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Lung inflammation, emphysema, and lung cancer development in A/J mice in response to chronic exposure to aerosol from a heated tobacco product and cigarette smoke

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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.

How to assess reduced risk of harm...

Cigarette Smoke

Exposure → Molecular Changes → Disruption of Biological Mechanisms → Cell/Tissue Changes → Disease → Population Harm

Reduced Emissions → Reduced Exposure → Reduced Adverse Health Effects

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18 Non-Clinical Studies and 10 Clinical Studies

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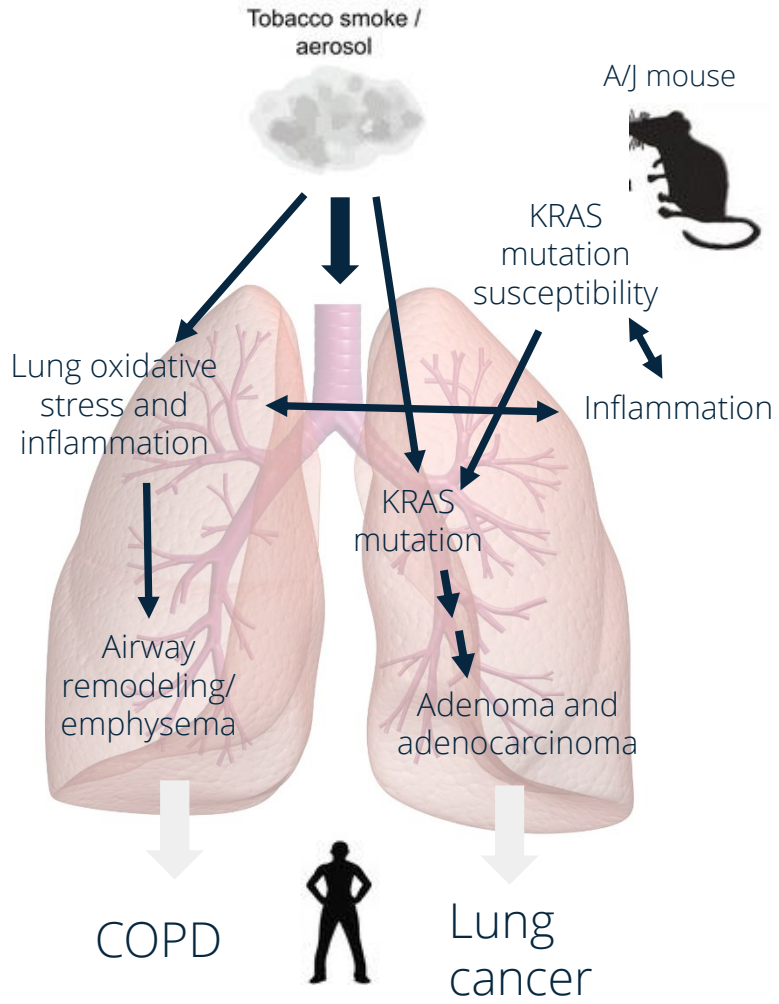
Study objective
Combined chronic toxicity and carcinogenicity study – A/J mice

- To assess the impact of lifetime exposure to Heating System (THS) aerosol, compared with that of 3R4F cigarette smoke, on systemic toxicity, development of lung inflammation, emphysema, and lung tumor incidence and multiplicity in an 18-month exposure study in A/J mice

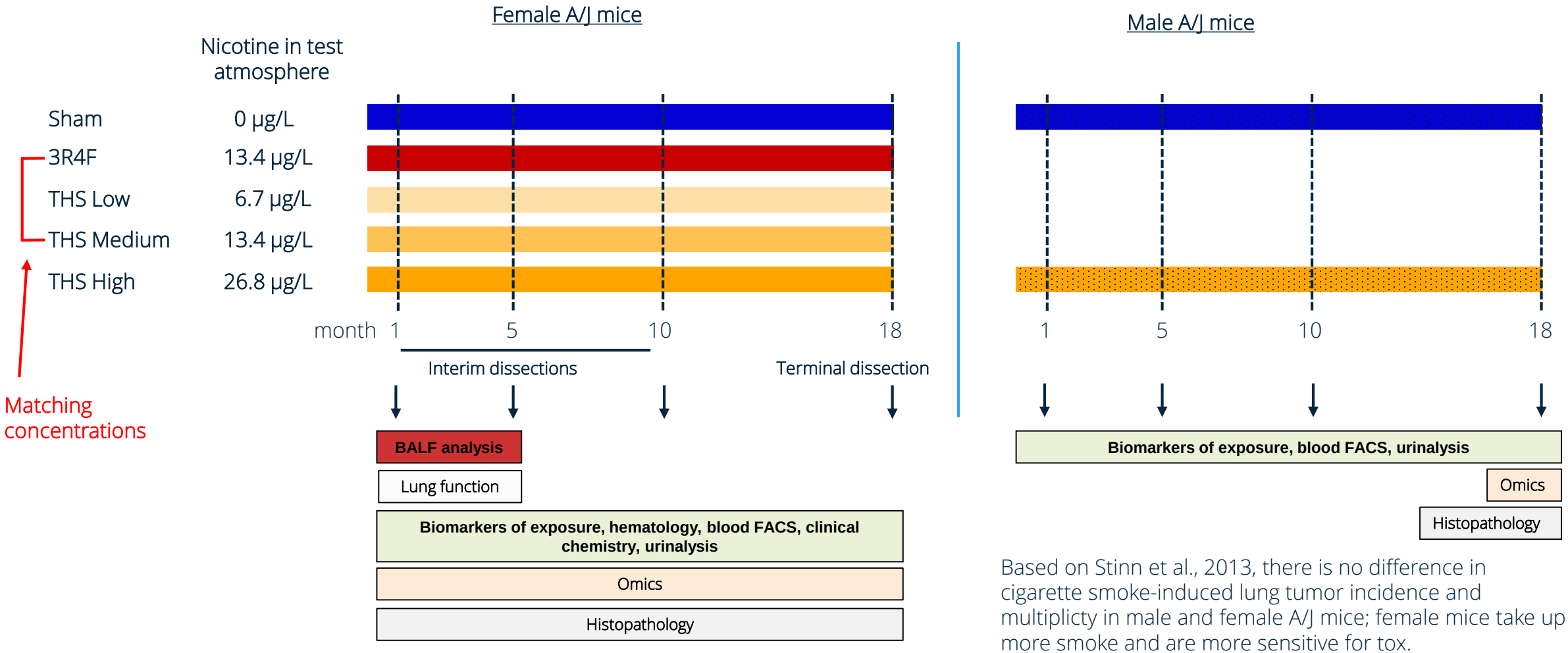
The A/J mouse model for lung cancer and emphysema



