PMI RESEARCH & DEVELOPMENT

# Non-Clinical Toxicology of Nicotine and Aerosol from a Heated Tobacco Product

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PMI SCIENCE

# *PMI's Goal for Harm Reduction*

## Offering adult smokers satisfying products that reduce risk

- 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 (RRPs) so that consumer acceptance can be best fulfilled
- Our **Heated Tobacco Product (HTP)** is a RRP.

*The Tobacco Atlas 3rd Edition. American Cancer Society, 2009
Figure adapted from Clive Bates presentation to E-Cigarette Summit (19 Nov 2013)

Reduced-Risk Products (“RRPs”) is the term the company uses to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. PMI’s RRPs are **in various stages of development and commercialization**, and we are conducting **extensive and rigorous scientific studies** to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when **compared to smoking cigarettes.**

Before making any such claims, we will **rigorously evaluate the full set of data** from the relevant scientific studies to determine whether they substantiate reduced exposure or risk. Any such claims **may also be subject to government review and authorization** as is the case in the US today.

# Outline & Scope

Short description of design and methods used in a series of *in vivo* inhalation studies assessing the effects of nicotine aerosol, conventional cigarette smoke and aerosols from potentially Risk-Reduced Product (pRRP) [or candidate Modified Risk Tobacco Product] – Heated Tobacco Product

Description of effects of nicotine (in combination with pyruvic acid) using pre-clinical and systems toxicology endpoints

Toxicity of cigarette smoke exposure and the effect of a HTP – interpretation of findings using nicotine-exposure knowledge

Application of a mouse disease model for cardiovascular and respiratory disease – effects of cigarette smoke exposure, switching to HTP aerosol exposure and cessation

HTP: Heated Tobacco Product

# Methods – *in vivo* studies

Regulatory rat inhalation studies (OECD protocols; 28-day, 90-day*, OECD GLP)

- Aerosol generation: using appropriate standards
  - Smoking machines: conventional cigarettes, reduced risk products
  - Collison nebulizers: nicotine/salt solution, nicotine containing liquids

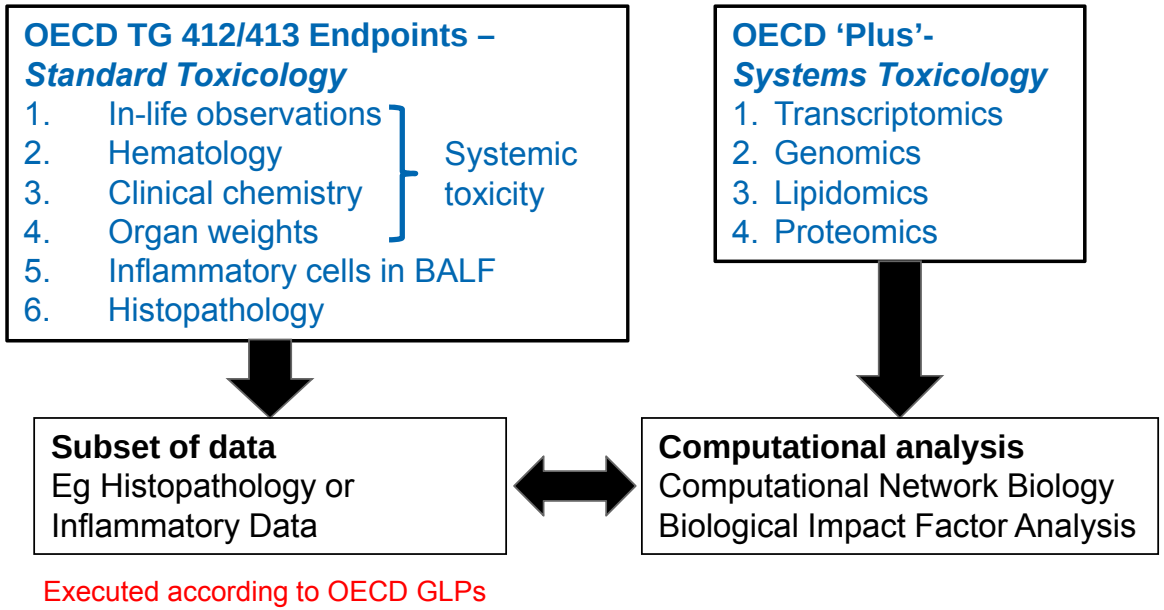
- Aerosol characterization: *demonstrate that aerosol is representative and follows guidelines*
  - Determination of aerosol markers (e.g. TPM, CO, carbonyls), breathing zone
  - Particle size distribution

- Bio-monitoring: *demonstrate that exposure is representative*
  - Respiratory physiology
  - Urinary particulate and gas phase biomarkers of exposure (e.g. nicotine metabolites, mercapturic acid metabolites from acrolein, benzene, acrylonitrile, NNK) blood carboxyhemoglobin)

***OECD Guidelines for the Testing of Chemicals, Section 4.**
Test No. 413: Subchronic Inhalation Toxicity: 90-day Study, OECD 2009
Test No. 412: Subacute Inhalation Toxicity: 28-Day Study, OECD 2009

# Methods - design

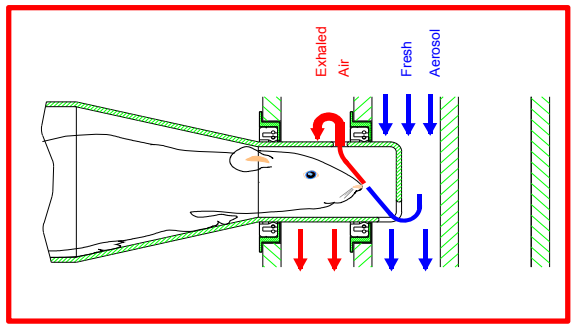
- Endpoints:




- Test atmospheres presented in low/medium/high concentration: up to MTD: 50 µg Nicotine/l* for nicotine-containing aerosols – 23 µg Nicotine/l for cigarette smoke; concentration-response

*50 µg/l nic corresponds to 13.6 mg/kg, daily dose; way above acute toxicity levels- or the nicotine amount from approx. 130 cig/day for a 60 kg human

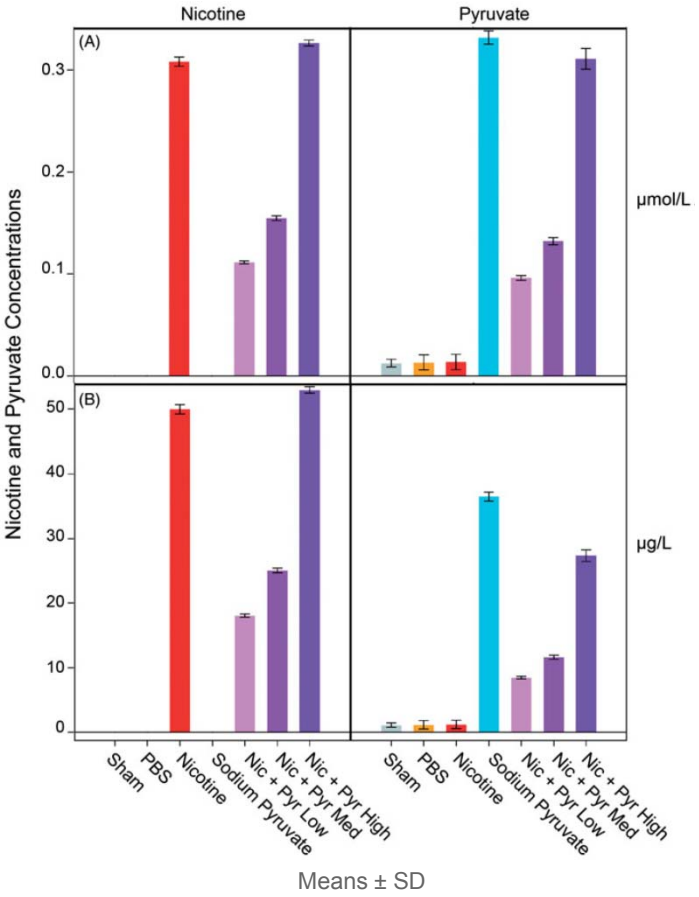
# Methods
## Exposure – nebulized nicotine –nicotine-salt solutions




# Effects of nicotine – 28-day inhalation study (OECD TG 412)
# Exposure and Bio-monitoring

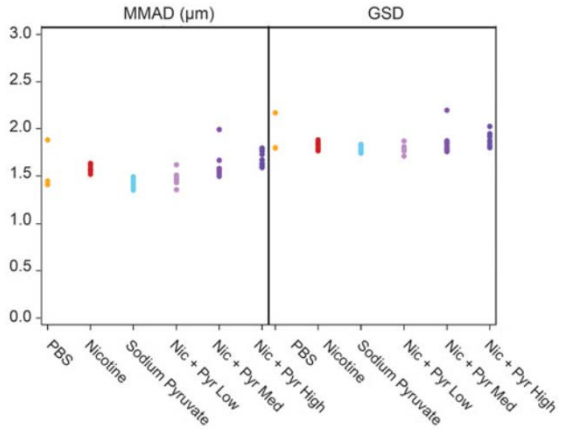
Urinary nicotine metabolites demonstrate appropriate exposure to ‘respirable’ aerosol

Test atmosphere concentrations

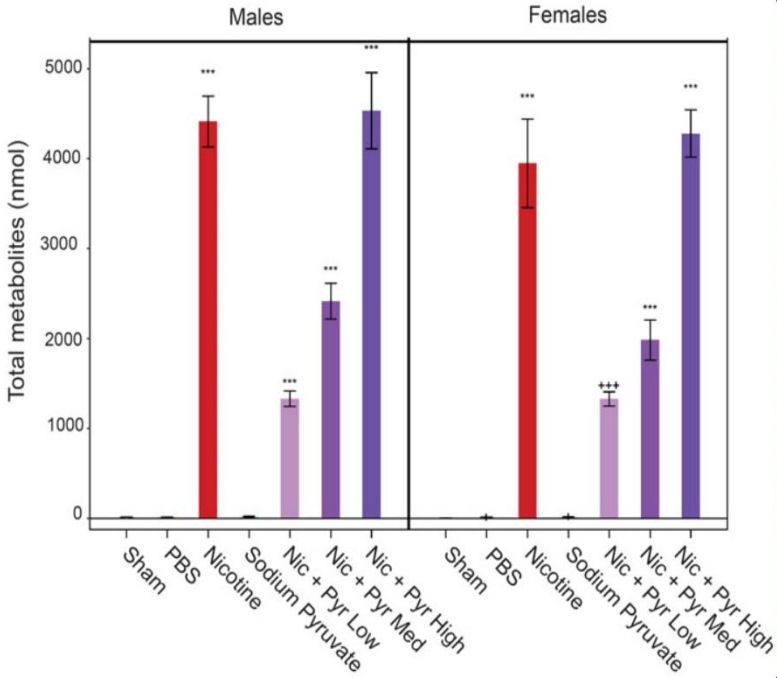



Means ± SD

Particle size determination




Urinary nicotine metabolites

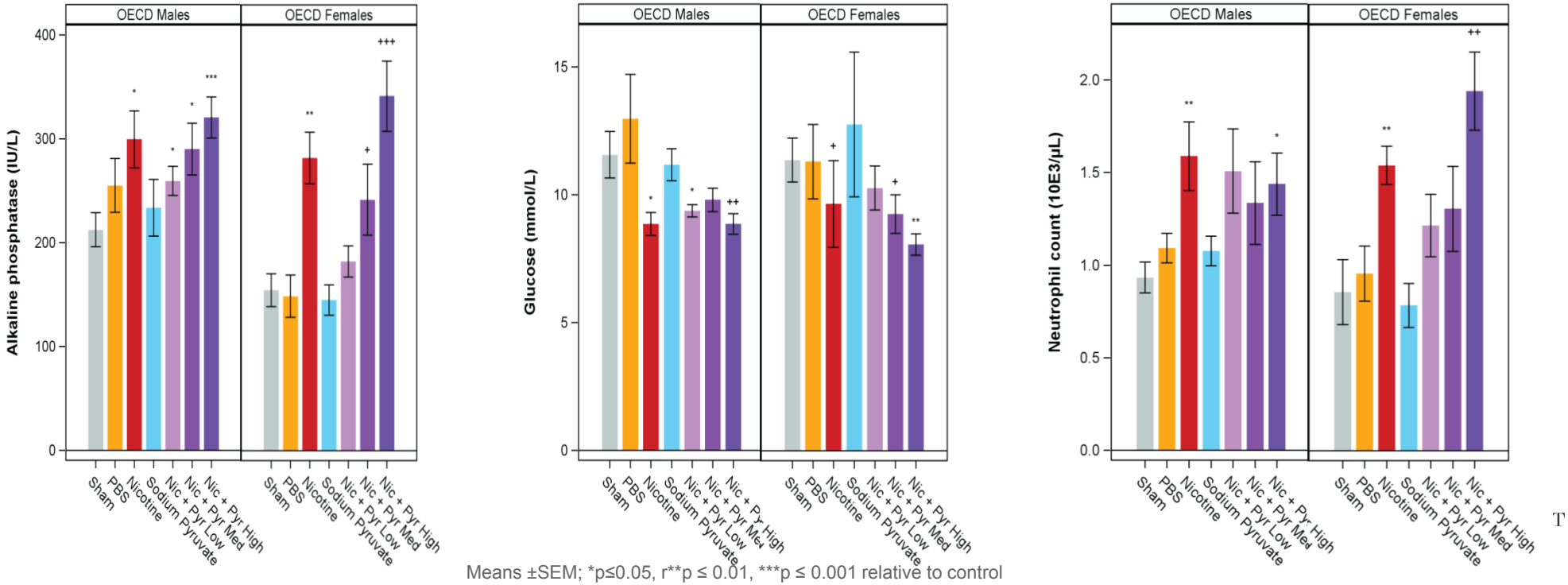



Means ± SEM; ***p ≤ 0.001

# Effects of nicotine – 28-day inhalation study (OECD TG 412)
# Systemic effects

Nicotine concentration-dependent changes in:

