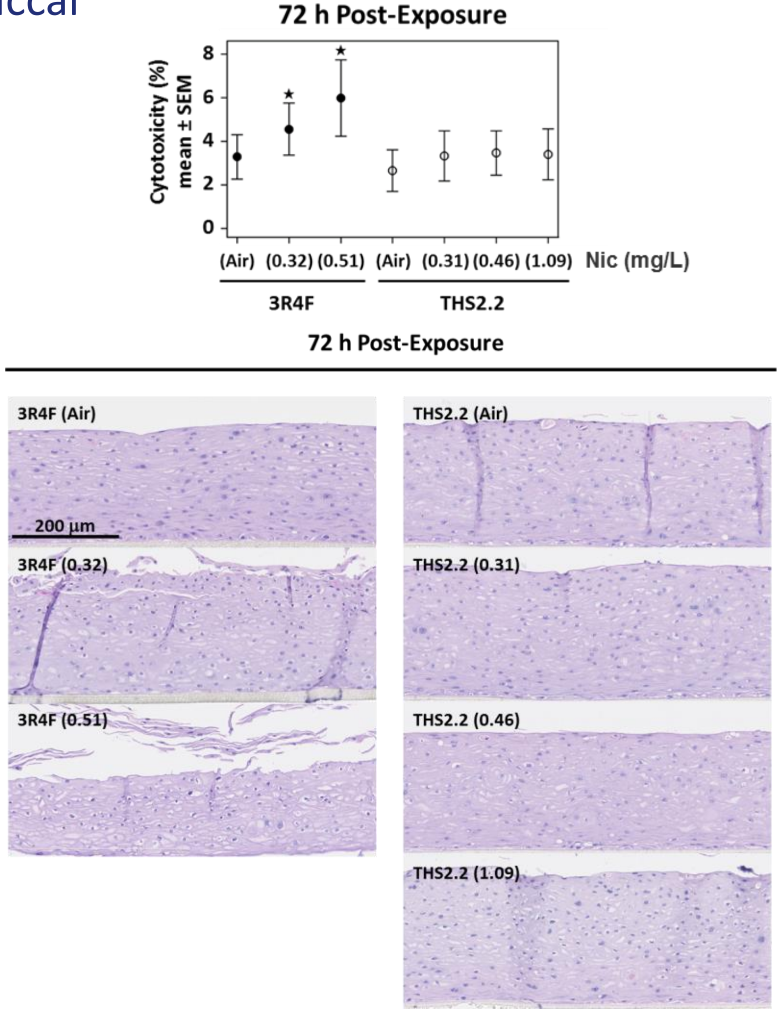
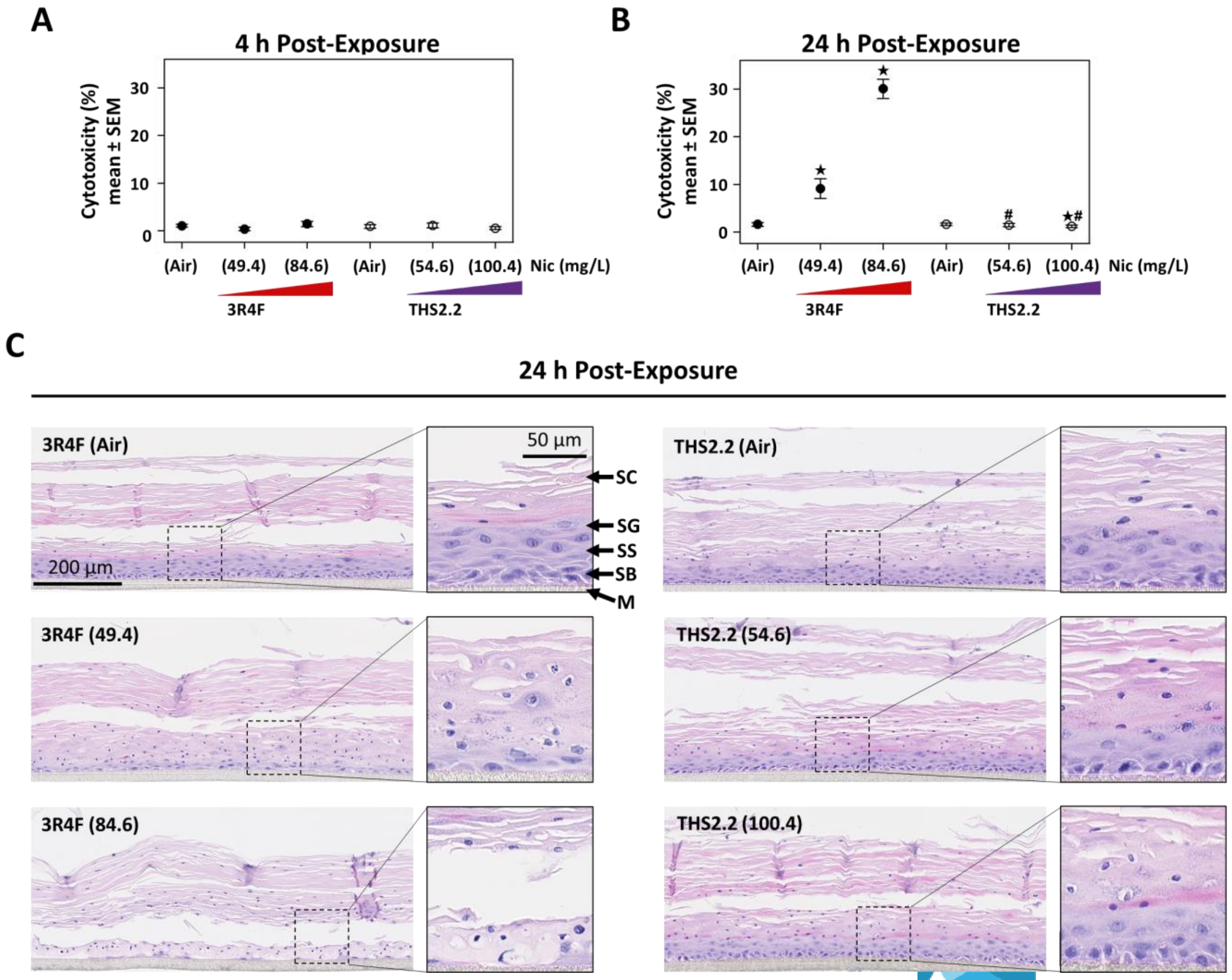
Tissue Damage Was Not Observed in Buccal and Gingival Cultures Following THS2.2 Aerosol Exposure