- Smoke-induced lung cancers in human:
  - Non-small cell lung carcinoma (NSCLC, 80%) and small cell lung carcinoma (SCLC, 20%)
  - Lung adenocarcinomas accounts for more than 50% of NSCLCs
- Human adenocarcinomas frequently carry KRAS mutations
- A/J mouse model develops cigarette smoke-induced lung adenocarcinomas, with increased transcription rate of mutated KRAS
- Suitable to study co-morbidities: inflammation and oxidative stress associated with pathogenesis of lung cancer and COPD

- Stinn et al., 2013: Lung Inflammatory effects, tumorigenesis, and emphysema development in a long-term inhalation study with cigarette mainstream smoke in mice, Tox, Sci. 131, 596-611; Manenti & Dragani, 2005: Pas1 haplotype-dependent genetic predisposition to lung tumorigenesis in rodents: a meta-analysis, Carcinogenesis 26:875-882;To et al., 2006: A functional switch from lung cancer resistance to susceptibility at the Pas1 locus in Kras2LA2 mice. Nature Genetics 38, 926-930

Study design
Combined chronic toxicity and carcinogenicity study – A/J mice



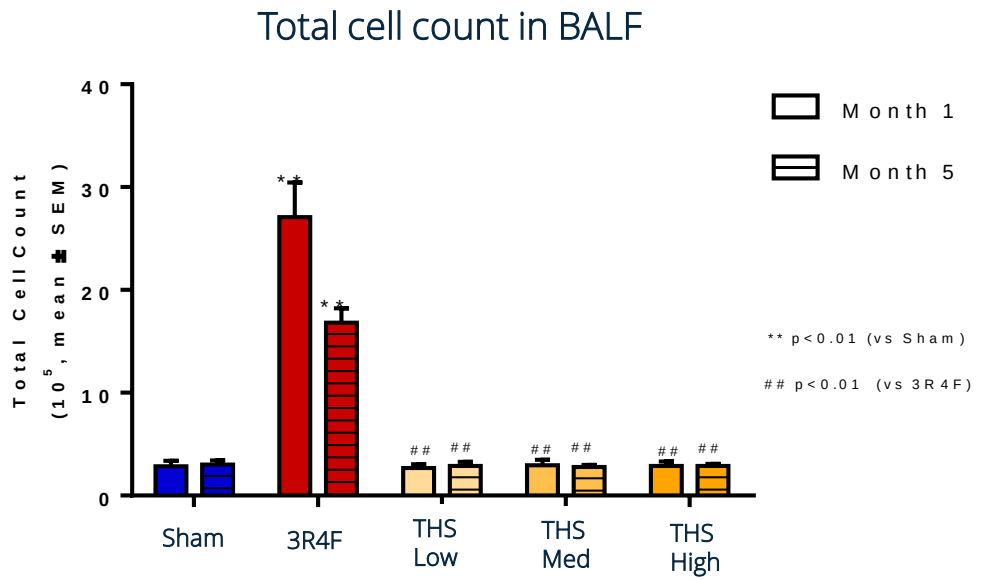
Based on Stinn et al., 2013, there is no difference in cigarette smoke-induced lung tumor incidence and multiplicty in male and female A/J mice; female mice take up more smoke and are more sensitive for tox.

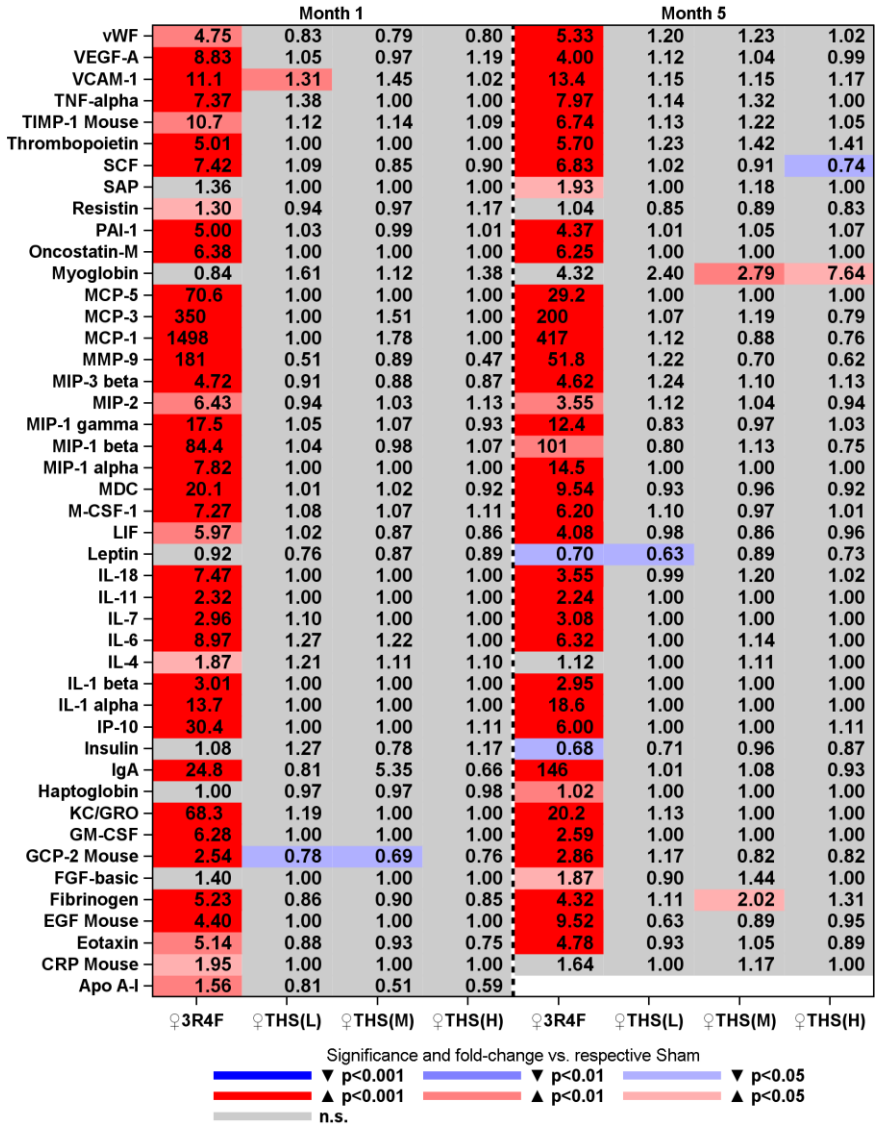
26.8 µg/L nicotine concentration in THS aerosol represents 56 Sticks/day (*FDA, 2005. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Food and Drug Administration, Washington, DC. http://www.fda.gov/cder/guidance.), Stinn et al., 2013, Toxicology. 2013, 305:49-64. doi: 10.1016/j.tox.2013.01.005