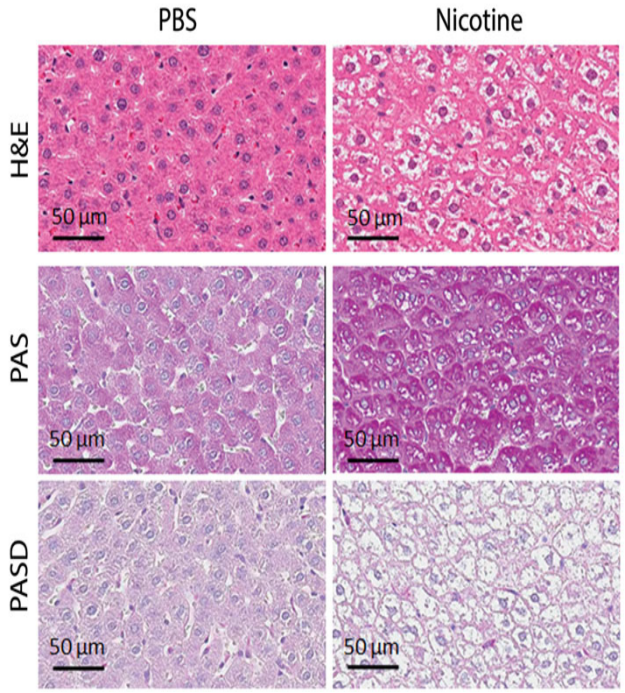
- Body weight development (approx. 5% body weight gain reduction in ‘high’ concentration group)
- Organ weights (relative to body weight): Liver, higher than controls; Spleen, lower
- Clinical chemistry: Alkaline phosphatase and alanine aminotransferase: higher than controls; cholesterol and glucose: lower than controls – all in normal ranges
- Hematology: neutrophils, higher than controls; lymphocytes, lower – all in normal ranges

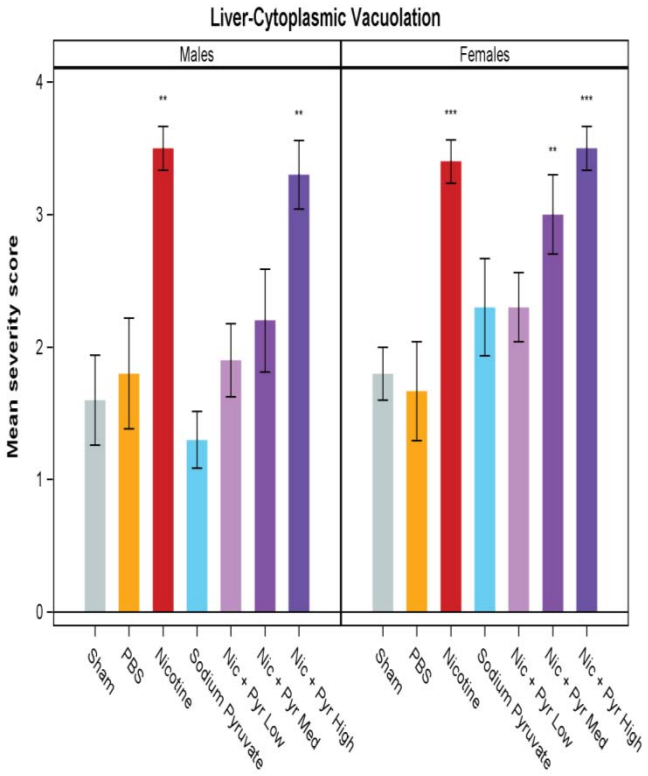


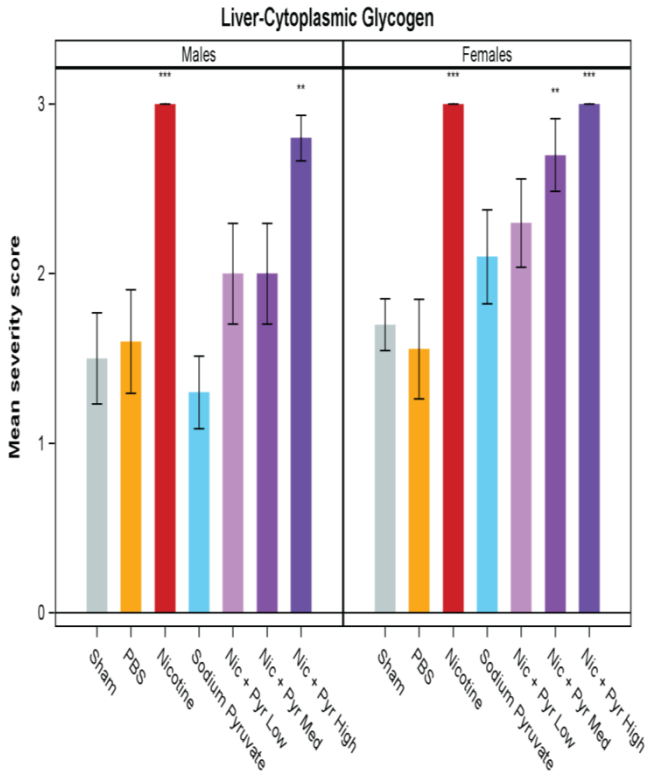

Means ±SEM; *p≤0.05, r**p ≤ 0.01, ***p ≤ 0.001 relative to control

# Effects of nicotine – 28-day inhalation study (OECD TG 412)
# Histopathology of non-respiratory tract organs

Liver effects: nicotine concentration-dependent cytoplasmic vacuolation – vacuoles contain PAS-positive material, likely to consist of glycogen granules




Means ±SEM; *p≤0.05, r**p ≤ 0.01, ***p ≤ 0.001 relative to control

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# Effects of nicotine – 28-day inhalation study (OECD TG 412)
# Summary & Conclusion

- Exposure to nicotine aerosols, alone or in combination with pyruvic acid, results in mild changes in toxicity in a 28-day inhalation toxicology study with respect to:
  - Systemic effects: increased liver weight, blood neutrophil counts, activity of liver enzymes and decreased blood levels of cholesterol and glucose
  - Histopathological effects: increased vacuolation of and glycogen content in hepatocytes

  Changes in liver gene expression and lipidomics provide mechanistic explanation for the changes observed, i.e. alterations in xenobioitic and lipid metabolism

***Toxicity of aerosols of nicotine and pyruvic acid (separate and combined) in Sprague–Dawley rats in a 28-day OECD 412 inhalation study and assessment of systems toxicology.***

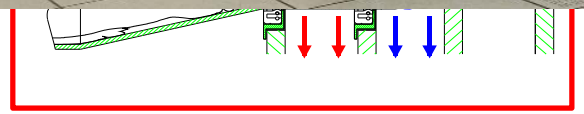
Blaine Phillips, Marco Esposito, Jan Verbeeck, Stephanie Boue, Anita Iskandar, Gregory Vuillaume, Patrice Leroy, Subash Krishnan, Ulrike Kogel, Aneli Utan, Walter K. Schlage, Monali Bera, Emilija Veljkovic, Julia Hoeng, Manuel C. Peitsch, and Patrick Vanscheeuwijck.

Inhalation toxicology 2015 Aug;27(9):405-31, PMID 26295358

# Methods – Conventional cigarette smoke and aerosol from a HTP 90-day inhalation study (OECD TG 413)

Approximately 1.2m

Exp



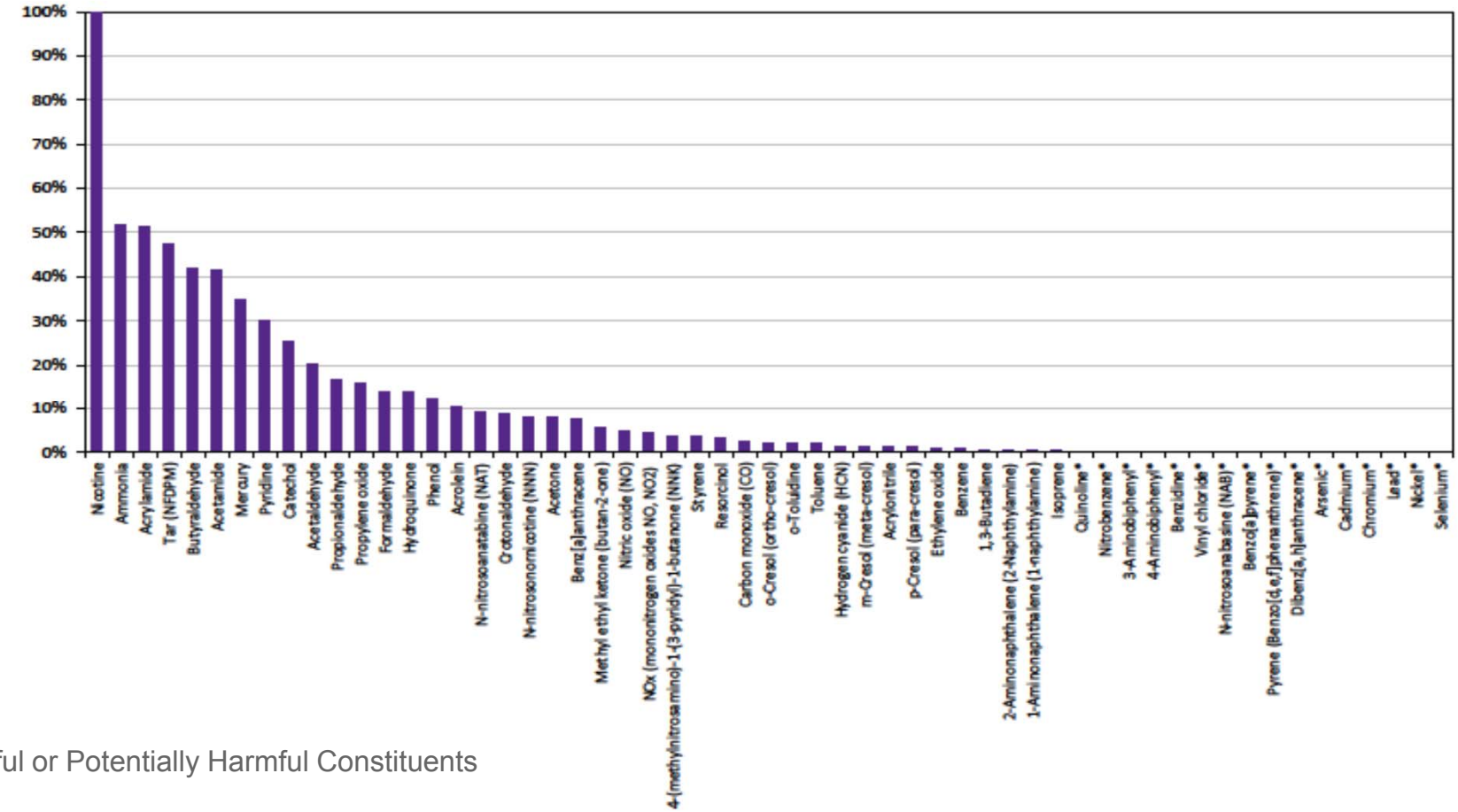
Assessment of smoke/aerosol – Health Canada Intense smoke protocol

Conventional cigarettes: **Smoke** from University of Kentucky Standard Reference Cigarette 3R4F

Potentially Reduced-Risk product: **Aerosol** from Heatsticks and Tobacco Heating System, **HTP**, commercialized as iQOS (also designated as P1 or THS 2.2)

# Smoke chemistry – determination of 58 constituents in conventional cigarette smoke and HTP (THS2.2) aerosol

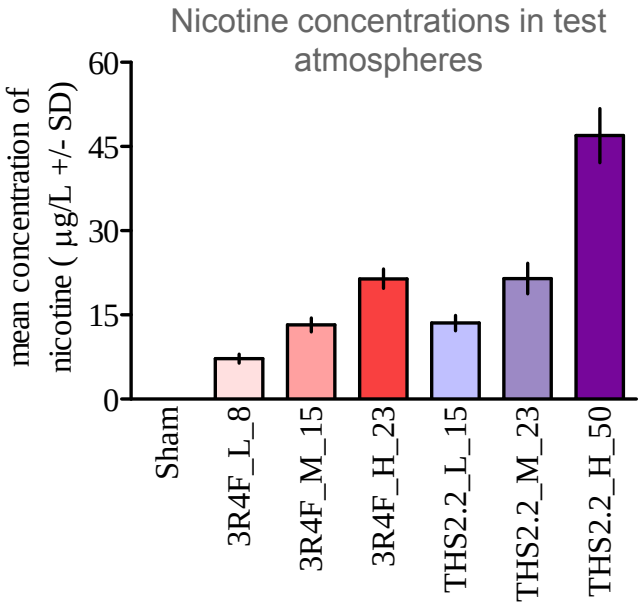
- Smoke chemistry data shows a strong reduction of HPHC* in THS2.2 relative to 3R4F (data per mg nicotine, 100 % is data from 3R4F)

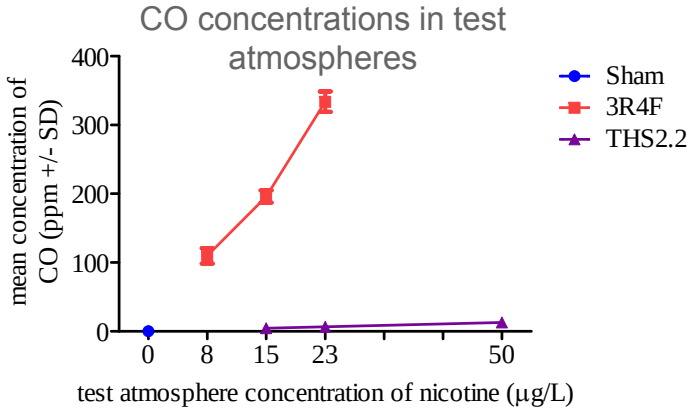


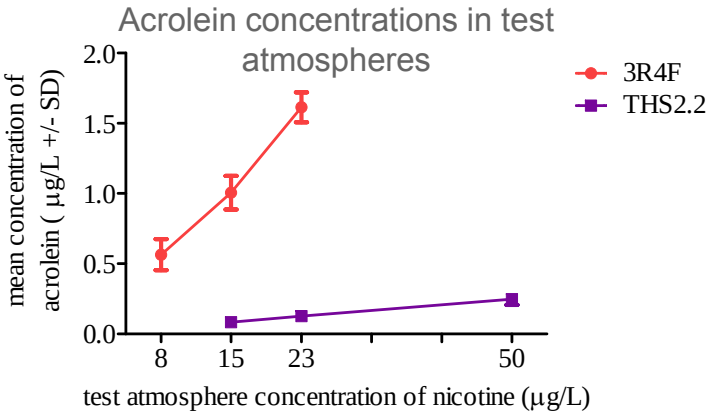

*HPHC: Harmful or Potentially Harmful Constituents

# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413) Exposure

- Test atmosphere characterization – gas phase and particulate matter markers indicate that:
  - Target nicotine concentrations met
  - HPHCs in test atmospheres reflect aerosol chemistry data