Buccal



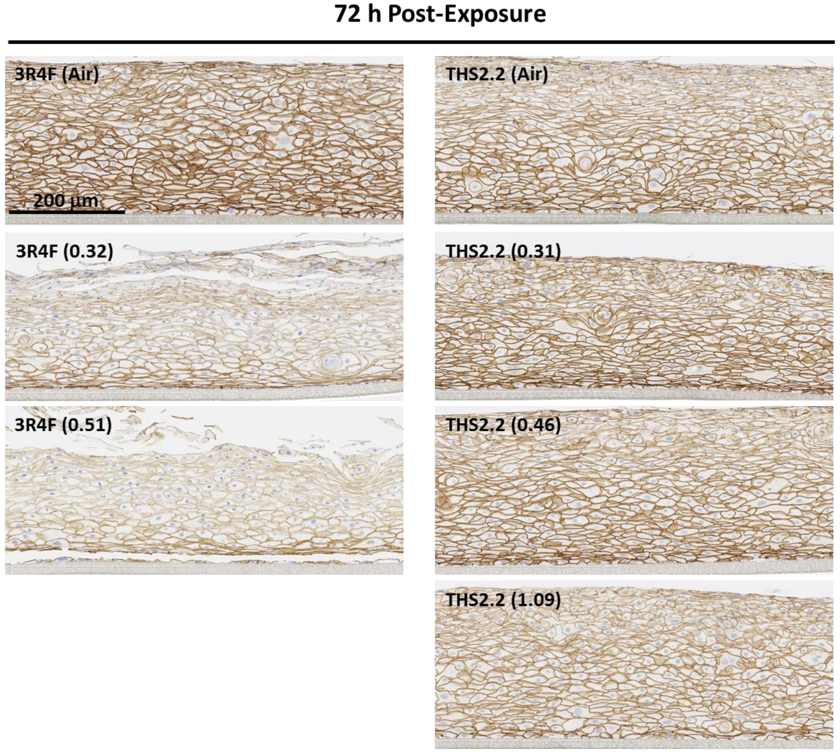
Gingival



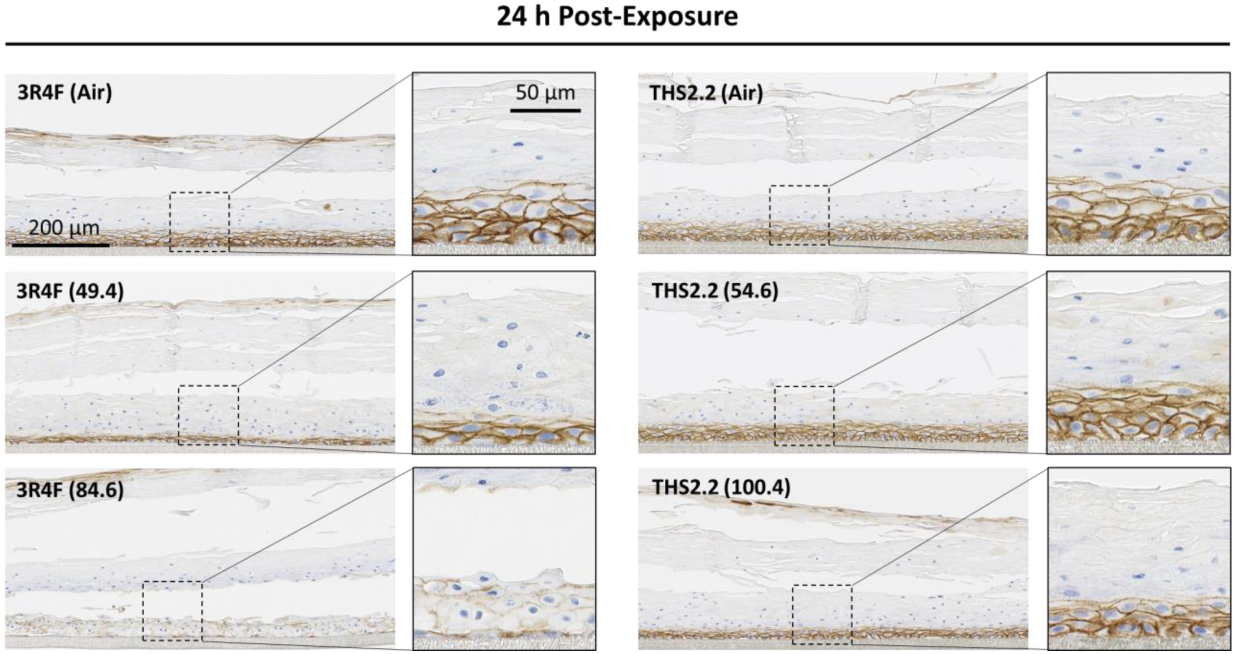
Zanetti F. et al., Food and Chemical Toxicology, 2017;
Zanetti F. et al., Chemical Research in Toxicology, 2016

Decreased Expression of E-cadherin Was Detected Following CS but Not THS2.2 Aerosol Exposure

Buccal



Gingival



A destabilization of E-cadherin expression by CS was demonstrated in oral mucosa cells

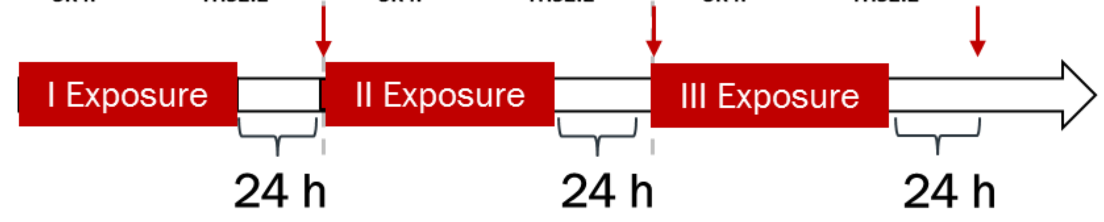
A significant reduction in E-cadherin levels was reported in periodontal disease compared with healthy conditions

Zanetti F. et al., Food and Chemical Toxicology, 2017;
Zanetti F. et al., Chemical Research in Toxicology, 2016

