Table 1. Biological system, items, and aerosol generation

	Phillips et al., 2016	Wong et al., 2020	Gu et al., 2023	Nitta et al., 2022	Wong et al., 2016	Qiu et al., 2023	Yoshida et al., 2020
Animal model	ApoE	A/J	C57BL/6 J	C57BL/6J	Sprague Dawley rats	Sprague-Dawley rats	Pregnant CD-1 mice
Sex	Female	Male and female	Male	Male	male and female	male and female	Female
Reference	3R4F	3R4F	5 Marlboro Red cigarettes	Peace nonfilter cigarettes	3R4F	Marlboro Red tobacco cigarettes	3R4F
Test item	THS2.2	THS 2.2 HeatStick	5 Marlboro heatsticks	Marlboro IQOS HeatStick Regular	THS2.2	IQOS	IQOS
Smoke/aerosol generation	30-port rotary smoking machine	30-port rotary smoking machine	Not clear	Cigarette smoke exposure system for small animals (Model SIS-CS)	30-port rotary smoking machine	Pulsatile smoke/aerosol	Tobacco smoke (TS) generator (SG-300, Shibata Scientific Technology, Tokyo, Japan)
Puffing reference item	to a butt length of 34.1 6 0.7 mm to 35.4 6 1.2 mm, with the resulting puff count averaging 10.5 puffs per stick	puff duration (2 s), puff frequency (one puff every 30 s), and closed ventilation holes	8 puffs/cigarette, 2 s puff duration, and 30 s interpuff interval	six puffs/min, and 3.5% CS diluted with compressed air.	puff duration (2 s), puff frequency (one puff every 30 s),	No details provided	2 s puff duration, 30 s puff interval, and 10 puffs/ heat stick or cigarette
Puffing test item	12 puffs	puff duration (2 s), puff frequency (one puff every 30 s), and closed ventilation holes	12 puffs/ heatstick, 2 s puff duration, and 30 s interpuff interval	six puffs permin, and 3.5% IQOS aerosol diluted with compressed air	puff duration (2 s), puff frequency (one puff every 30 s),	No details provided	2 s puff duration, 30 s puff interval, and 10 puffs/ heat stick or cigarette

(Table continues)

Puff volume	55-ml	55 ml	55 mL	15-mL stroke volume	55 ml	No details provided	55 mL
Regimen	Health Canada Intensive Smoking Protocol based on ISO standard 3308 (revised in 2000)	Health Canada Intensive Smoking Protocol	Health Canada Intense standard	N/A	Health Canada Intensive Smoking Protocol based on ISO standard 3308	No details provided	Health Canada Intense (HCI)

Table 2. Exposure

	Phillips et al., 2016	Wong et al., 2020	Gu et al., 2023	Nitta et al., 2022	Wong et al., 2016	Qiu et al., 2023	Yoshida et al., 2020
Exposure	Whole body	Whole body	Whole body	Nose only (?)	Nose-only	Nose-only	Whole body
Reference item smoke	600 mg TPM/m3, equivalent to 29.9 mg nicotine/m3 (µg/L)	Nicotine 13.4 µg/L	Not evaluated	Not evaluated	8, 15 and 23 µg nicotine/l	Not evaluated	Not evaluated
Test item aerosol	nicotine-matched to 3R4F, 29.9 mg/ m3	Nicotine 6.7 µg/L, 13.4 µg/L, and 26.8 µg/L	Not evaluated	Not evaluated	15, 23, and 50 µg nicotine/l	Not evaluated	Not evaluated
Exposure duration	3 h per day, 5 days per week, for up to 8 months (Intermittent daily exposure to fresh filtered air for 30 min after the first hour of smoke exposure and for 60 min after the second hour of exposure for 3R4F group to avoid a buildup of excessive COHb	6 h/day, 5 days/week, and for up to 18 months.	30 min, twice/day (4–6 h between), 5 days/week, for 24 weeks	30 min/day for 5 days/week over 6 mo.	6 h per day for 90 days with 42 day recovery	5 days per week for 2 months, 1 session per day, with each session consisting of 10 cycles spread over 5 minutes, to approximate the consumption of a single cigarette or a single vaping session	four heat stick/cigarettes a day with 20-min smoke exposures on days 7 and 14 of gestation