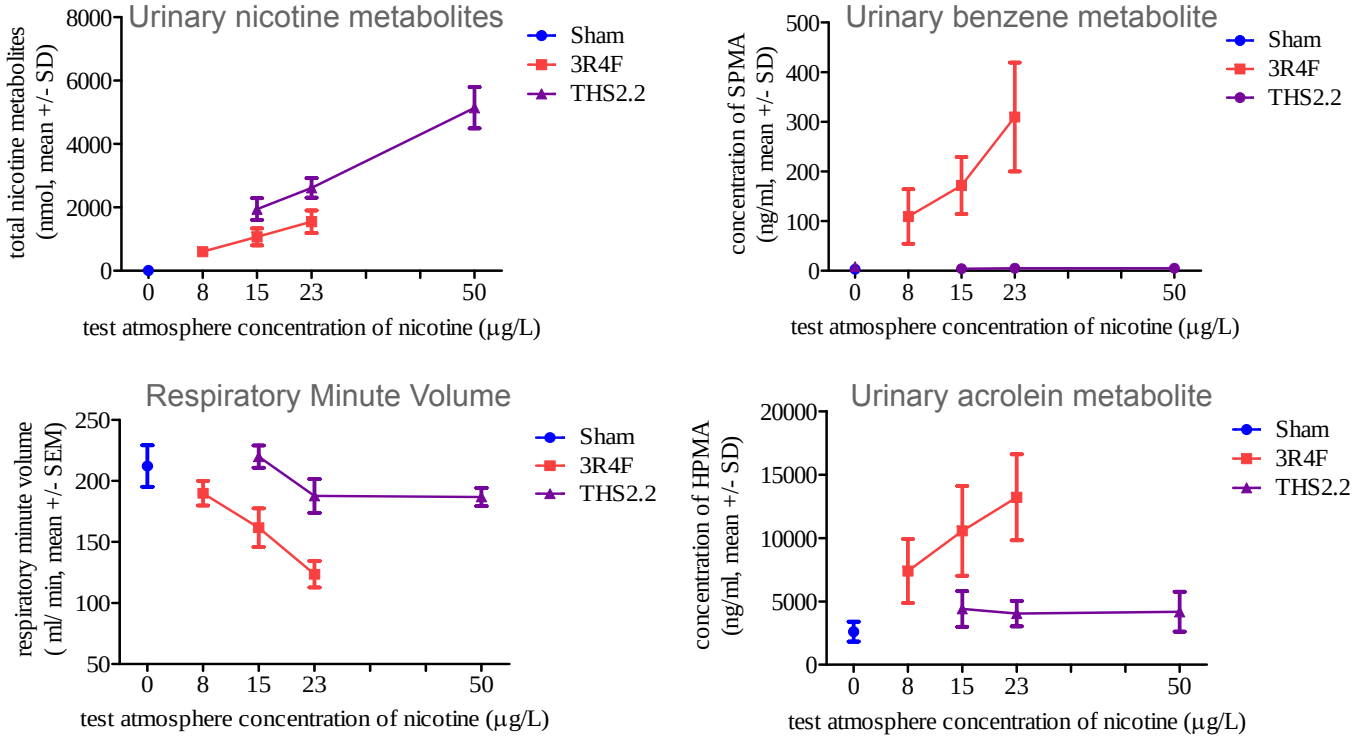


# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413) - Biomarkers of exposure

- Bio-monitoring parameters indicate that:
  - Urinary biomarkers of nicotine correlate with nicotine levels in test atmosphere
  - Urinary biomarkers of HPHC correlate with chemical composition of aerosols
  - The HTP causes much less upper respiratory tract irritation resulting in higher uptake of aerosol



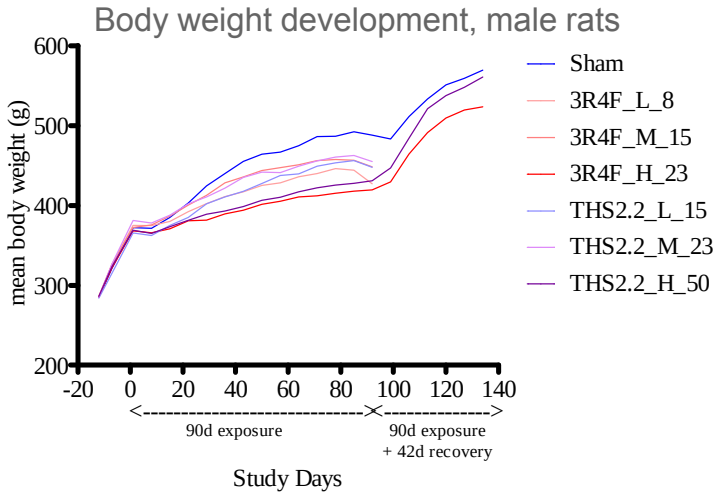

Data for male rats only, after 4 weeks of exposure. The data for female rats and different collection time points are very similar.

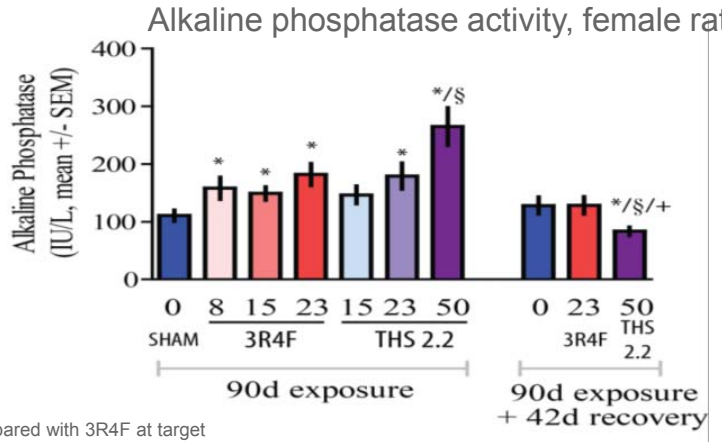
# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413) - Systemic toxicity

- No remarkable toxic effects in THS 2.2
- Body weight effect in males only

- Nicotine concentration-related increases in
  - Neutrophil count in blood
  - Relative weight of liver
  - Liver enzyme activity (normal range)
- Mild liver effects

→ Effects similar to those of nicotine exposure

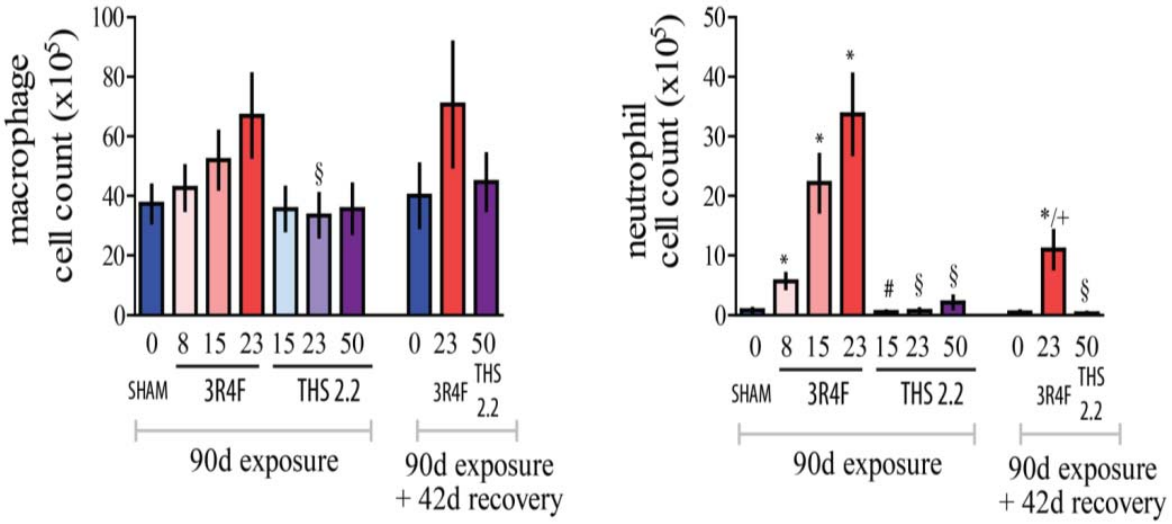



* p < 0.05 as compared with SHAM at a given time point, # p < 0.05 as compared with 3R4F at target nicotine 15 µg/l at a given time point, § p < 0.05 as compared with 3R4F at target nicotine 23 µg/l at a given time point, and + p < 0.05 as compared with the same group at the 90-day exposure time point

# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413) - Lung inflammation

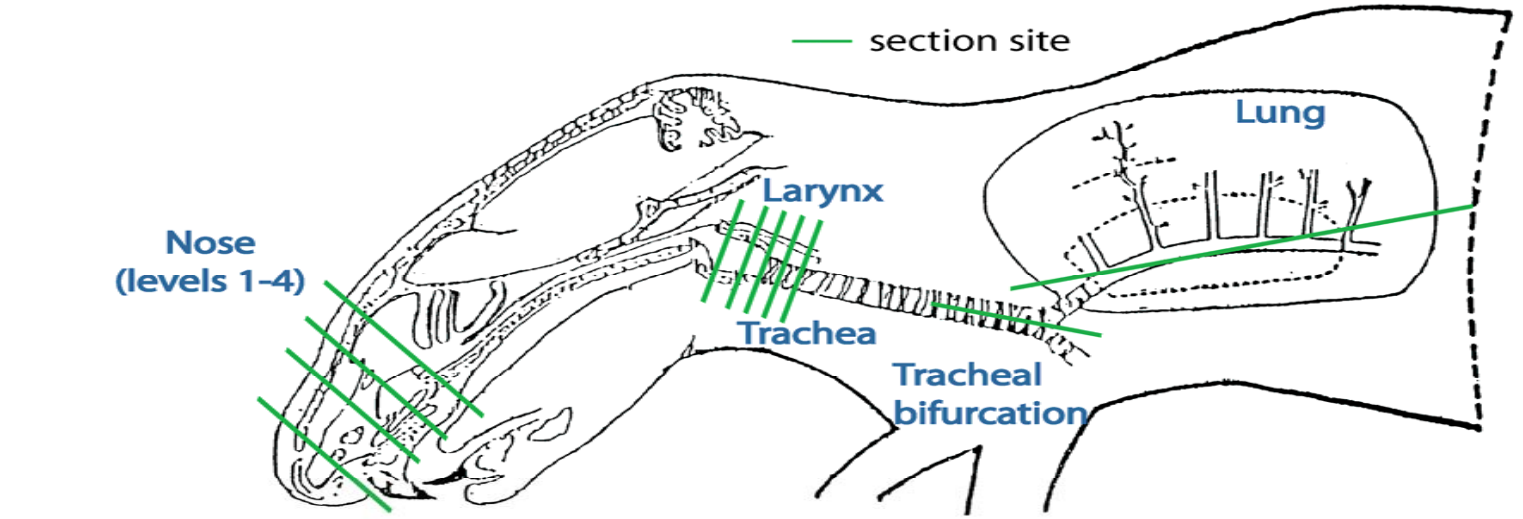
- Very low numbers of inflammatory cells are recruited in the lungs (BALF) of THS2.2 aerosol-exposed rats




Remarks:
•Data for male rats; the data for female rats are very similar.
•* $p < 0.05$ as compared with SHAM at a given time point, # $p < 0.05$ as compared with 3R4F at target nicotine 15 μg/l at a given time point, § $p < 0.05$ as compared with 3R4F at target nicotine 23 μg/l at a given time point, and + $p < 0.05$ as compared with the same group at the 90-day exposure time point

Means ± SEM

# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413) Histopathology of respiratory tract organs



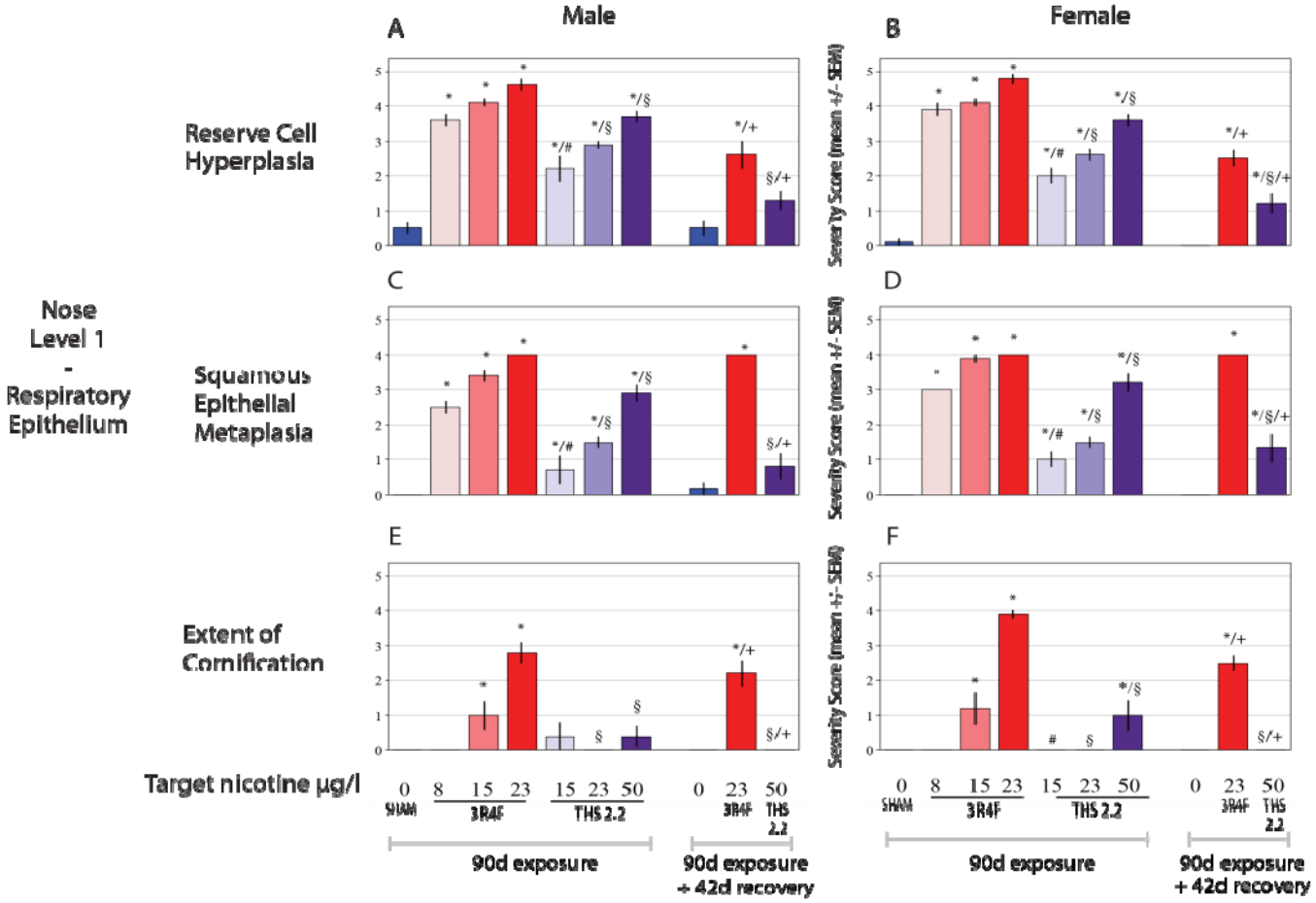

Strong reduction of nasal effects in P1-exposed rats, from moderate at nose level 1 to absent at nose level 4