Systemic toxicity – lung inflammation

Combined chronic toxicity and carcinogenicity study – A/J mice

- No lung inflammation in THS-exposed mice
- 3R4F effects:
  - Clear lung inflammation, including higher total cell count and higher macrophage, neutrophil, and lymphocyte counts



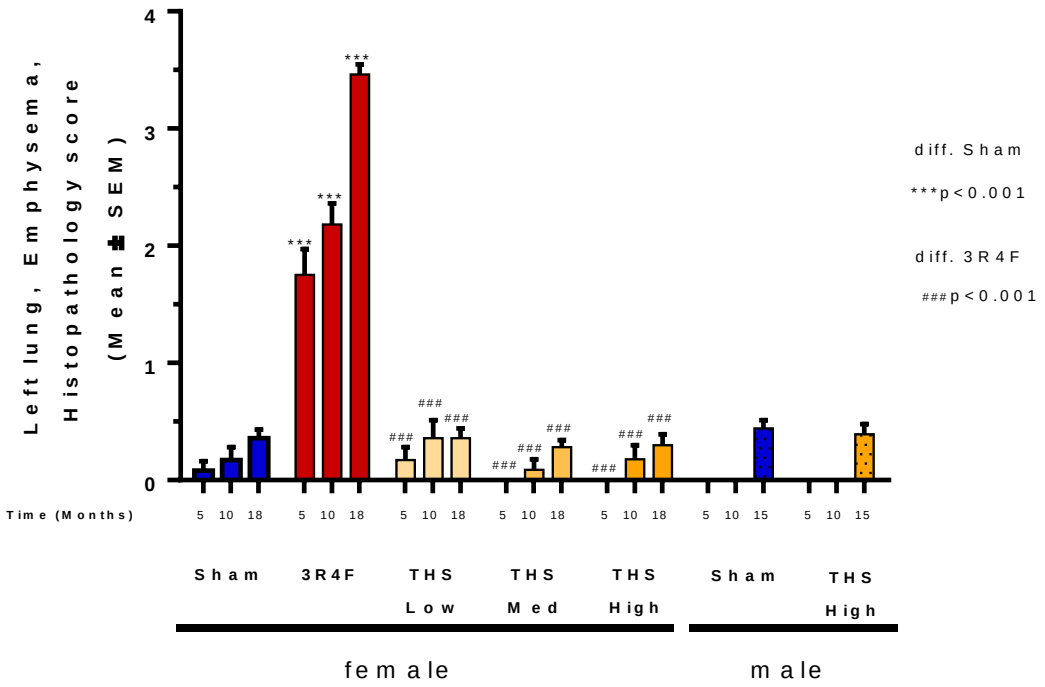


Emphysema – histopathology & morphometry

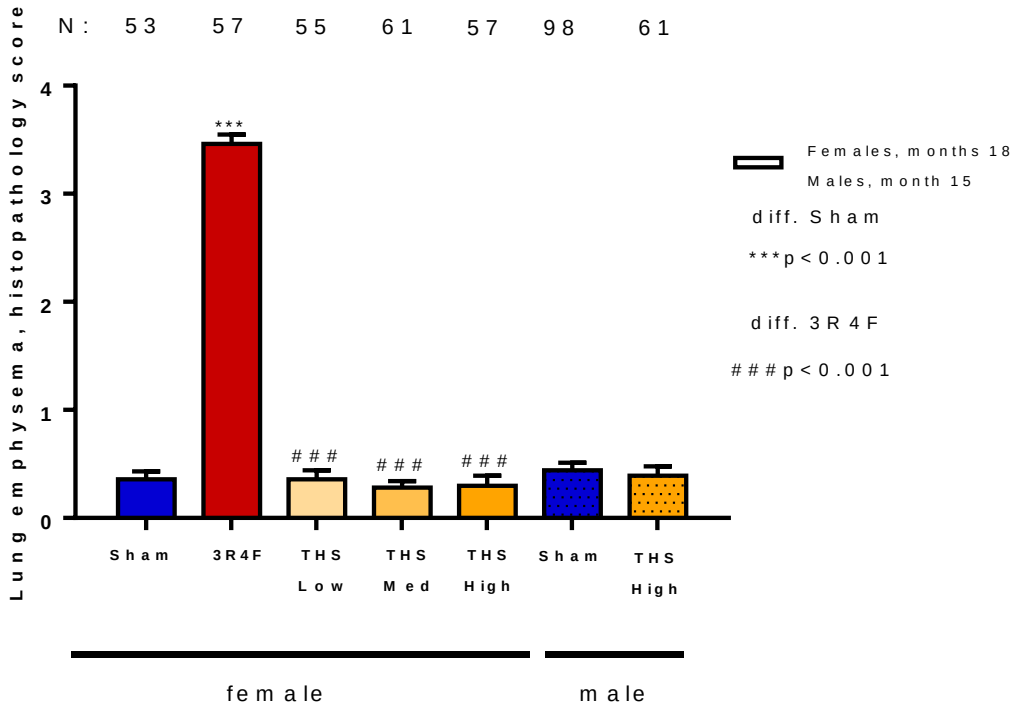
Combined chronic toxicity and carcinogenicity study – A/J mice

- **No pulmonary emphysema** observed in THS-exposed mice upon histopathological analysis, while upon 3R4F smoke exposure there is a clear progression
- Results confirmed by extended morphometric analysis