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

Assessment of THS 2.2 using human organotypic gingival cultures

I exposure				II exposure				III exposure				
3R4F (49.4)	3R4F (84.6)	THS2.2 (54.6)	THS2.2 (100.4)	3R4F (49.4)	3R4F (84.6)	THS2.2 (54.6)	THS2.2 (100.4)	3R4F (49.4)	3R4F (84.6)	THS2.2 (54.6)	THS2.2 (100.4)	Nic (mg/L)
0.9*	1	1	1.2	1.1	1.5*	1.1	1.3*	0.7*	0.5*	0.8*	0.9	VEGFA
0.8	0.7	0.8	0.8	0.9	0.6	0.7	0.9	5.9*	3.8*	1	1	TNFA
0.8	0.6*	0.6*	0.8*	0.3*	0.2*	0.5*	0.6*	0.5*	0.4*	0.5*	0.6*	CCL5
0.9	0.9	0.8	1	0.6*	0.5*	0.8	0.9*	0.5*	0.5*	0.8	0.8	MMP-9
1.5*	1.5*	1.2*	1.4*	1.9*	2*	1.3*	1.5*	7.5*	8.3*	1.8*	2.5*	MMP-1
0.4*	0.9	0.5*	0.6	1.1	1.4	0.7	0.5	1	1.4	0.8	1.2	CCL2
1.1	1.1	0.9	0.8	1	0.8	0.7	0.8	1.4	1.4	0.9	1.1	IP-10
0.9	1	0.8*	0.8	1.1	1.2*	0.9	1	4.1*	3.7*	1	1.2	IL8
0.5	0.5*	1	1	0.5*	0.3*	0.8	0.6	0.6	0.5*	0.7	0.8	IL6
1.5	1.2	1	1.5	1.2	1.3	0.8	0.7	0.9	1.2	1	0.8	IL1B
1.1	1.6*	0.7	0.7	2*	2.7*	1	1.2	11*	16*	1.1	1.3	IL1A
0.8*	0.8*	0.8*	0.8*	0.8*	0.8*	0.8*	0.8*	2*	2.2*	0.9	1	CXCL1
0.9	1	0.9	0.9	2*	3*	1.2	1.4	14*	11*	1.5	1.8*	CSF2
1.1	1.1	1	1.1	1.4	2*	0.7	1.3	4.4*	4.6*	1.4	1.5*	CSF3



- 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]

Zanetti F. et al., Food and Chemical Toxicology, 2017;

[1]Giannopoulou et al., 2003 [2]Romanelli et al., 1999 [3]Kim et al., 2006 [4]De Nardin et al., 200 [5]Bostrom et al., 1998; Bostrom et al., 1999; Ojima and Hanioka, 2010, [6]Tymkiw et al., 2011