Strong reduction of laryngeal effects in P1-exposed rats

Strong reduction of tracheal effects in P1-exposed rats

Very low pulmonary effects in P1-exposed rats

# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413)
# Histopathology of respiratory tract organs - Nose

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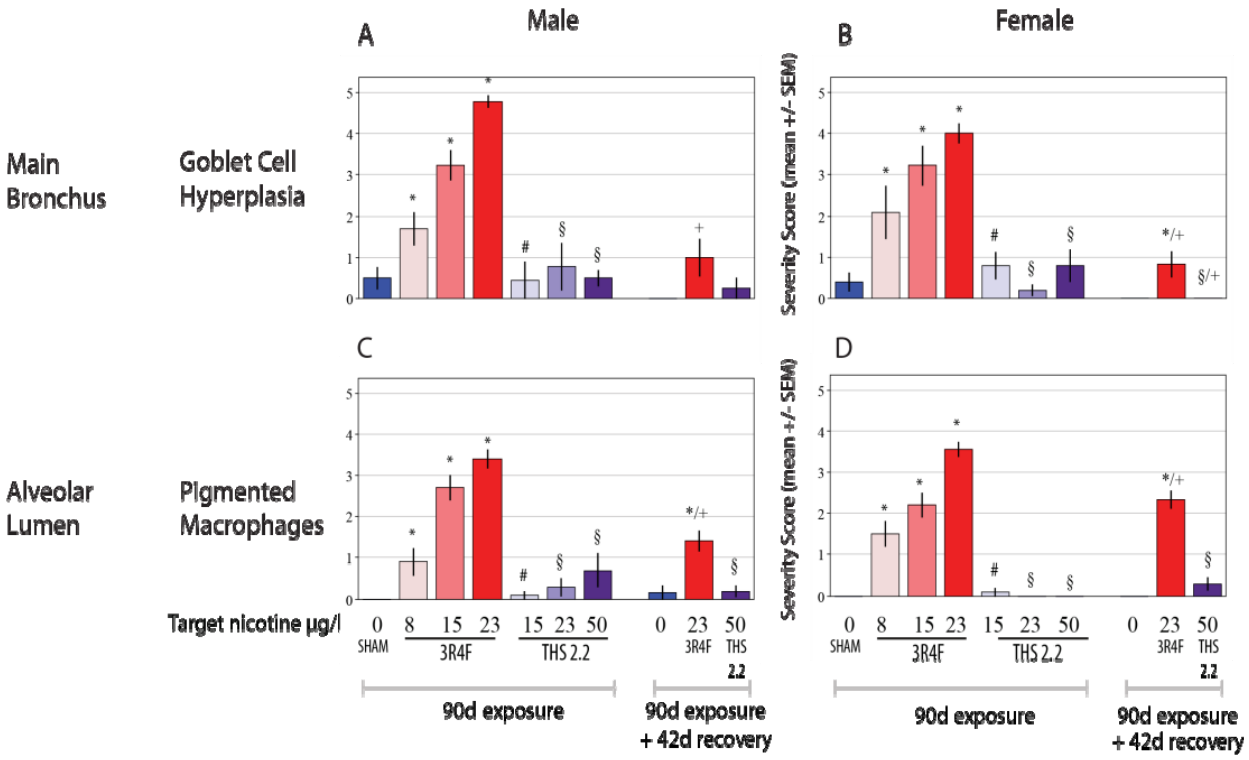
# Cigarette smoke and HTP aerosol– 90-day inhalation study (OECD TG 413)
# Histopathology of respiratory tract organs – Bronchi and lungs

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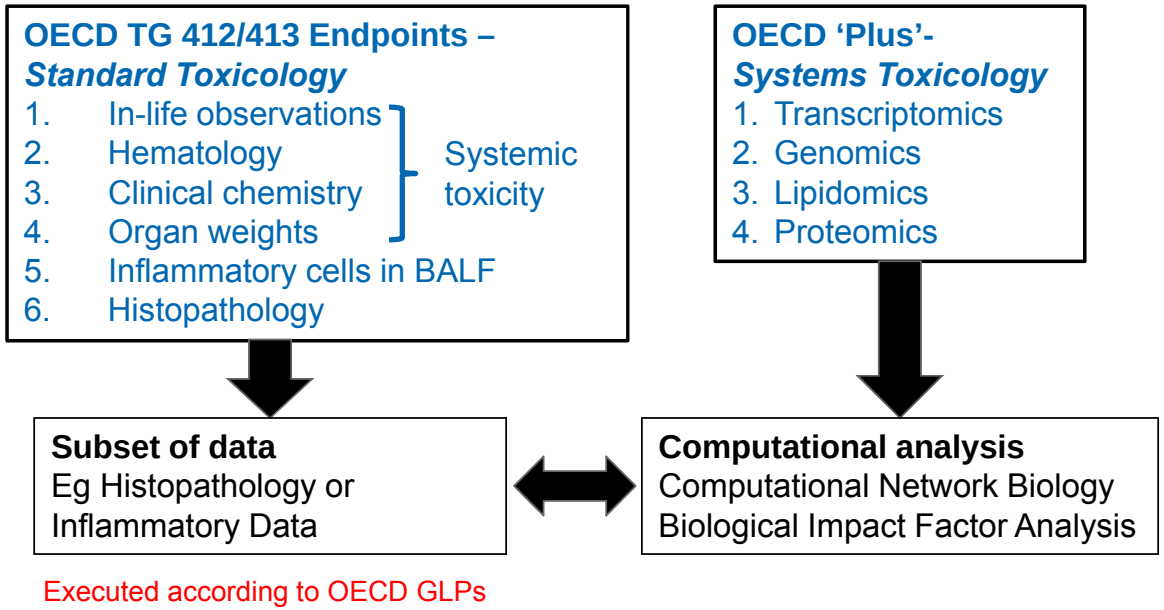
# Methods - design

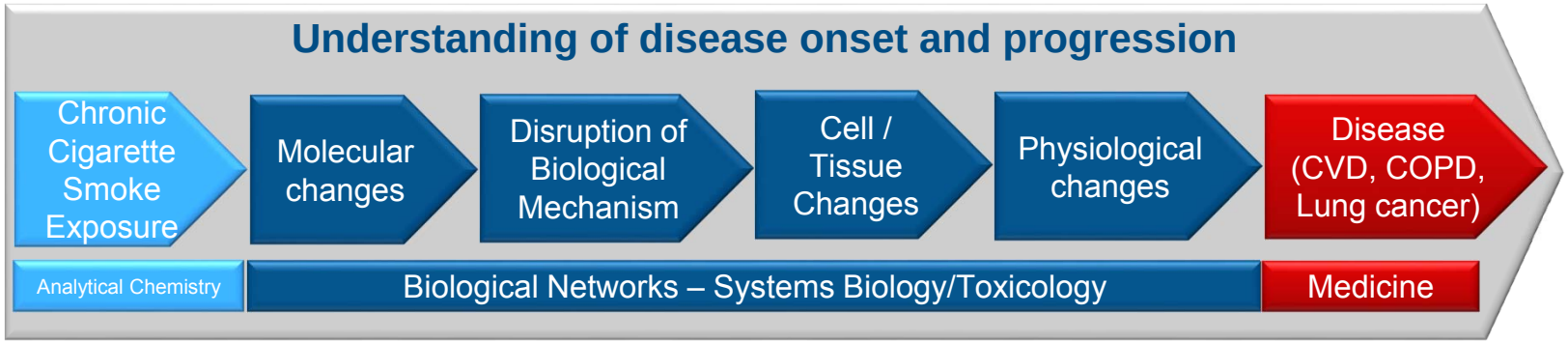
- Endpoints:




- Test atmospheres presented in low/medium/high concentration: up to MTD: 50 µg Nicotine/l* for nicotine-containing aerosols – 23 µg Nicotine/l for cigarette smoke; concentration-response

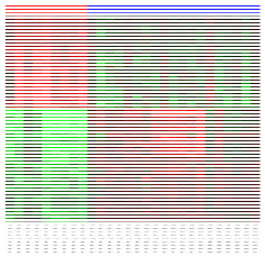
*50 µg/l nic corresponds to 13.6 mg/kg, daily dose; way above acute toxicity levels- or the nicotine amount from approx. 130 cig/day for a 60 kg human

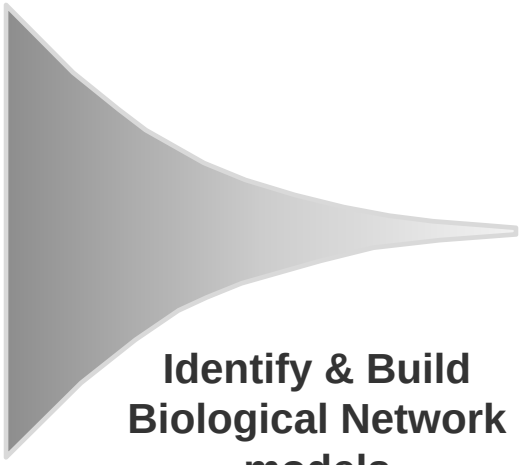
# System Toxicology Research
## *Identify and Represent Disease Mechanisms*



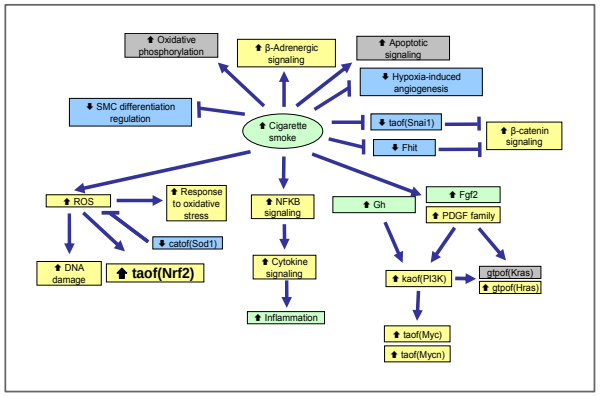

Literature-derived knowledge


Data-derived knowledge



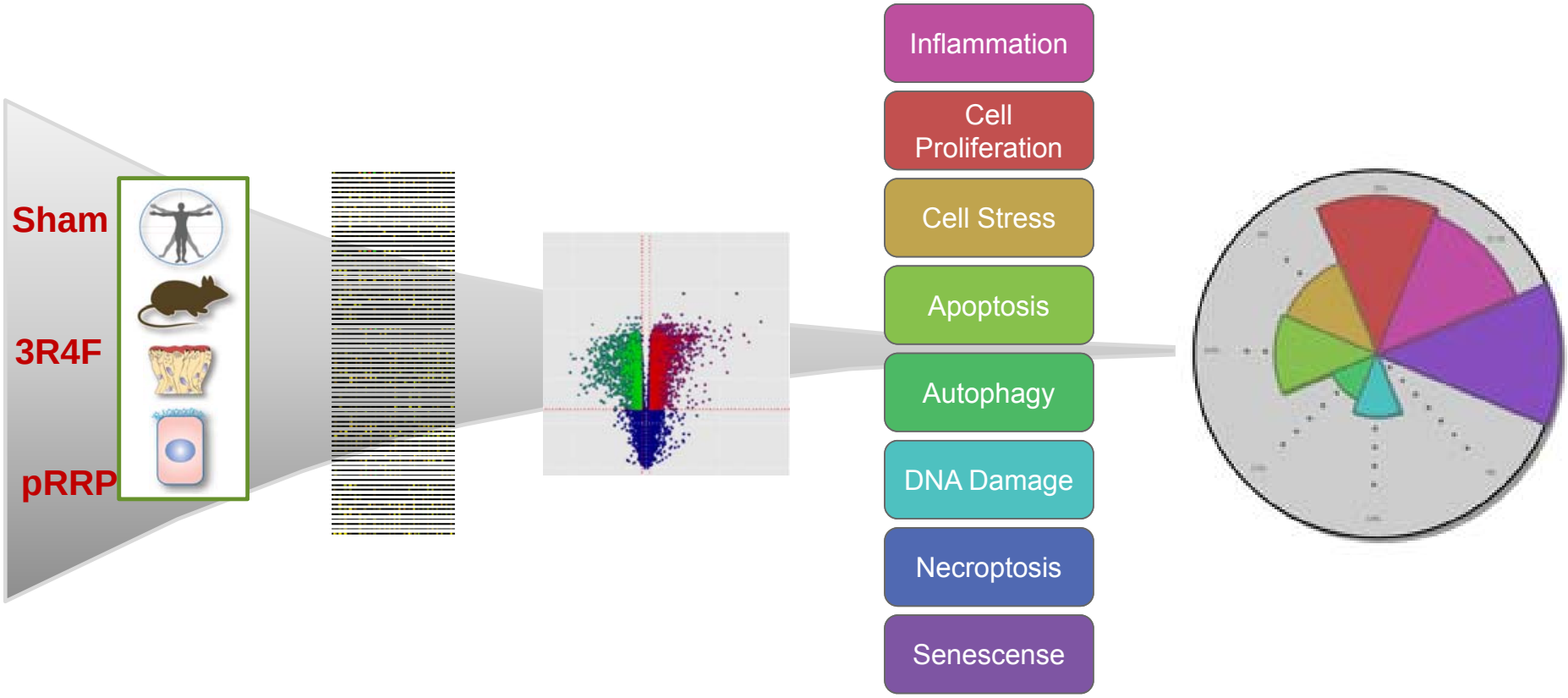


**Identify & Build Biological Network models**




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

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




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)