Lung emphysema, histopathology, time course

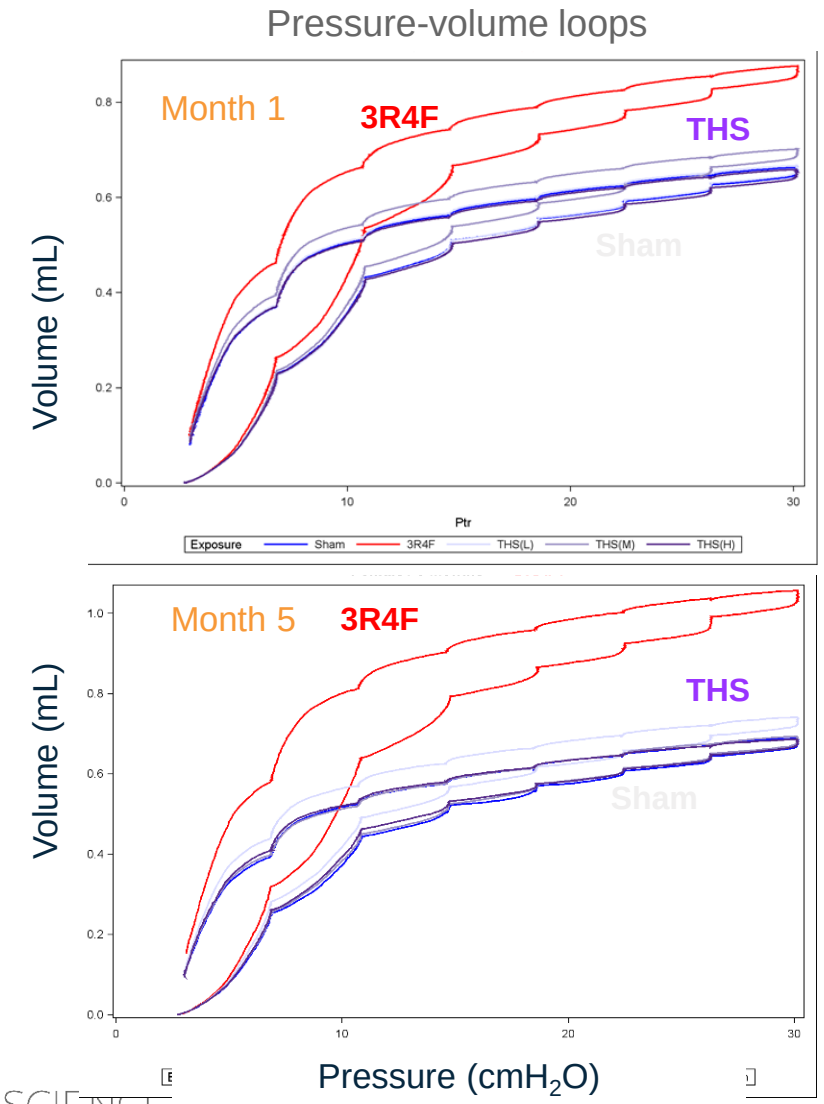


Lung emphysema, histopathology, terminal dissection



Emphysema – lung function

Combined chronic toxicity and carcinogenicity study – A/J mice

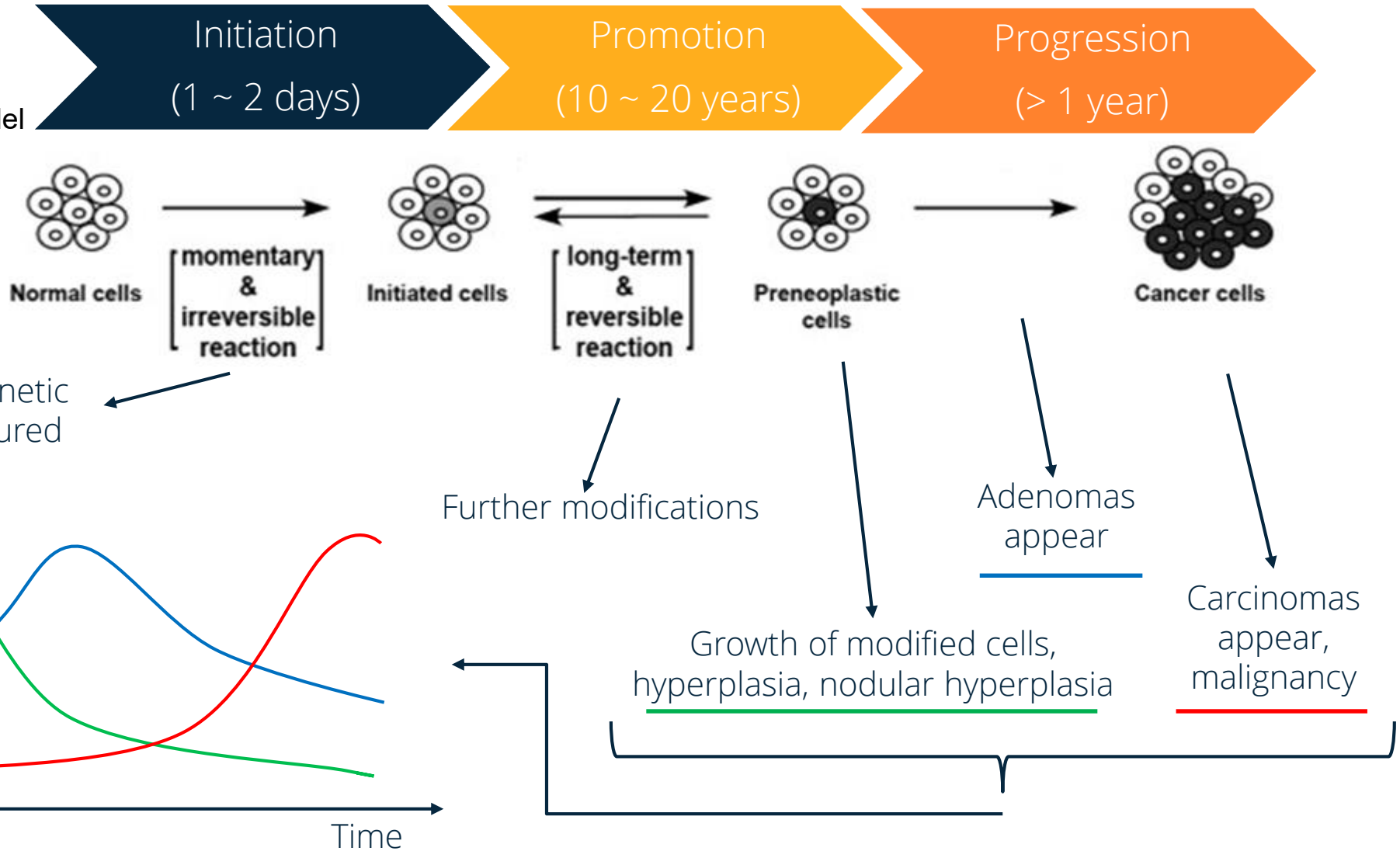


- No change in lung function in THS aerosol-exposed mice relative to Sham
- No changes in compliance and airway resistance in THS groups
- 3R4F exposure-related changes
  - Leftward & upward shift of the pressure-volume loops for both the inflation and deflation phases
  - Higher lung volumes at specified pressure; greater ease with which the lungs may be extended at a specified pressure

