Materials and Methods

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  - Cigarette smoking
  - Cigarette mainstream smoke
  - Lung nodule incidence

- **Methods**
  - Transgenic Mouse Strains, rasH2 and p53
  - An Exploratory Inhalation Toxicity Study with Cigarette Mainstream Smoke in Two Transgenic Mouse Strains, rasH2 and p53

Background and Objective

The ILSI/HESI Collaborative Evaluation Program on Alternative Models for Carcinogenicity Assessment from 1996-2001 has demonstrated the feasibility of using short- or medium-term in vivo rodent test systems, such as transgenic and knockout animal models, in place of a second 2-year rodent bioassay (ILSI/HESI Alternatives to Carcinogenicity Testing Project, 2001).

Establishing reproducible and validated animal models for lung cancer induced by tobacco has proven difficult, despite the causality between lung tumors and cigarette smoking in humans (IARC, 2004). The ILSI/HESI Collaborative Evaluation Program, 2001).

We investigated the suitability of the transgenic mouse model expressing the human c-Ha-ras proto-oncogene (rasH2) and the heterozygous tumor suppressor p53 knockout mouse model (p53") for studying cigarette-mainstream smoke-induced carcinogenicity.

### Test Atmosphere Characterization

- **Diluted mainstream smoke (MS)**: from the University of Kentucky Reference Cigarette 2R4F
- **Dilution** to 240 µg total particulate matter (TPM)/l with conditioned fresh air, continuous flow of aerosol

### Results

#### Body Weight Development

- **Body weight development** during exposure period; not dose-related.

#### Lung Nodule Multiplicity

- **No statistically significant MS-mediated increases.**

#### Lung Nodule Incidence

- **No statistically significant MS-mediated effects.**

#### Urethane Treatment

- **Micronuclei** in peripheral blood induced in both strains, most pronounced 48 hours after urethane treatment.

#### Lung Nodule Incidence and Multiplicity in Urethane-Treated Mice

- **Increase in lung nodule incidence, but no statistically significant differences between sham and smoke groups.**

#### Histopathological Evaluation of Lungs

- **RasH2**: Presence of broncho-alveolar adenomas and carcinomas, irrespective of urethane treatment and time point.

#### Materials Generation

- Cigarette smoking according to ISO protocol (15 min puff in 240 µg TPM/l, each cigarette puffed once every minute, butt length 3 cm; Vanscheeuwijck et al., 2002).

#### Micro and Treatment


#### Body Weight and Organ Weight Data

- One way analysis of covariance (using pretreatment weights as covariates).

### Conclusion

- **MS did not induce lung tumors in rasH2 and p53" mice at the end of the 6-month exposure period or the 3-month post-exposure period.**

- **MS did not promote lung tumor development in urethane treated rasH2 and p53" mice at the end of the 6-month exposure period or the 3-month post-exposure period.**

- The findings are different from earlier observations by Stimm et al. (2006), who reported an increase in tumor multiplicity in A/J and SWR mice (with a slightly different exposure/post-exposure duration). The findings are different from earlier observations by Stimm et al. (2006), who reported an increase in tumor multiplicity in A/J and SWR mice (with a slightly different exposure/post-exposure duration).