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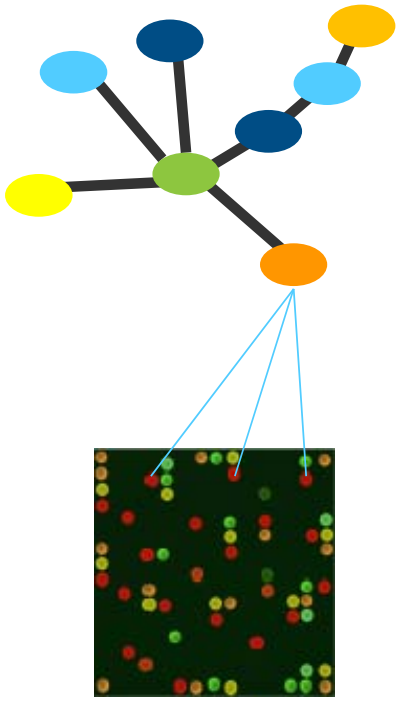
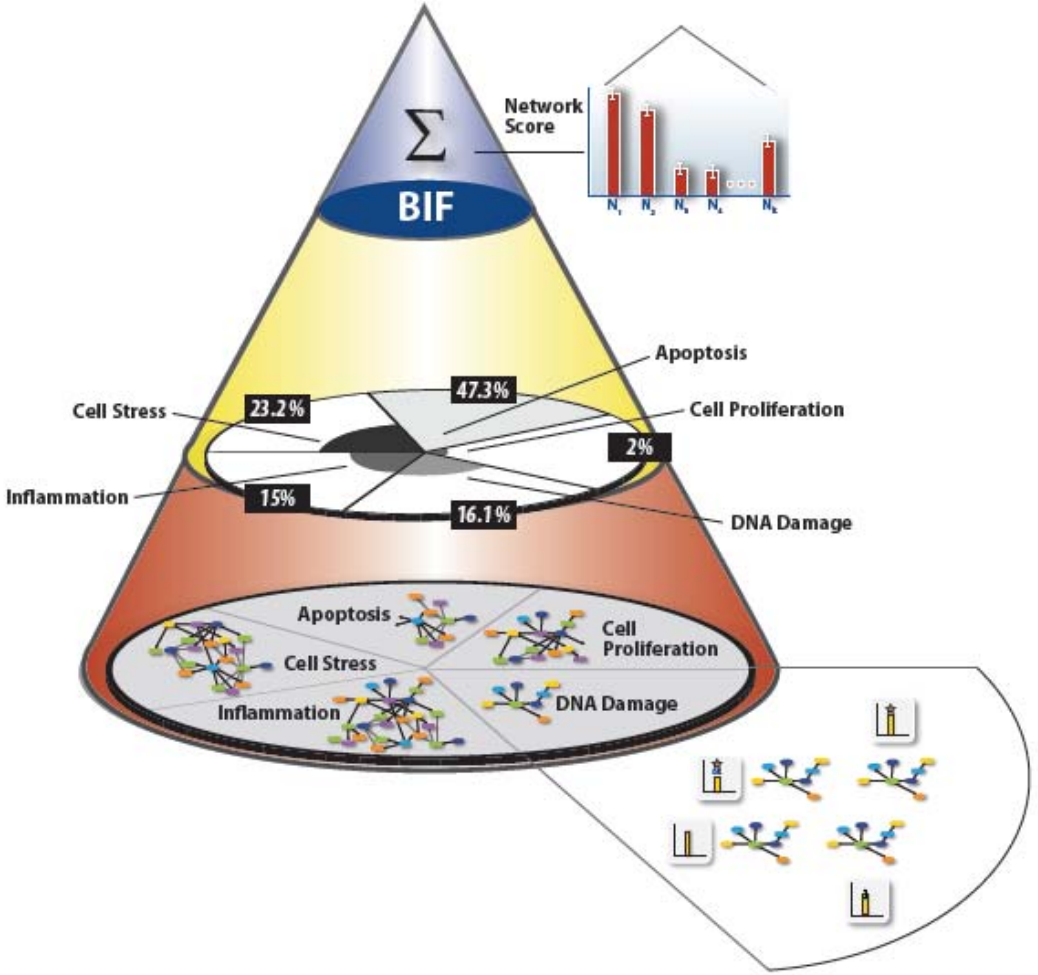
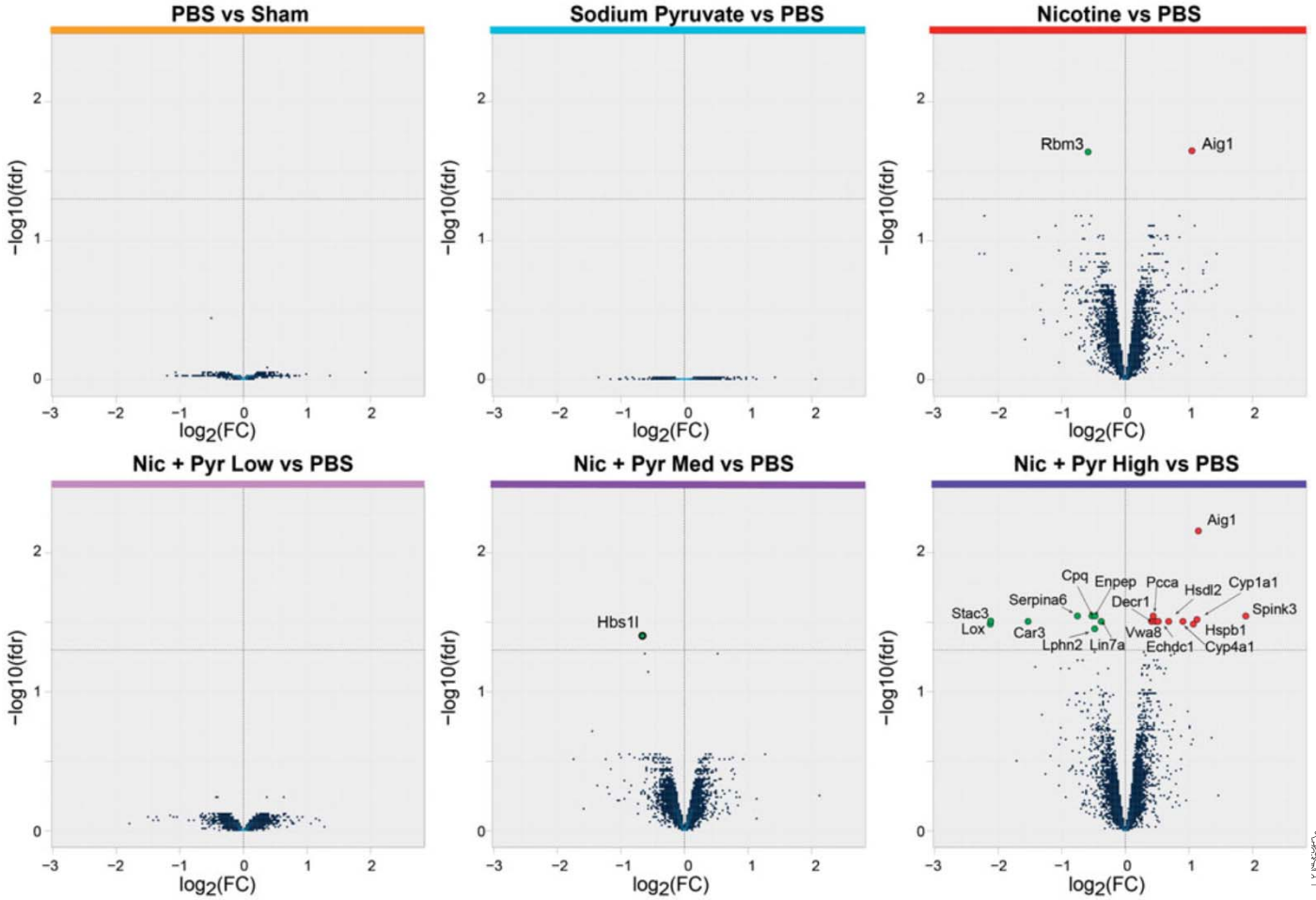
# Methods
## From Gene Expression Profiles to Biological Impact Factor





# Effects of nicotine – 28-day inhalation study (OECD TG 412)
# Application of systems toxicology on liver changes

System response profile of liver transcriptomics reveal up- and down-regulated gene expression




Up-regulated:

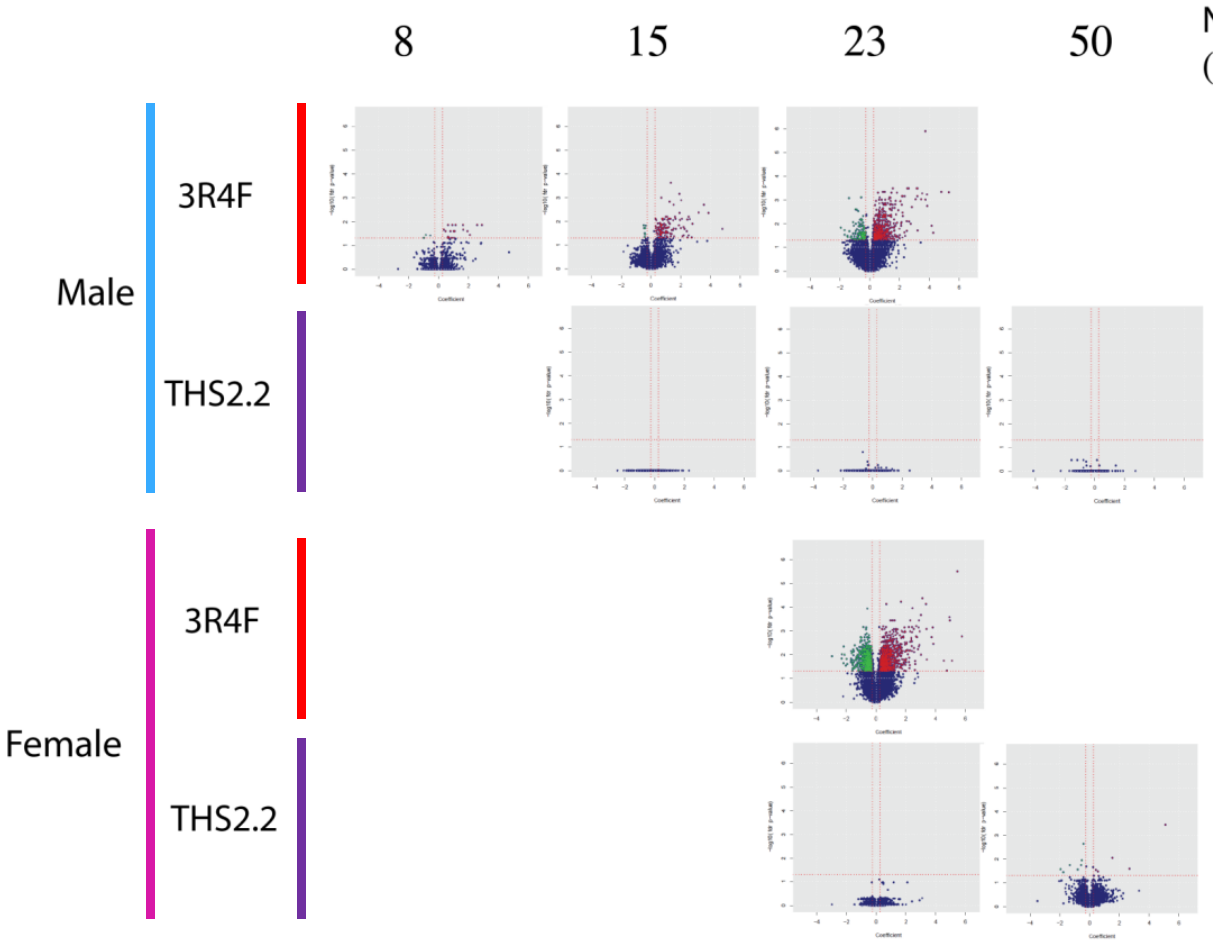
- Xenobiotic metabolism
- Oxidative phosphorylation
- Fatty acid/cholesterol metabolism
- Cell cycle

Down-regulated:

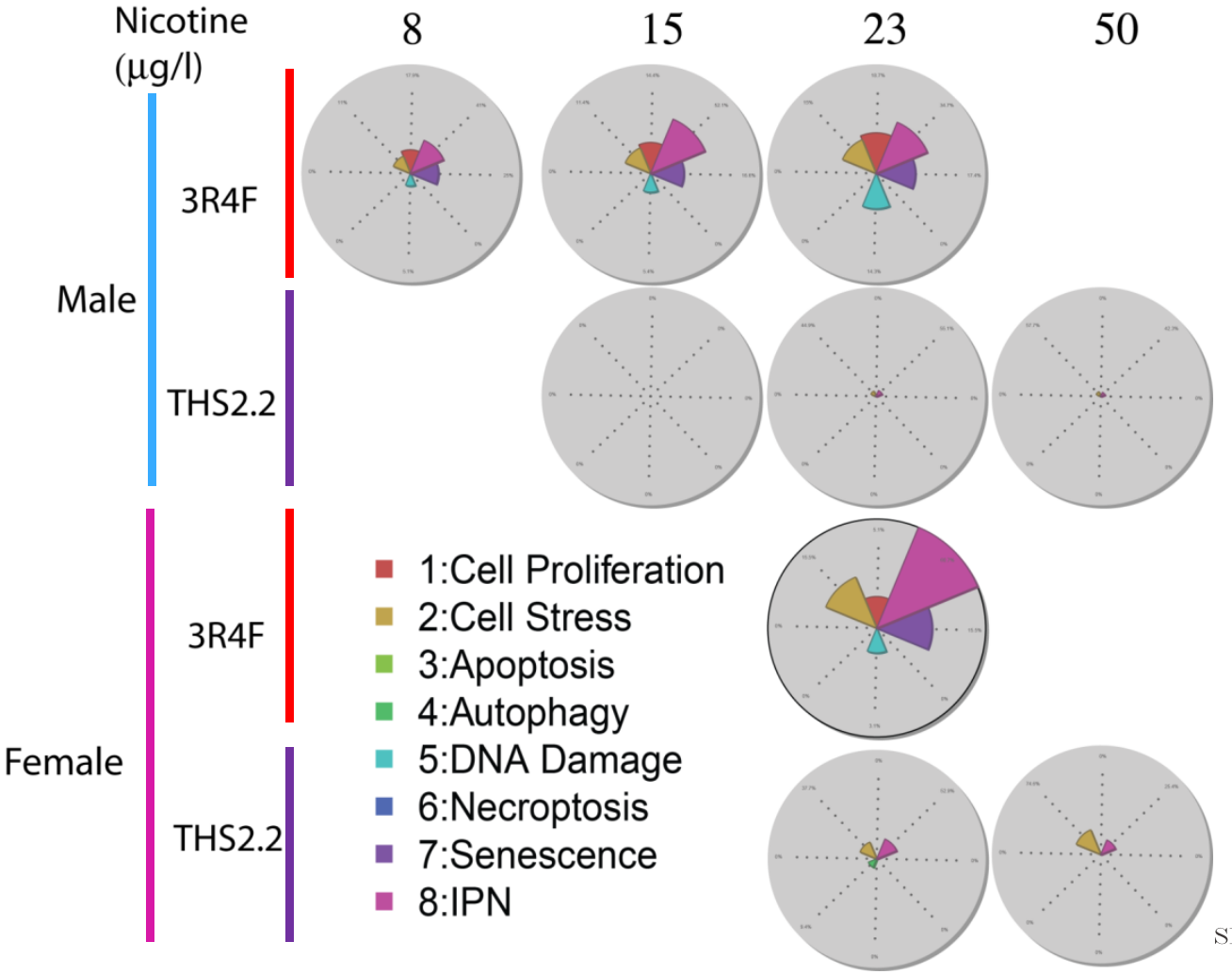
- Intracellular signaling

Lipidomics confirm downregulated glycerolipids and sterols

# Cigarette smoke and HTP aerosol– 90-day inhalation study
# Systems biology evaluation – Systems Response Profile analysis of lung (90 days)