Neoplastic changes in the lung

Combined chronic toxicity and carcinogenicity study – A/J mice

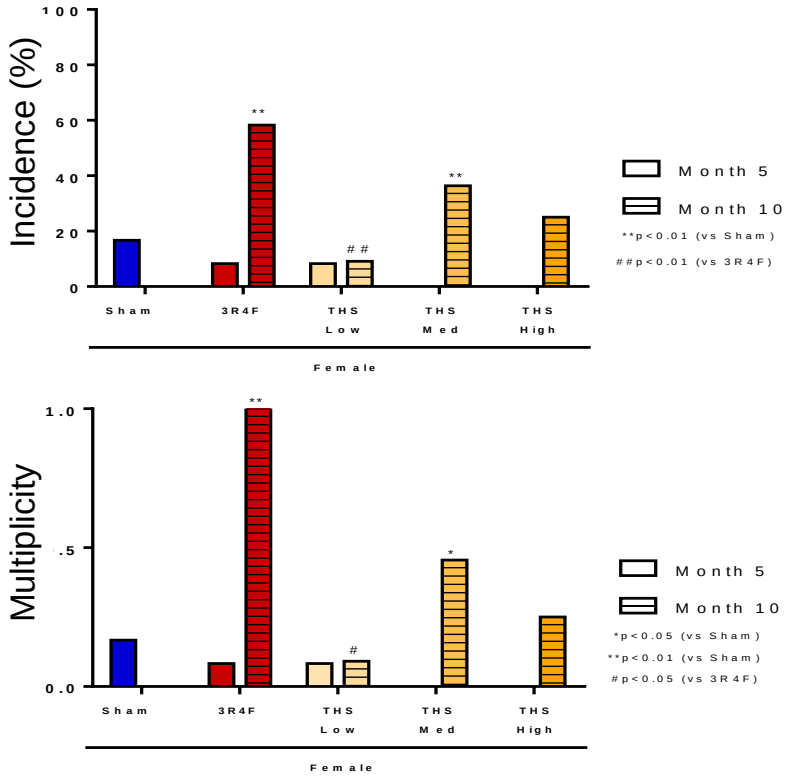
- Events in carcinogenesis:
  - Three-step carcinogenesis model



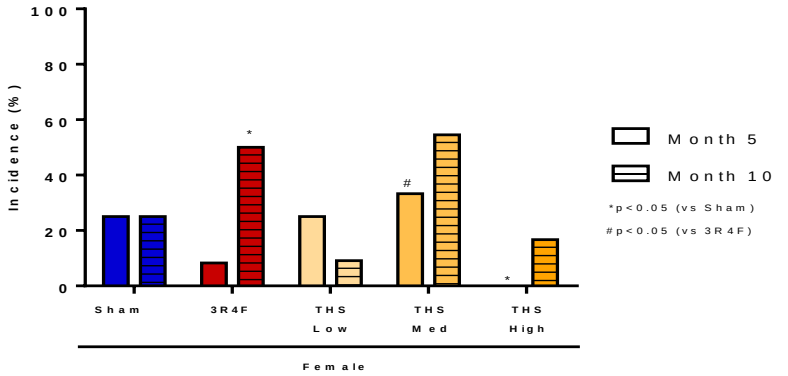
Neoplastic changes in the lung

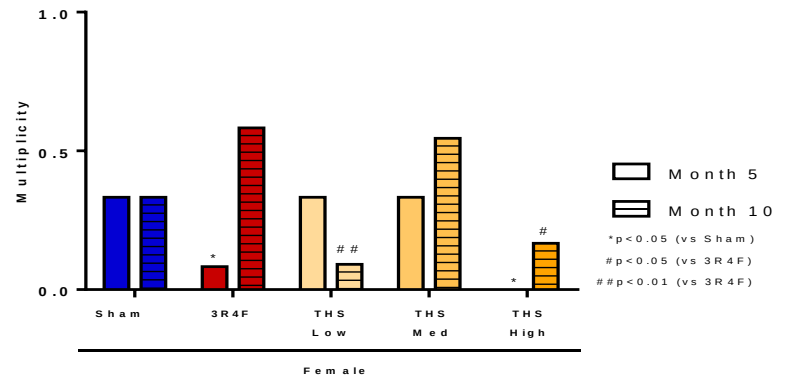
Combined chronic toxicity and carcinogenicity study – A/J mice

Hyperplasia



Adenoma and adenocarcinoma





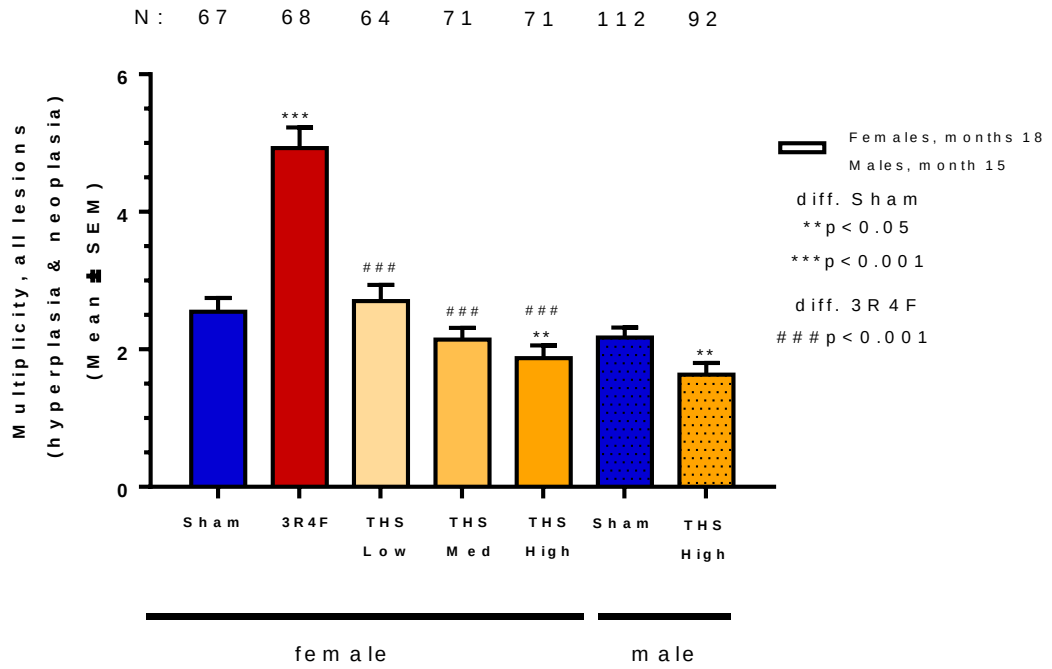
- Early time points:
  - Lower incidence and multiplicity of hyperplastic changes in the THS aerosol-exposed lungs
  - Similar incidence of pre-neoplastic/neoplastic changes in lungs from all groups, low multiplicity
  - Too early to conclude – 10 months of exposure is not sufficient to detect significant changes in adenomas and adenocarcinomas