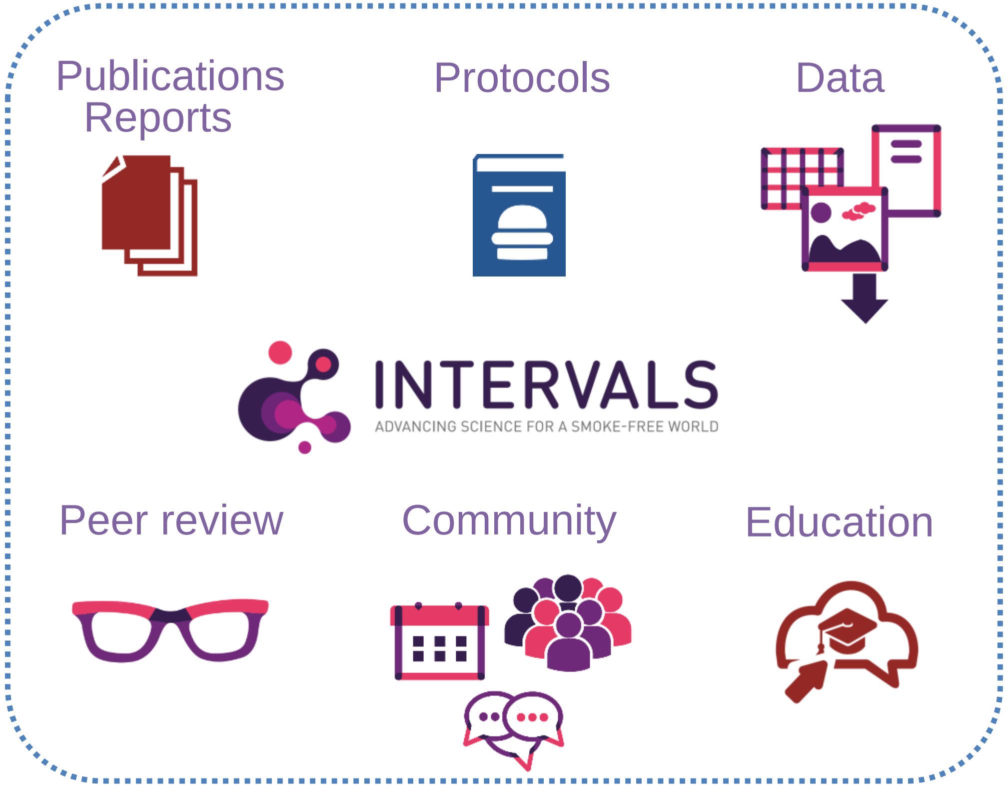
Conclusions Part II

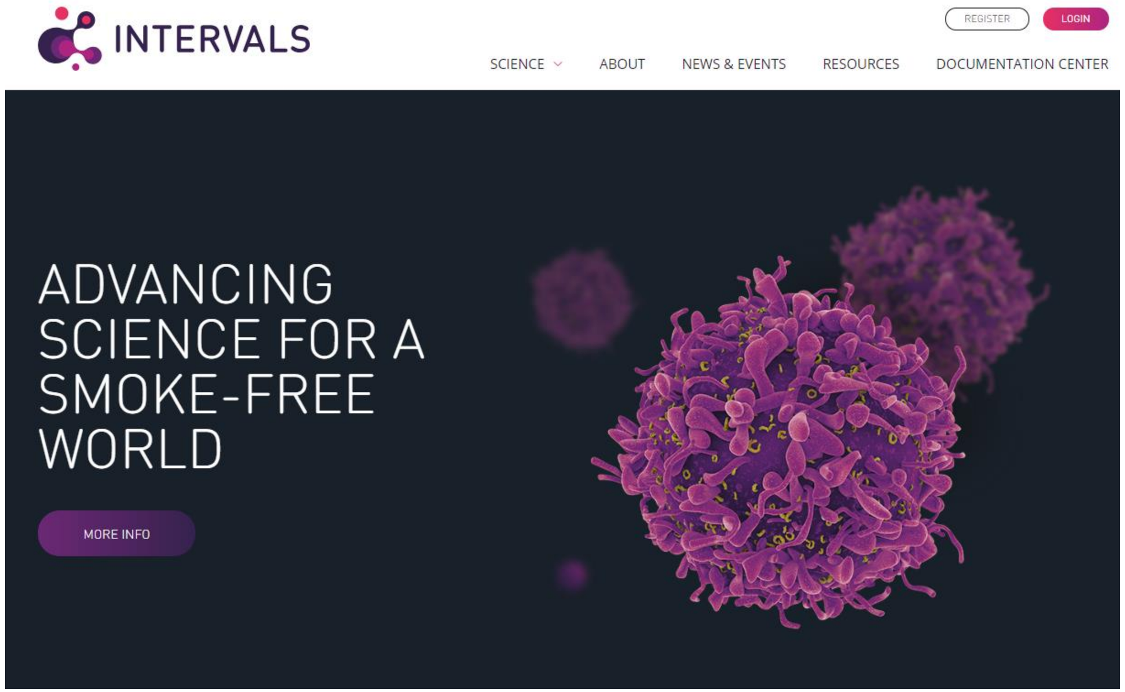
- Minor histopathological alterations and minimal cytotoxicity were observed upon THS 2.2 aerosol exposure compared with exposure to CS in both oral and gingival organotypic cell cultures.
- Five inflammatory markers exhibited mild alterations with THS 2.2 exposure compared with 11 upon CS exposure in the gingival cell cultures.
- Exposure to THS 2.2 aerosol had a lower impact on the pathophysiology of human gingival and buccal organotypic cultures than CS.