# Cigarette smoke and HTP aerosol– 90-day inhalation study
# Systems biology evaluation – Network Perturbation Analysis of lung (90-days)



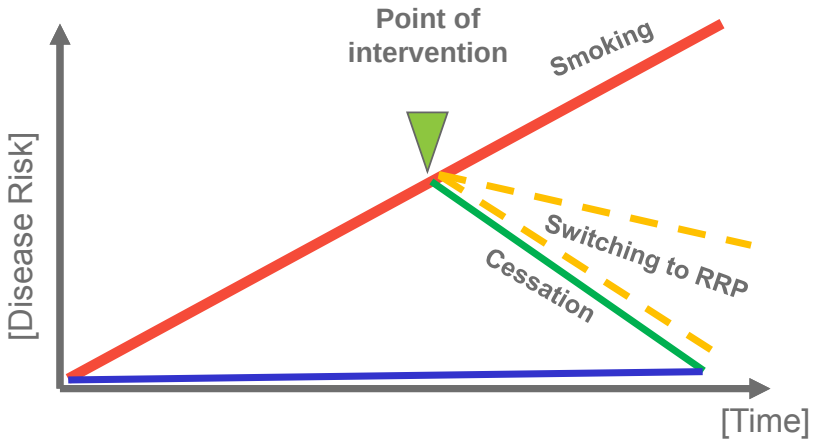
# Cigarette smoke and HTP aerosol– 90-day inhalation study
# Summary and conclusions

- *Pre-clinical toxicology*: Exposure of rats to aerosol from THS2.2 – even at a multiple of the aerosol concentration - results in a dramatically lower biological effects as compared to exposure to 3R4F in
  - Systemic toxicity; where effects were observed, they are related to nicotine exposure
  - Lung inflammation
  - Histopathology of respiratory tract organs

- *Systems toxicology*: Exposure of rats to aerosol from THS2.2 results in a dramatically lower biological network perturbations in the transcriptomes of the nasal epithelium and lung tissue

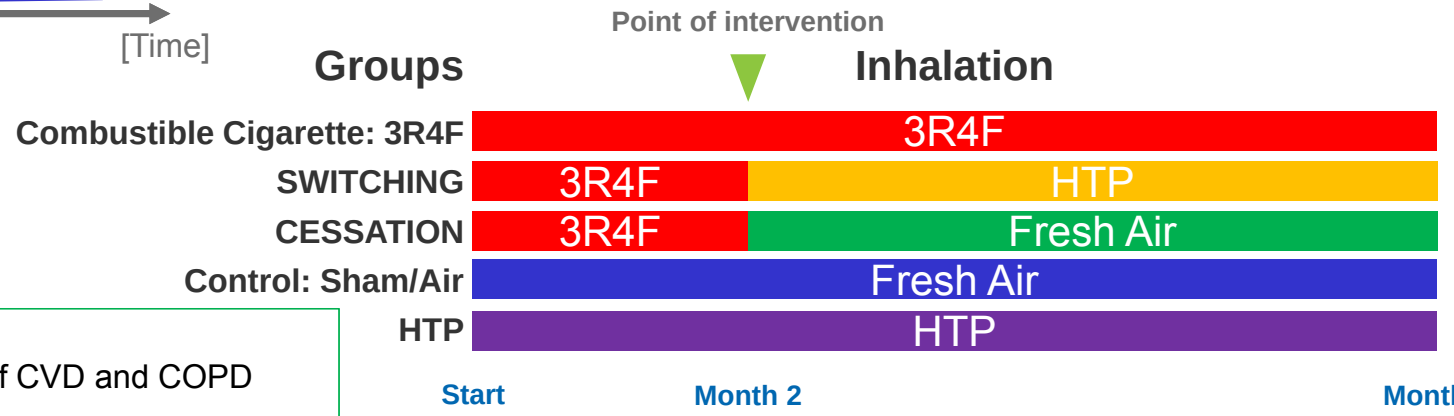
# Methods
## Switching Study in an Animal Model of Disease – cardiovascular disease/emphysema

**Comparing switching to the HTP P1* with ongoing smoking and benchmarking against cessation**




Non-clinical study

**ApoE-P1 Switching Study Design**




**Mice**:
- ApoE$^{-/-}$ mice: animal model for aspects of CVD and COPD

**Exposure**:
- 8 months with dissections at 1,2,3,6 and 8 months
- Test atmosphere nicotine concentrations: approx. 30 µg/l
- Whole body, 3 x 1 h/day

*P1: HTTP:THS2.2 or iQOS

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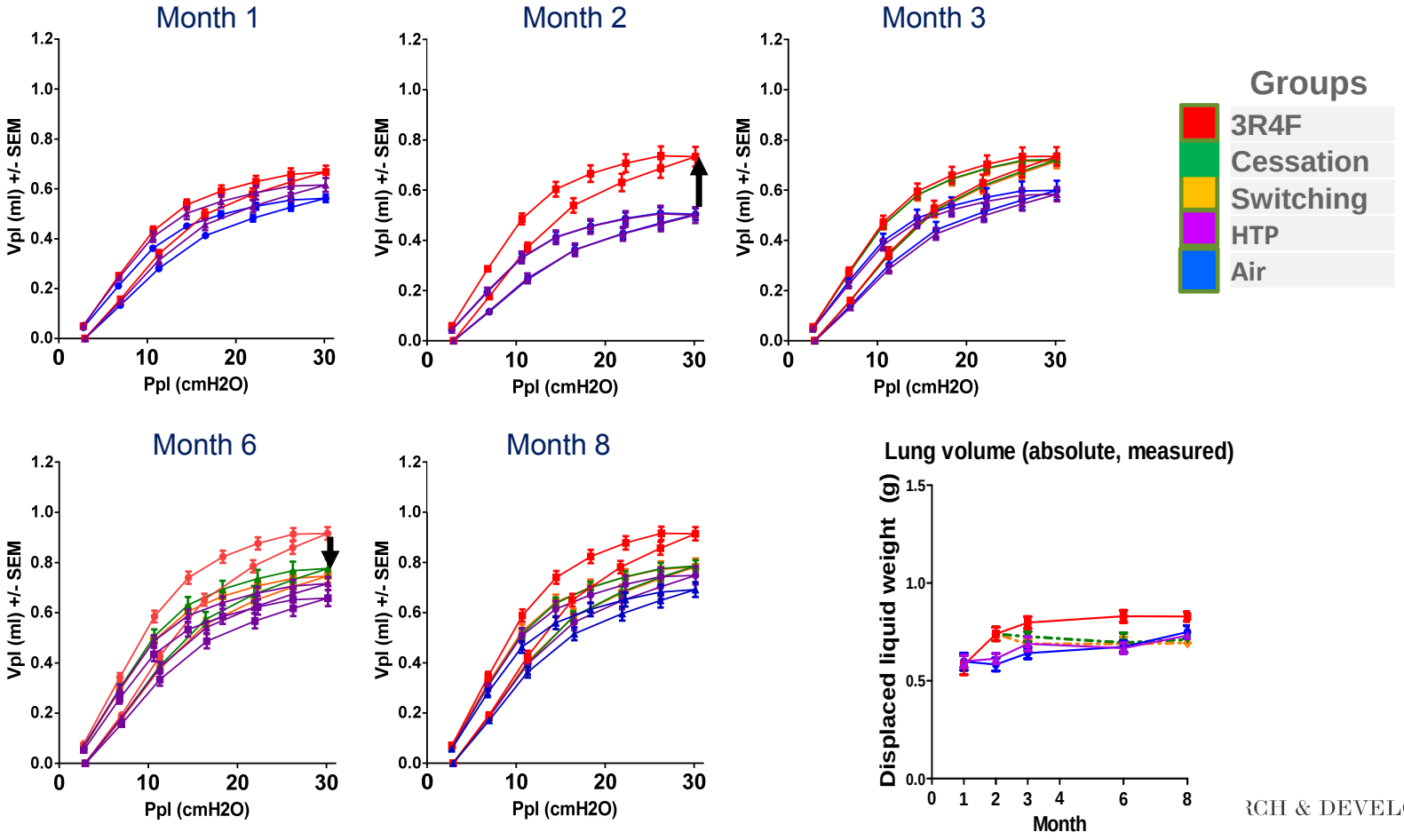
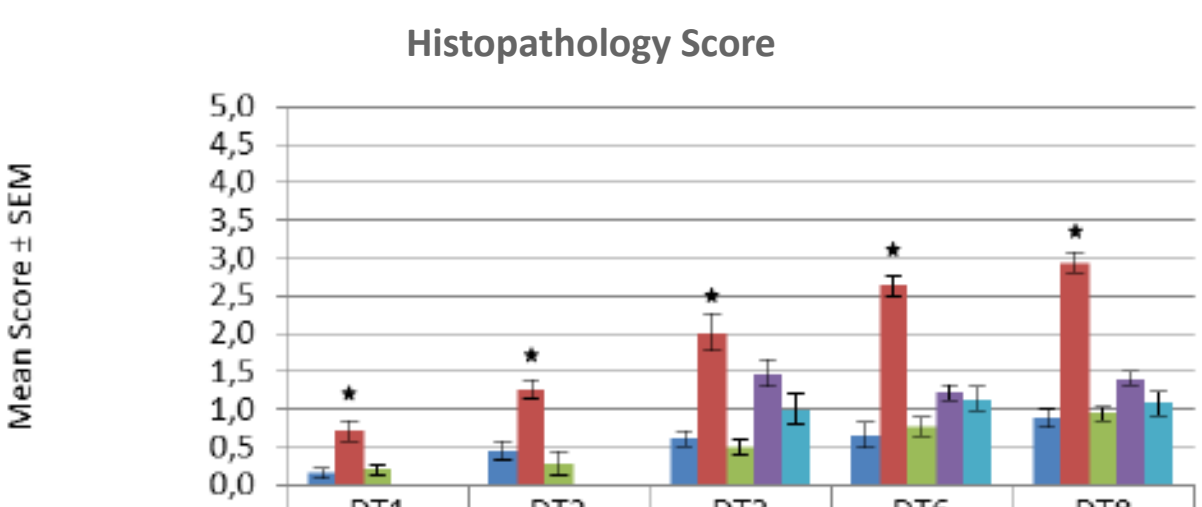
# Switching Study in an Animal Model of Disease
# Result Summary: Disease Endpoint - Lung function and Lung Volume