Neoplastic changes in the lung

Combined chronic toxicity and carcinogenicity study – A/J mice

- At the terminal dissection (18 months or 15 months), the incidence of all pre-neoplastic and neoplastic lesions together was very similar in all groups: 80%–95% in the female mice and 73%–90% in the males.
- The multiplicity of all lesions (survival adjusted) is higher in 3R4F smoke-exposed mice; THS exposure resulted in similar or lower multiplicity than Sham-exposed mice.

Multiplicity, all lesions: nodular hyperplasia, bronchioloalveolar adenoma and carcinoma, terminal dissection



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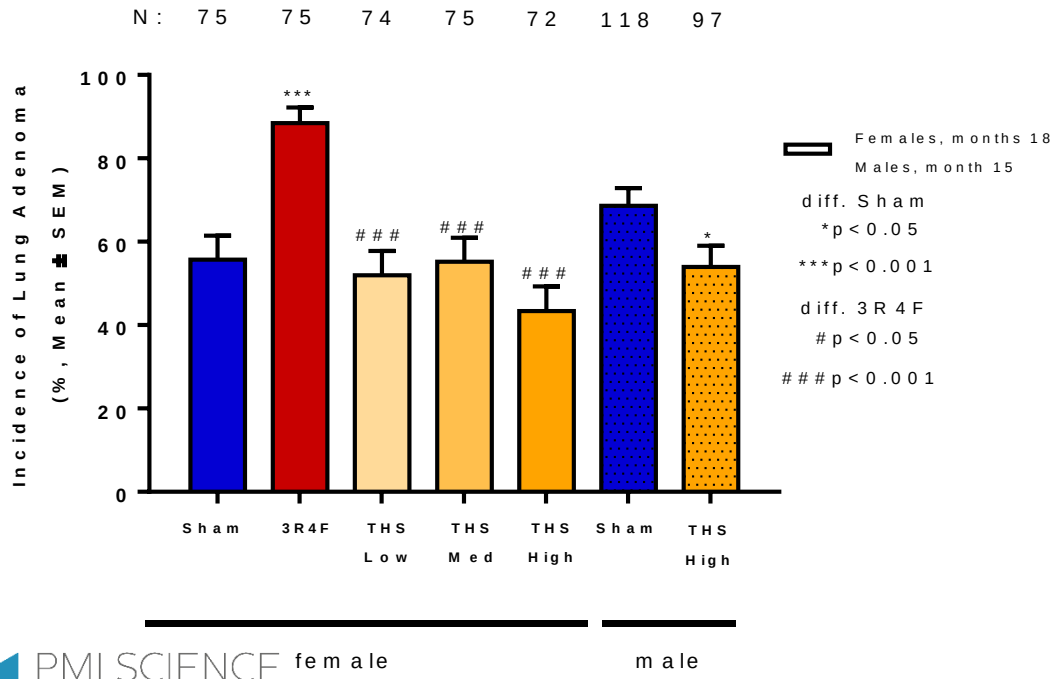
Neoplastic changes in the lung

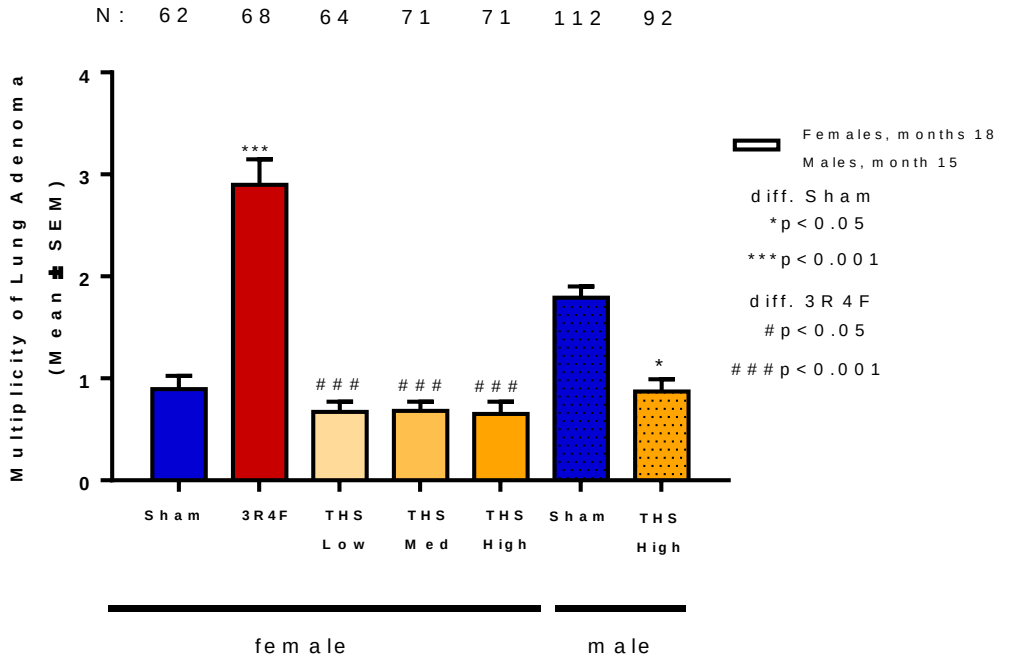
Combined chronic toxicity and carcinogenicity study – A/J mice

- At the terminal dissection, there was a lower incidence and multiplicity (survival-adjusted) of **bronchioloalveolar adenomatous** lesions in the THS aerosol-exposed mice than in 3R4F smoke-exposed mice.
- Incidence and multiplicity of **bronchioloalveolar adenomas** in THS-exposed mice was similar or lower than upon air exposure (Sham).