Zanetti F. et al., Food Chem Toxicol. 2017, Zanetti F. et al., Chem Res Toxicol. 2016

INTERVALS – Live Demo

Aim: establish a **community** and a public **repository** for 21st-century preclinical and clinical (systems) **inhalation toxicology assessment** data and results that supports open data principles.





www.intervals.science

Boué S, Exner T, Ghosh S et al. [Supporting evidence-based analysis for modified risk tobacco products through a toxicology data-sharing infrastructure] [version 2; referees: 2 approved] F1000Research 2017, 6:12 (doi: 10.12688/f1000research.10493.2)

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Thank you

Backup

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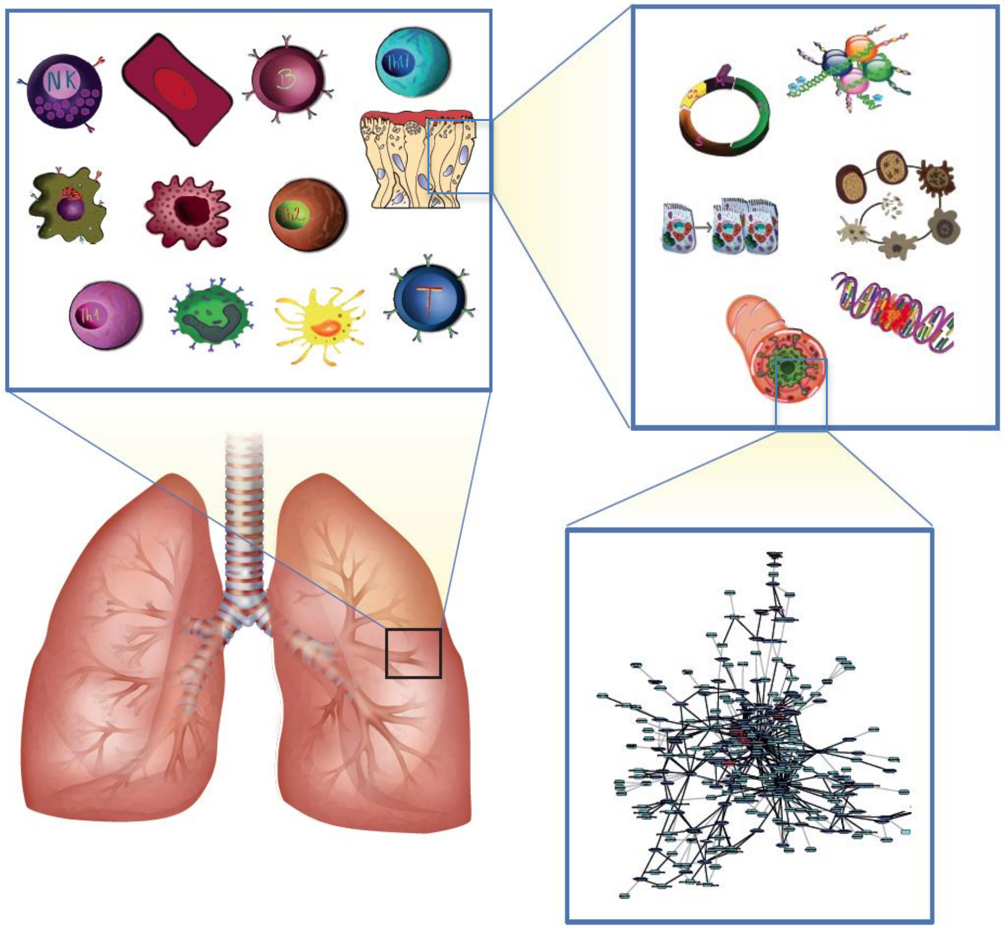
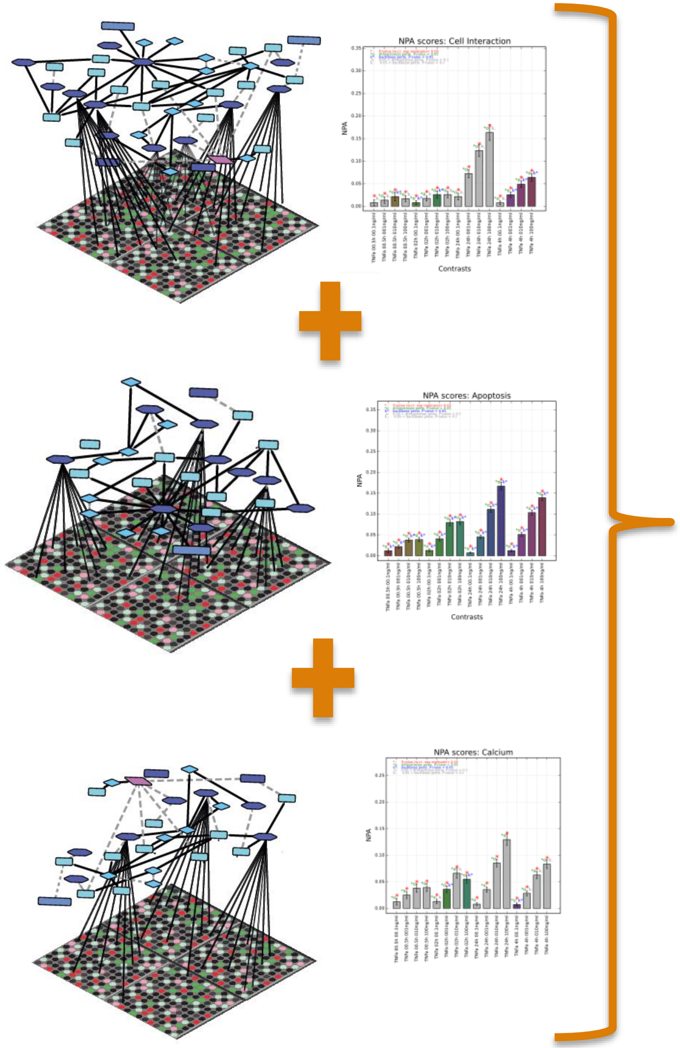
- The gloss of the resins was assessed with the Novo-Curve glossmeter (Rhopoint Instrumentation, East Sussex, UK) and recorded as gloss units. Surface roughness was assessed with 3D scanning microscopy (Infinite Focus G4, Alicona Imaging GmbH, Graz, Austria).
- The effects of 3R4F CS and THS 2.2 aerosol on the gloss of composite resins was largely **resin type-dependent**. Indeed, neither 3R4F nor THS 2.2 caused clear variations of **DVS** (Durafill VS) resin surface gloss. Conversely, in **TEC** (Tetric EvoCeram BulkFill) and **FSU** (Filtek Supreme Ultra) resin types, THS 2.2 exposure caused a decrease in gloss, whereas 3R4F caused an increase. These trends were maintained throughout the three weeks entailed by the exposure protocol.
- At baseline, the surface roughness was higher in TEC resins compared with FSU and DVS. The exposure to 3R4F CS and THS 2.2 aerosol did not affect the surface roughness in any of the three composite resins tested. Moreover, no differences between 3R4F- and THS 2.2-exposed resin discs were detected in terms of surface roughness after three weeks of exposure.

