Introduction and Objective

**Background:** Chronic exposure to cigarette smoke (CS) is the leading cause of chronic obstructive pulmonary disease (COPD) and lung cancer. The chronic inflammatory state observed in COPD is one contributing factor linking COPD and lung cancer development. Emphysema changes in the peripheral lung, which arise in the context of chronic inflammation and an impaired pro tease-anti-pro tease balance, may create a susceptible microenvironment and confer a growth advantage to lung epithelial cells. Impaired clearance of carcinogenic substances resulting from chronic airflow obstruction can also contribute to lung carcinogenesis. Candidate modified risk tobacco products (mRTTs) have been developed with the aim of reducing the level of harmful constituents in smoke from conventional cigarettes. However, the impact of mRTTs on disease development and the mechanisms linking chronic lung inflammation, COPD, and lung cancer development are largely unknown. The AU mouse is highly susceptible to lung tumor development and has been widely used as a screening system in carcinogenic testing, including that of CS (Streim et al., 2013).

**Objective:** This study was performed to evaluate and compare the impact of lifetime exposure to CS from the 3HR reference cigarette and aerosol from the Tobacco Heating System (THS) 2.2, a mRTT, on lung tumor incidence and multiplicity, the extent of lung inflammation and emphysema changes, and systemic toxicity in AU mice.

Study Design and Endpoints

Female AU mice were exposed to filtered air (Sham), to three concentrations of THS 2.2 aerosol (6.7, 13.4, 26.8 µL nicotine), and to one concentration of 3HR CS (13.4 µL nicotine) for six hours/day, five days/week, for 18 months (Figure 1). Additional male mice were exposed to Sham and THS 2.2 aerosol (6.7 µL nicotine) for 15 months. Care and use of the mice were in accordance with the National Advisory Committee for Laboratory Animal Research (NACSLAR) (2004). All animal experiments were approved by the Institutional Animal Care and Use Committee. Intraperitoneal injections of subgroups of female mice were performed after one, five, and 10 months of exposure. Terminal dissections were performed at Months 15 and 18 for the male and female mice, respectively. At selected time points, animals were allocated for the analysis of the endpoints: bronchoalveolar lavage fluid (BALF) analysis by flow cytometry and multi-analyte (cytokines/chemokines) profiling, histopathological evaluation of the lungs, lung function tests, lung morphology, lung tumor analysis, and an extensive systems toxicological analysis (transcriptomics, proteomics, DNA sequencing).

Aerosol Exposure and Uptake

The daily monitoring of aerosol components indicated that the aerosol/CS was generated and delivered to the inhalation chambers in a consistent manner, with mean nicotine test atmosphere concentrations very close to the target concentrations. Aerosol generated from THS 2.2 sticks contained lower concentrations of total particulate matter, carbon monoxide, and carbonyl compared with 3HR CS at the same nicotine concentration (Table 1).