Means ± SEM

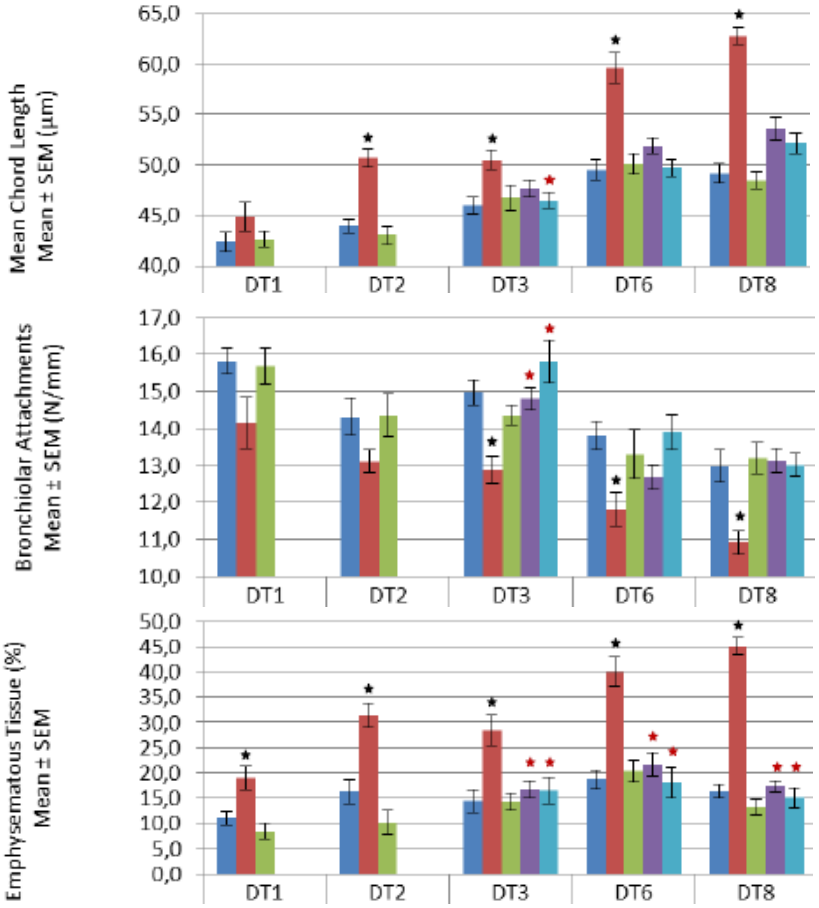
RCH & DEVELOPMENT

# Switching Study in an Animal Model of Disease
# Result Summary: Tissue changes – Lung Emphysema



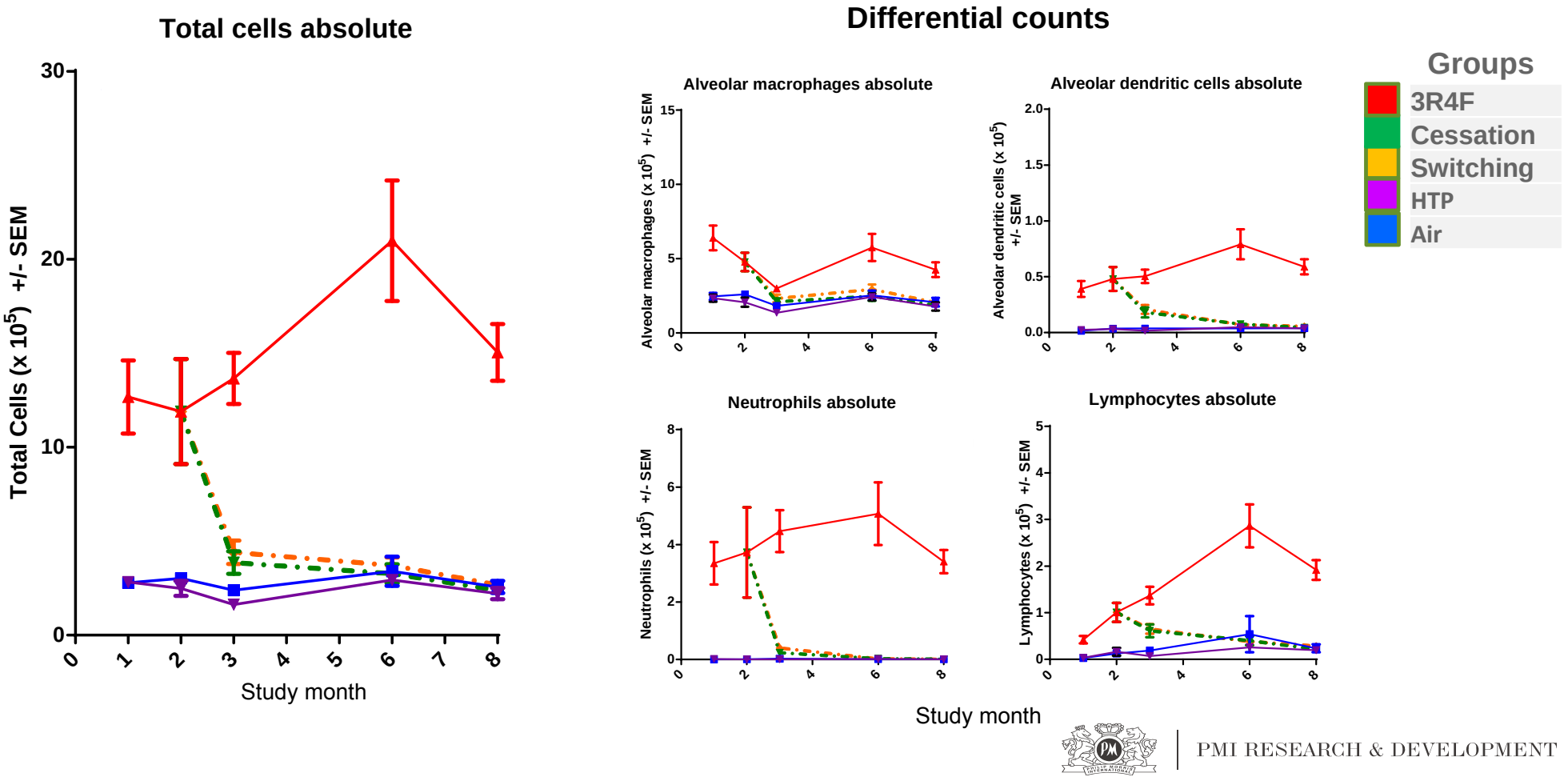

| | DT1 | DT2 | DT3 | DT6 | DT8 |
|---|---|---|---|---|---|
| SHAM | 0,2 | 0,5 | 0,6 | 0,7 | 0,9 |
| 3R4F_CONT | 0,7 | 1,3 | 2,0 | 2,6 | 2,9 |
| THS 2.2_CONT | 0,2 | 0,3 | 0,5 | 0,8 | 0,9 |
| Cessation | | | 1,5 | 1,2 | 1,4 |
| THS 2.2_Switch | | | 1,0 | 1,1 | 1,1 |

*: Statistically significant compared to sham
*: Statistically significant compared to 3R4F:CONT at DT2



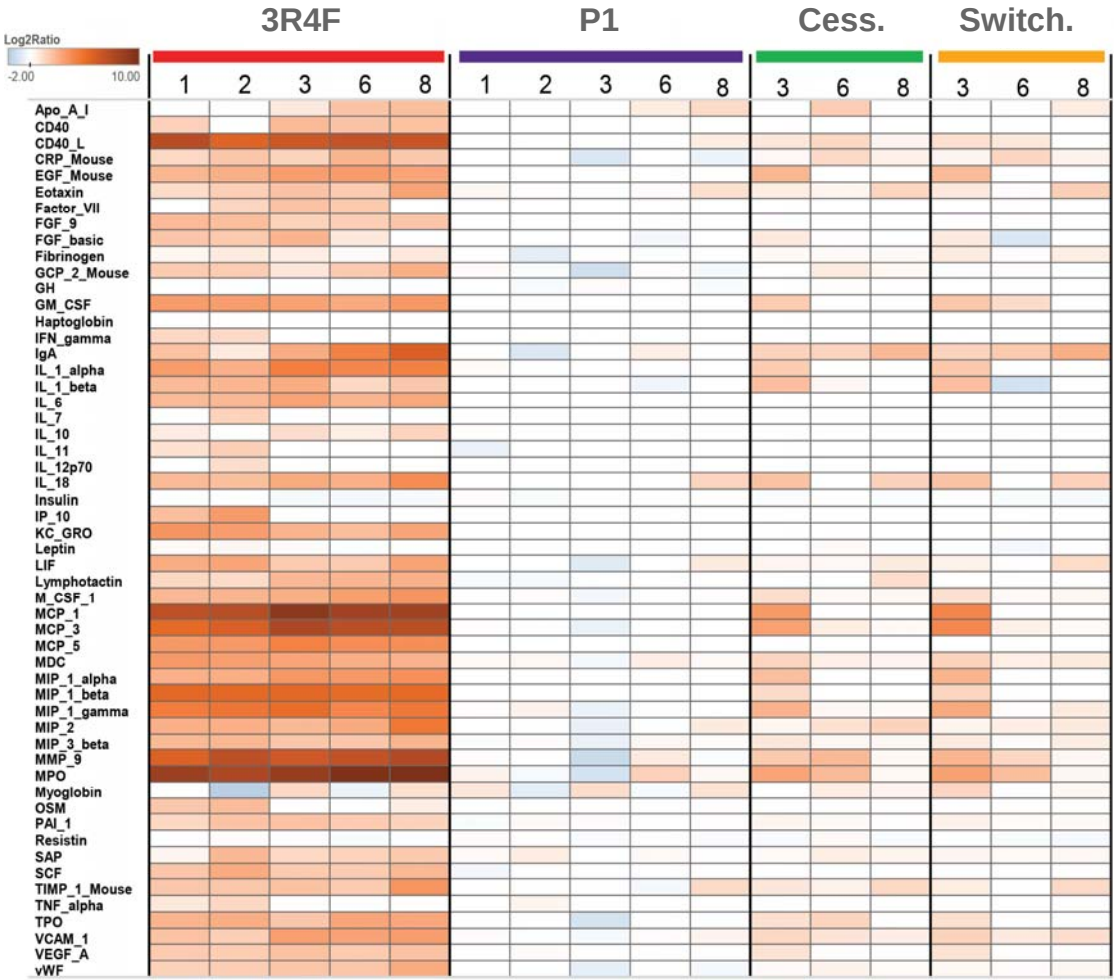
# Switching Study in an Animal Model of Disease
# Result Summary: Disease Mechanism - Lung Inflammation

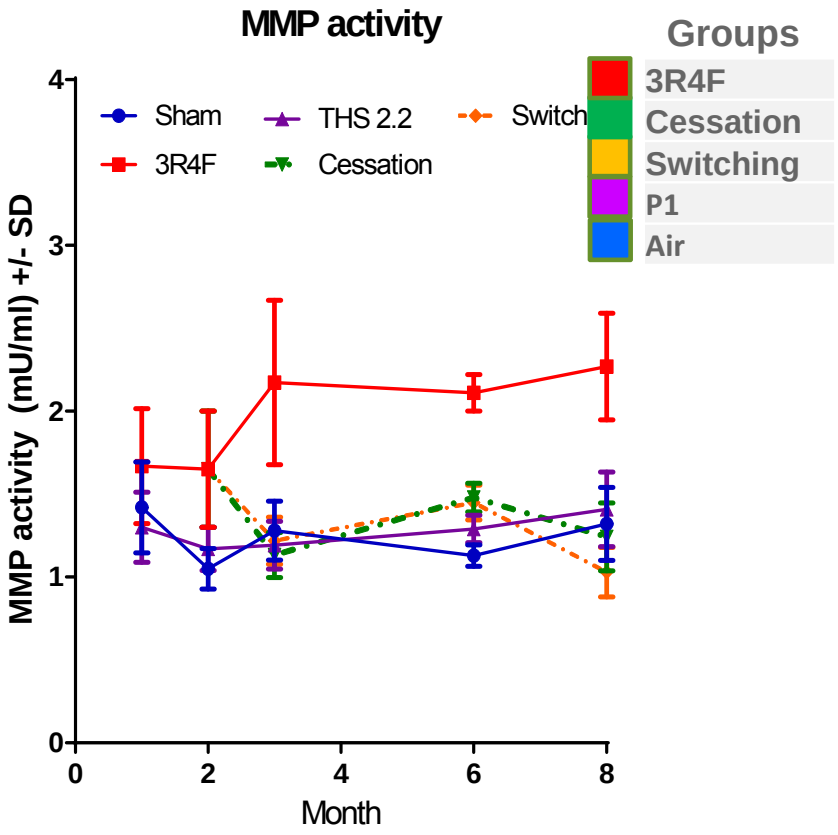
Free lung cells in Broncho-alveolar lavage fluid (BALF)



# Switching Study in an Animal Model of Disease
# Result Summary: Disease Mechanism - Lung Inflammation

Multiple analyte profiling in Broncho-alveolar Lavage Fluid

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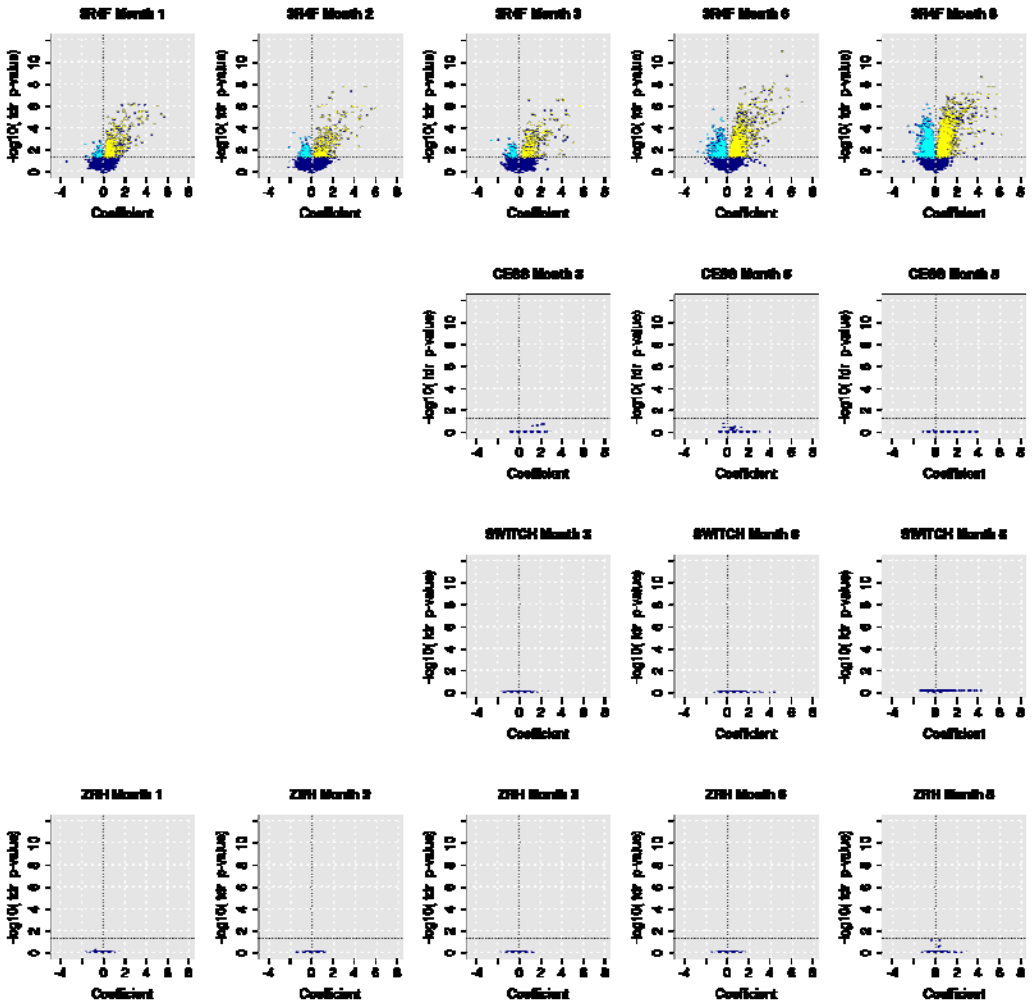
# Switching Study in an Animal Model of Disease
# Result Summary: Differential Gene Expression - Lung