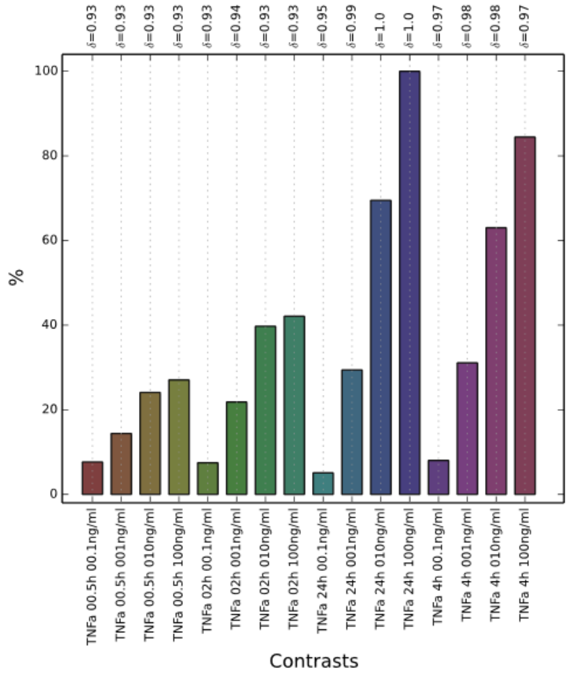
Systems Toxicology Approach for Pre-Clinical RRP Assessment



CBN CAUSAL BIOLOGICAL NETWORKS DATABASE



Network perturbation amplitude



Biological impact factor

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Hoeng et al.: A network-based approach to quantifying the impact of biologically active substances. Drug discovery today 2012, 17 (9), 413-418.
Hoeng et al.: Toxicopanomics: applications of genomics, transcriptomics, proteomics and lipidomics in predictive mechanistic toxicology. FL: 2014.