Incidence bronchioloalveolar adenoma



Multiplicity bronchioloalveolar adenoma

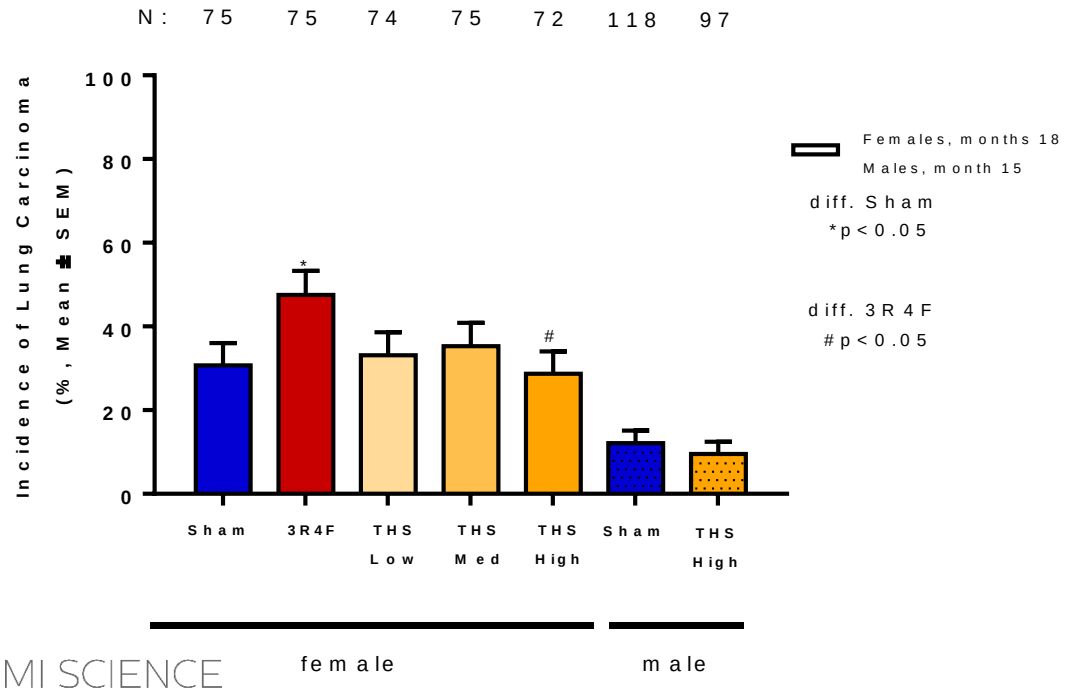


Neoplastic changes in the lung

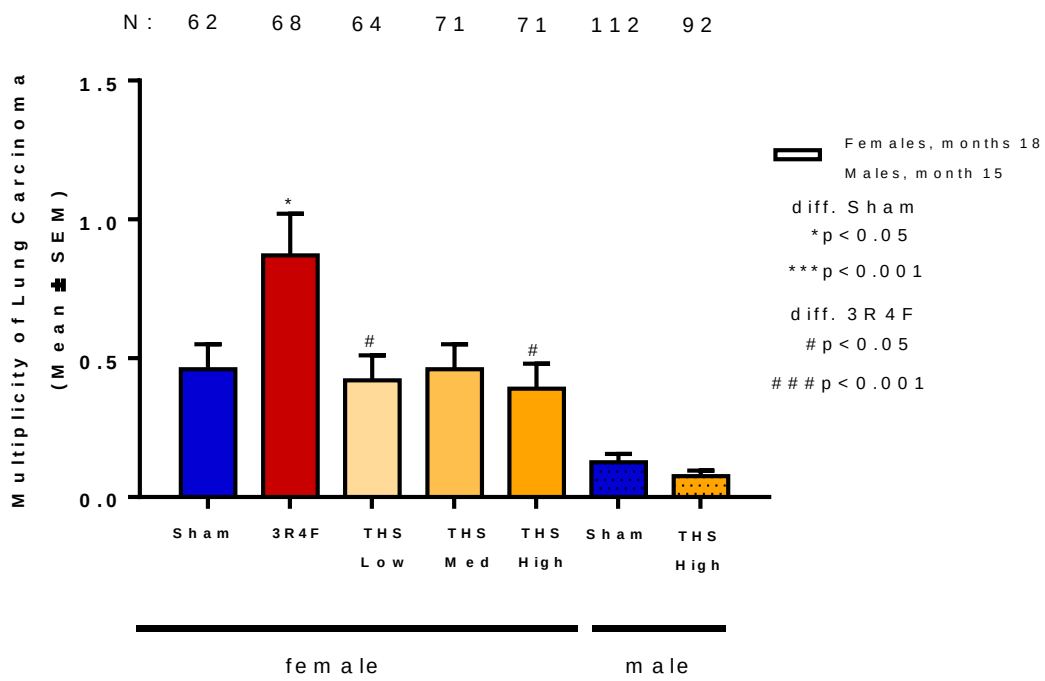
Combined chronic toxicity and carcinogenicity study – A/J mice

- At the terminal dissection, there was a lower incidence and multiplicity (survival adjusted) of **bronchioloalveolar malignant carcinomas** in the THS aerosol-exposed mice than in 3R4F smoke-exposed mice.
- Incidence and multiplicity of **bronchioloalveolar carcinomas** in THS-exposed mice was similar or lower than upon air exposure (Sham).