TPM, total particulate matter; COHb, carboxyhemoglobin

Table 3. Exposure monitoring

	Phillips et al., 2016	Wong et al., 2020	Gu et al., 2023	Nitta et al., 2022	Wong et al., 2016	Qiu et al., 2023	Yoshida et al., 2020
Atmosphere in exposure chambers	Flow rate, temperature, relative humidity, concentration and particle size of TPM, and the concentrations of CO, formaldehyde, acetaldehyde, and acrolein, PSD	Particle/droplet size distribution and TPM, nicotine, CO, formaldehyde, acetaldehyde, and acrolein concentrations	Not evaluated	Not evaluated	TPM, CO, nicotine, formaldehyde, acetaldehyde, acrolein, and PSD	Not evaluated	Not evaluated
Animal monitoring	daily basis, body weight weekly, and exposure parameters (COHb in blood and nicotine metabolites in urine) 3 times during the study	in-life monitoring	Not evaluated	Body weight monthly	Body weights, food consumption, ophthalmoscopy, and health status	Not done	Not evaluated
Exposure biomarkers	Plasma levels of nicotine and cotinine, blood COHb, urinary biomarkers of CS exposure, oxidative stress, and inflammation: HPMA, S-phenylmercapturic acid, CEMA, NNAL, 4-HNE, MDA, tetranor PGE-M, 2,3-dinor-8- iso-PGF2a, 8-iso-PGF2a, 2,3-dinor-TBX2, 11-dehydro-TBX2, and 12-hydroxyeicosatetraenoic acid	blood and urine biomarkers of exposure	Not evaluated	Serum cotinine	carboxyhemoglobin in blood, and nicotine metabolites HOCOT, NCOT, cotinine, NNO, NNIC) in 24-h urine other metabolites of aerosols constituents: (HPMA, NNAL, SPMA and CEMA)	Not evaluated	Not evaluated
Hematology and clinical chemistry			Not evaluated	Not evaluated	Plasma and serum PT and APTT	Not evaluated	Not evaluated

TPM, total particulate matter; PSD, particle size distribution; CO, carbon monoxide; HPMA, hydroxypropylmercuric acid; CEMA, 2-cyanoethylmercapturic acid; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; HOCOT, trans-30-hydroxycotinine; NCOT, norcotinine; NNO, nicotine-N'-oxide; NNIC, nornicotine; SPMA, S-phenylmercapturic acid; PT, prothrombin time APTT, activated partial thromboplastin time.

Table 4. Respiratory endpoints

	Phillips et al., 2016	Wong et al., 2020	Gu et al., 2023	Nitta et al., 2022	Wong et al., 2016	Qiu et al., 2023	Yoshida et al., 2020
Lung function	Flexi Vent rodent ventilator system	flexiVent FX equipment and Flexiware v7	FlexiVent FX system	FlexiVent system	Respiratory physiology	Not evaluated	Not evaluated
Lung lavage	Absolute number of free lung cells, alveolar macrophages, dendritic cells, neutrophils, and lymphocytes, concentrations of inflammatory mediators	Total free lung cell, lymphocytes, macrophages, and neutrophils, inflammatory mediators, MMP activity.	Markers of inflammation, MDA and SOD activities	Total cell, macrophage, neutrophil, and lymphocyte numbers and percentages	60 selected proteins (RodentMAP®v3 .0;) in supernatant, number and viability of the free lung cells alveolar macrophages, neutrophils, lymphocytes, and eosinophils	Not evaluated	Not evaluated
Histopathology	left lung (serial sections), nose (3 representative levels, L1, L2, and L4), and liver was performed in a blinded fashion by a board-certified veterinary pathologist	microscopy and scanned digital slides evaluted by study pathologist in a blinded manner	Not evaluated	Not evaluated	According to the OECD 413 specifications.	Not evaluated	Not evaluated
Morphometry	DI, MCL, BAs	DI, MCL, BAs	MLI	MLI and DI		Not evaluated	Not evaluated

(Table continues)

Other	Lung volume, Transcriptomics	Total lung volume, total volume of air total alveolar duct air volume volume-weighted mean volume of alveoli, alveolar surface density in septum, total number of alveoli	collagen deposition around the airways, E-cadherin (α-SMA) immunohistochemistry, MDA and SOD activities in serum and lung homogenates	Apoptosis, apoptosis-related proteins, lung transcriptomics	Not evaluated	Not evaluated	Not evaluated

MCL, mean chord length; DI, destructive index; BAs, Bronchiolar attachments; MLI, mean linear intercept; α-SMA, α-smooth muscle actin; MDA, malondialdehyde; SOD, superoxide dismutase

Table 5. Cardiovascular endpoints

	Phillips et al., 2016	Wong et al., 2020	Gu et al., 2023	Nitta et al., 2022	Wong et al., 2016	Qiu et al., 2023	Yoshida et al., 2020
Morphometry	Aortic arch plaque formation (confirmed with micro-CT)	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Heart analysis	Not evaluted	Not evaluated	Not evaluated	Not evaluated	Histopathology according to OECD guidelines	Microvessel density and area percentage from subepicardium, midmural, and subendocardium for each section to calculate mean optical area in heart	Not evaluated
Other	Lipidomics of aortic arches and plasma	Not evaluated	Not evaluated	Not evaluated	Not evaluated	SBP, echo-cardiography, electrocardiographic telemetry, arrhythmia inducibility testing, and opticalmapping	Not evaluated

SBP, systolic blood pressure

Table 6. Other endpoints

	Phillips et al., 2016	Wong et al., 2020	Gu et al., 2023	Nitta et al., 2022	Wong et al., 2016	Qiu et al., 2023	Yoshida et al., 2020
Reprotox	Not evaluated	Histopathology of non-respiratory tract organs	Not evaluated	Not evaluated	Histopathology of reproductive organs	Not evaluated	Sperm characteristics and daily sperm production, serum hormones
General and systemic effects	Histopathology of liver and kidney	Histopathology of all organs	Markers of inflammation in serum	Not evaluated	Necropsy, gross pathology and organ weights	Not evaluated	Not evaluated