3R4F

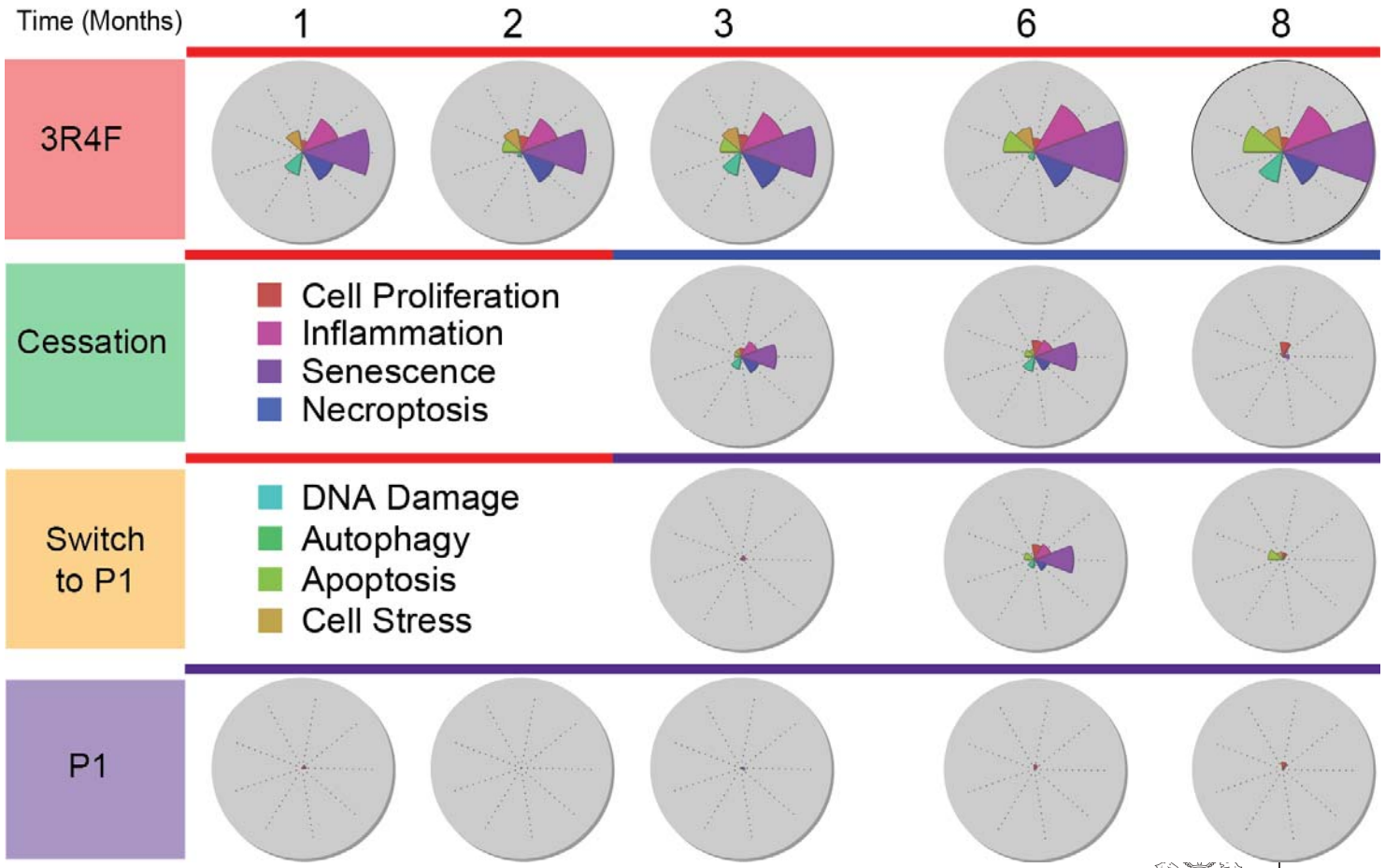
Cessation

Switch

P1

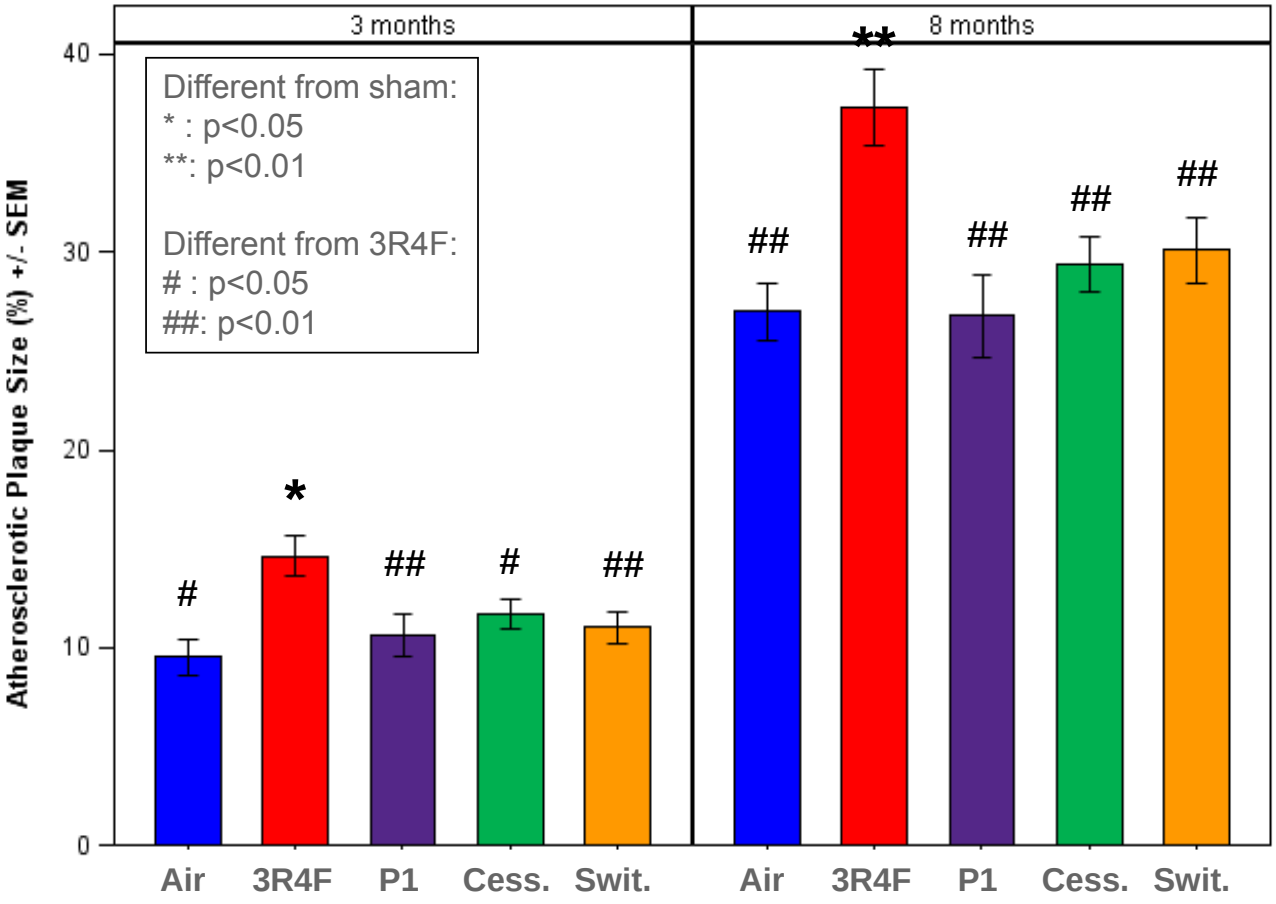


# Switching Study in an Animal Model of Disease
# Result Summary: Disease Mechanisms – Network Perturbations - Lung

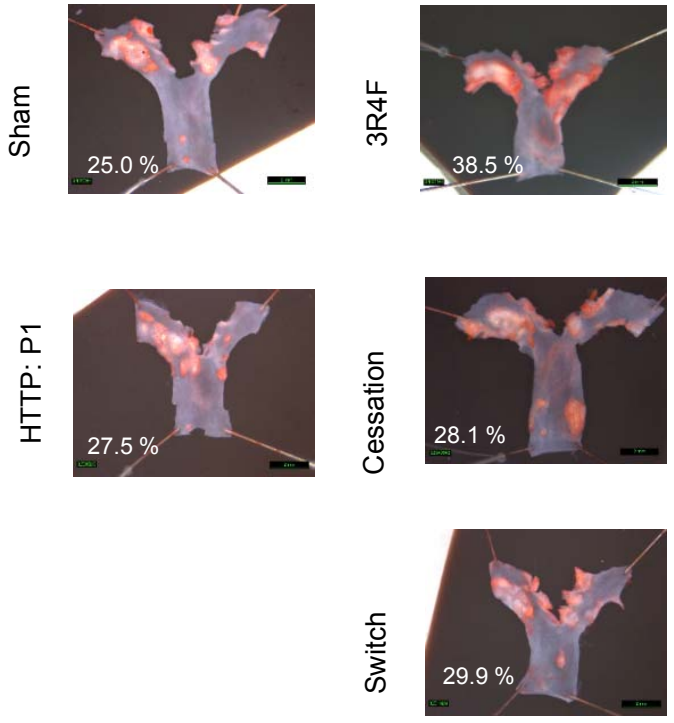


# Switching Study in an Animal Model of Disease
# Result Summary: Disease Endpoint - Aortic Plaque Growth

## Image analysis of stained atherosclerotic plaques




Aortic Arches at month 8

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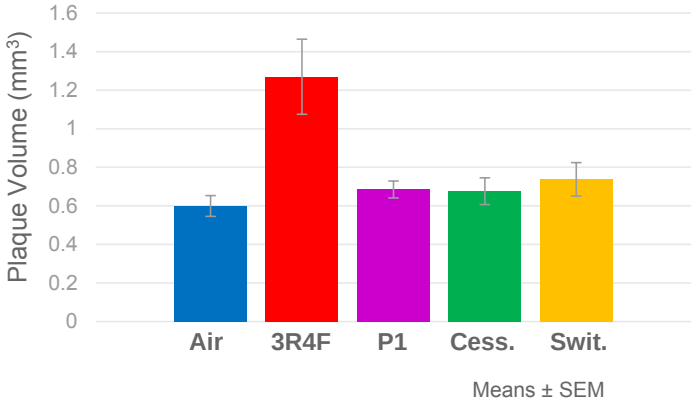
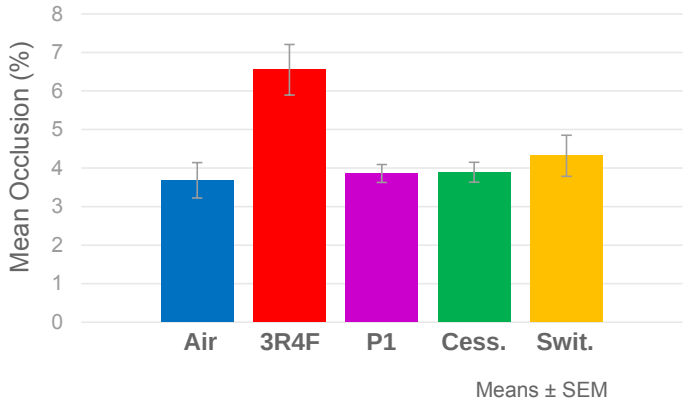
# Switching Study in an Animal Model of Disease
# Result Summary: Disease Endpoint - Aortic Plaque size at Month 8

## 3D Reconstruction from High Resolution micro-Computed Tomography

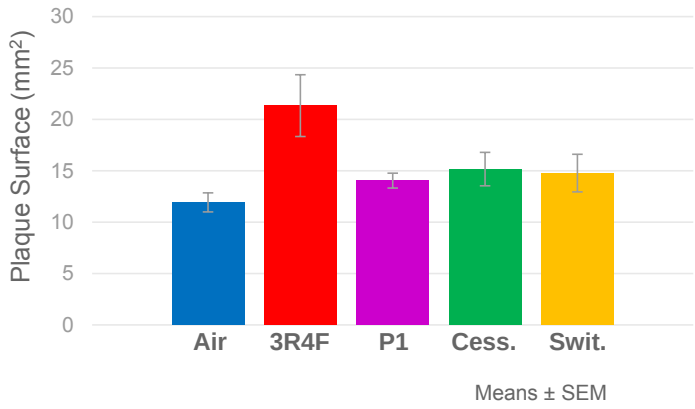
### Plaque Volume



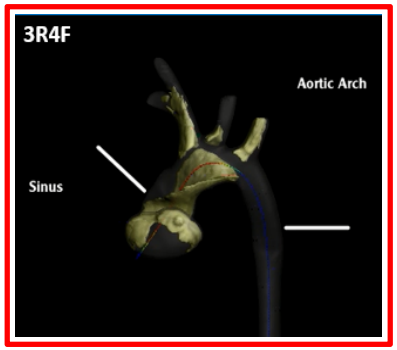

### Aortic Arch Occlusion

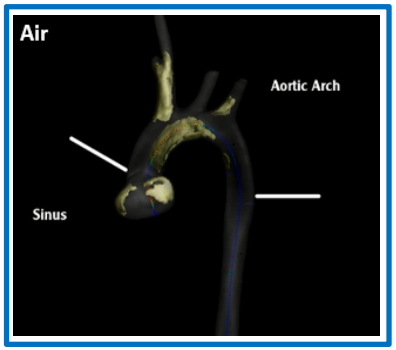


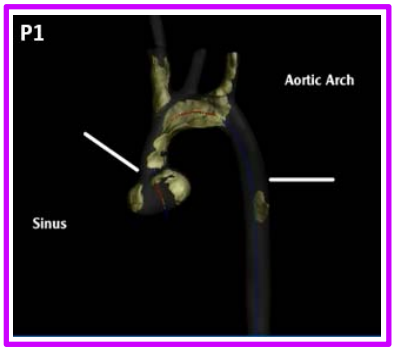

### Plaque Surface

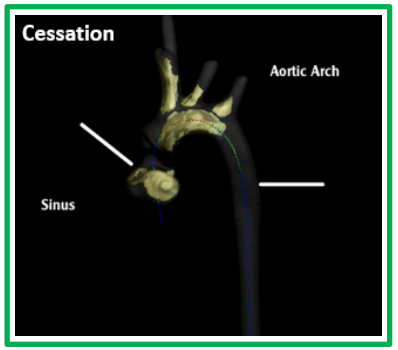


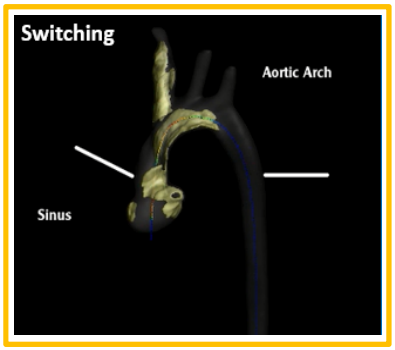

## 3D-Reconstructions



# Switching Study in an Animal Model of Disease
# Summary and Conclusions

- The ApoE$^{-/-}$ mouse model is suitable for studying smoke-related aspects of cardiovascular disease and COPD
- Continuous exposure to aerosol from a pRRP for up to 8 months does not increase cardiovascular disease, inflammation and emphysema
- Switching from cigarette smoke exposure after 2 months to fresh air exposure reverses the onset of disease as measured in apical, functional, and molecular endpoints
- Switching from cigarette smoke exposure to pRRP aerosol exposure reverses the onset of disease in a similar manner as cessation

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

Phillips B, Veljkovic E , Boué S , Schlage WK, Vuillaume G, Martin F, Titz B , Leroy P, Buettner A, Elamin A, Oviedo A, Cabanski M, Guedj E, Schneider T, Talikka M, Ivanov NV , Vanscheeuwijck P, Peitsch MC, Hoeng J.

Toxicological Sciences (2016) 149, 411-32 (PMID 26609137)

# Overall conclusion

- Exposure of rodents to cigarette smoke results in clear toxicological effects and development of known diseases
- Nicotine, when administered via inhalation exposure, causes limited toxicological effects after 28 or 90 days
- Aerosol from a pRRP, HTP elicits much lower toxicity (overall more than 80%) than cigarette smoke, even at a multiple of the nicotine exposure concentration. Most effects can be attribute to nicotine
- Aerosol from a pRRP, HTP does not  lead to smoke-related disease and switching from smoke to pRRP aerosol exposure is almost identical to cessation.

# THANK YOU!

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