Incidence bronchioloalveolar carcinoma



Multiplicity bronchioloalveolar carcinoma



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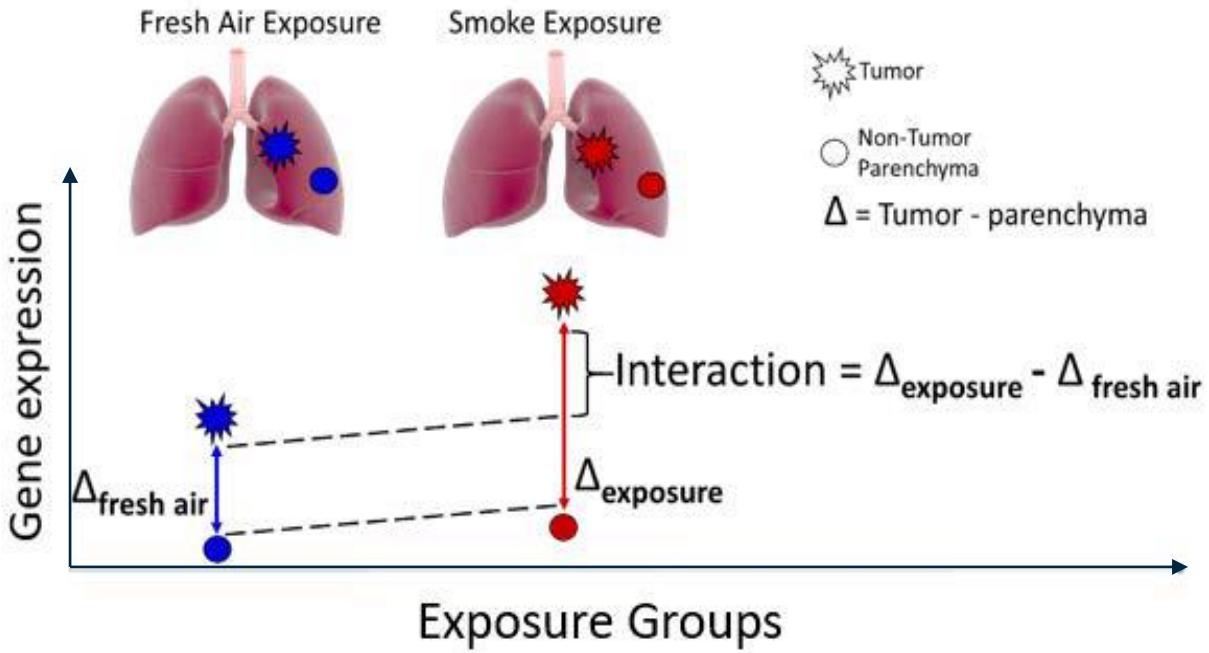
Neoplastic changes in the lung
Combined chronic toxicity and carcinogenicity study – A/J mice

- Tumor gene expression analysis – **tumor signature**

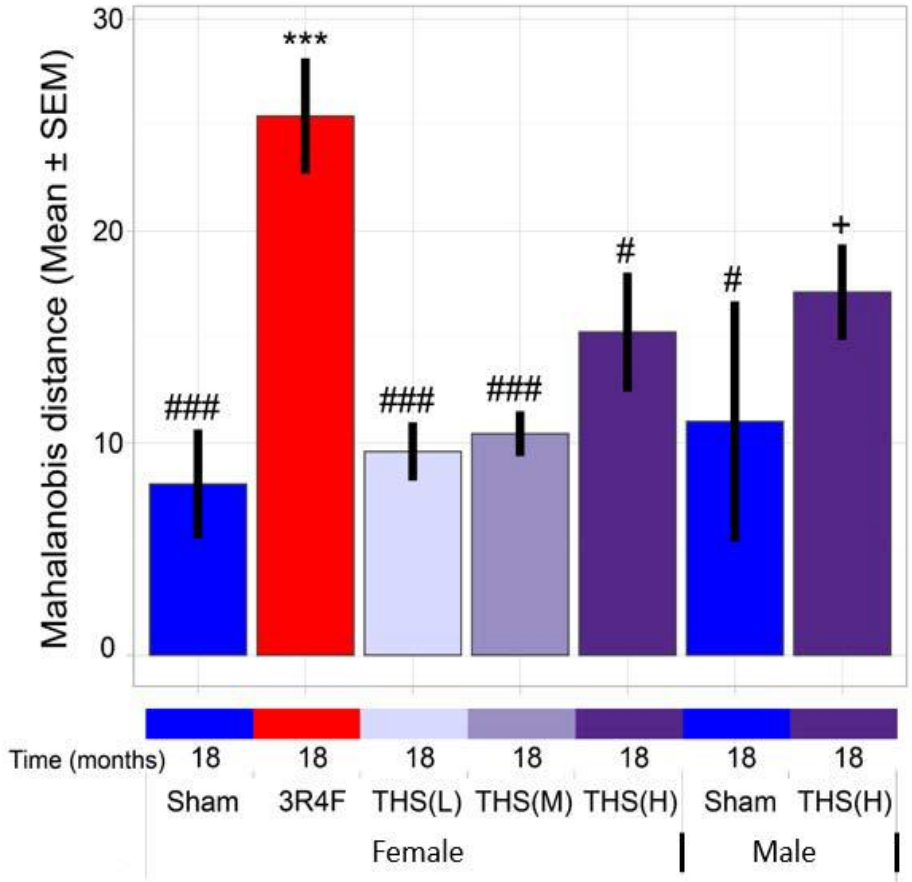


- **Δ**: difference in gene expression between excised tumor and parenchyma tissue
- Interaction defined as $\Delta_{exposure} - \Delta_{fresh\ air}$
- Interaction term estimates how differently genes behave in tumors of spontaneous vs. smoke-exposed mice
- 13 genes with the greatest interaction values were used for the gene signature*

Luettich et al., 2014, Systems toxicology approaches enable mechanistic comparison of spontaneous and cigarette smoke-related lung tumor development in the A/J mouse model, Interdiscip Toxicol. 2014 Jun; 7(2): 73–84, doi: 10.2478/intox-2014-0010

Neoplastic changes in the lung
Combined chronic toxicity and carcinogenicity study – A/J mice

- Tumors found in THS-exposed mouse lungs are spontaneous tumors



- Gene signature clearly distinguishes spontaneous tumors from smoke exposure tumors ($p<0.001$)
- All tumors found in THS aerosol-exposed mice were found to be more similar to spontaneous tumors than smoke-induced tumors

***: $p<0.001$ versus Sham (Fresh air); #: $p<0.05$; ###: $p<0.001$ versus 3R4F; +: Only 2 tumor samples found

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Summary and conclusions

Combined chronic toxicity and carcinogenicity study – A/J mice

- Reproducible exposure was achieved; target concentrations were met.
- **Systemic toxicity** changes reflect that stress-related effects and nicotine effects are less pronounced or absent in THS aerosol-exposed mice, even at twice the concentration of nicotine in the aerosol.
- No lung **inflammation** and **emphysematous changes** were observed in THS-exposed mice, even at twice the concentration of nicotine in the aerosol; clear inflammatory and emphysematous changes were observed upon 3R4F smoke exposure.
- No increased incidence and multiplicity were observed in **pre-neoplastic and neoplastic changes** in the lungs of THS-exposed mice, even at twice the concentration of nicotine in the aerosol; clear effects were observed upon 3R4F smoke exposure.


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