APPENDIX A6.1.3.26 – BOEXP NON-CLINICAL RESULTS

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6.1.3.26 Summary of Results for Non-Clinical Studies

6.1.3.26.1 Introduction

Although the main purpose of determining BoExp in non-clinical studies was to demonstrate proper exposure of the animals to cigarette smoke or THS aerosol, the exposure results in the non-clinical studies add evidence to the demonstration of exposure reduction and supplements the the clinical results.

In the non-clinical studies performed to assess standard toxicology and systems toxicology endpoints in inhalation studies, COHb and urinary nicotine metabolites have been used for many years to verify appropriate exposure levels to the particulate and gas vapor phase of aerosols. When studying the effects of THS aerosol compared with those elicited by cigarette smoke, the BoExp were augmented with the primary BoExp used in the clinical program. As shown in this section, the animals exposed to 3R4F show a dose response to these markers in line with the HPHC concentrations in smoke, while animals exposed to THS aerosol show a much reduced exposure across all exposure concentrations, except for exposure to nicotine, which is for some exposure groups matched to the exposure concentrations of the reference cigarette 3R4F. In study 15025, rats were exposed aerosol from a mentholated THS as well as to smoke form mentholated reference cigarettes. Therefore, menthol and one of its metabolites has also been investigated, to demonstrate proper exposure to the aerosol from the mentholated products.

Table 1 shows an overview of the studies discussed in this section.

BoExp to selected HPHCs were assessed in 2 non-clinical safety toxicology studies conducted in rats according to the Organization for Economic Co-operation and Development (OECD) Testing Guideline 413 (OECD (Organization for Economic Co-operation and Development) 2009), as well as in 2 longer-term studies conducted in mice. The first mouse study was designed to assess the harm reduction potential of THS in an animal model of both cardiovascular disease and COPD using Apoe$^{-/-}$ mice. The second study was conducted in an animal model of lung cancer and COPD using A/J mice. Study reports for these studies can be found in [section 7.2].

The subset of BoExp used in the non-clinical studies were selected from the list used in the clinical studies and are indicated below. As availability of toxicokinetic data in rodent models is very limited and the availability of sufficient samples quantity is limited, 18-h or 24- h urine was used for the analysis of (water soluble) metabolites of the following smoke constituents:

- 3-HPMA for acrolein
- S-PMA for benzene
- CEMA for acrylonitrile
- Total NNAL for NNK.

Urine was collected during the 6h-exposure period and during 16h after completion of the 6h-exposure period for the 2 rat studies (15006 and 15025). Urine samples collected during

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exposure and post-exposure were combined per rat to create the 24-h samples. In study 15006, urine collection occurred 3 times during week 2, week 4-5 and week 12-13; whereas in study 15025, urine was collected only once during the study (week 12).

The same BoExp were assessed in an 8-month Apoe$^{-/-}$ mouse inhalation study (study 15015) (Phillips 2016). Urine was not collected during the exposure phase due to technical limitations; however, post-exposure 18-h urine was collected from female mice during the exposure months 2, 3, 6 and 8. Furthermore these BoExp were also measured in 24-h urine collected during months 1-2, 5-6, and 8-9 in the A/J mouse study (study 15020).

In addition to these urine markers, blood COHb was also assessed in the rat and mouse studies. Blood was collected during the last hour of the daily exposure period at different time points during the studies. In the studies 15006 and 15025, blood was collected once during the exposure period during week 7. In the mouse study 15015, blood was collected during month 2, 4 and 7, whereas in the A/J mouse study 15020, blood was drawn from the mice during month 3 and 7.

Finally, Menthol and its metabolite M-I (p-menthane-3,8-diol) were analyzed in the blood from THS Menthol (THSM)- and mentholated reference cigarette (MRC)-exposed rats in order to verify exposure levels of menthol.

Table 1 Overview of Non-Clinical Studies Performed

Study	Study Number	Type	Design	Study report
Rat OECD THS2.2	15006	*In vivo*	TG413 (air (sham), 3R4F [a], THS2.2)	15006
Rat OECD THS2.2M	15025	*In vivo*	TG413 (air (sham), 3R4F, MRCL[b] and MRCH[b], THS2.2M)	15025
ApoE THS2.2	15015	*In vivo*	Parallel-group, switching (air (sham), 3R4F[a], THS2.2, cessation, switching)	15015_CVD_Resp_ApoE_SW_SR
A/J THS2.2	15020	*In vivo*	TG453 (air (sham), 3R4F, THS2.2)	15020

[a] 3R4F reference cigarette serves as an international standard for research purposes and provides a basis for comparing data collected in various laboratories (Chepiga 2000). It is the third production run and is considered to be representative of the US market cigarettes.

[b] Mentholated reference CCs produced by PMI, with the non-mentholated 3R4F reference cigarette specifications. MRC L was designed to yield 1.2 mg menthol/stick and MRC H to yield 2.4 mg menthol/stick (under ISO smoking regimen).

In the summary section, 6.1.3.26.2, a representative subset of BoExp data from the rat and mouse studies is shown (not from all collection time points), along with the ratios of these BoExp measured in animals exposed to THS aerosols to those in animals exposed to 3R4F

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smoke or mentholated reference cigarettes. This representation of the data allows to reflect the reduced levels of HPHCs in animals exposed THS aerosol compared with those exposed to smoke, and are consistent with the reduced exposure that has been found in the clinical studies.

Detailed data per BoExp for the rat and mouse studies are presented in the section 6.1.3.26.3, d.

6.1.3.26.2 Summary of BoExp Assessment in Non-Clinical Studies – THS vs. 3R4F

Table 2 shows a representative subset of the BoExp data obtained from 24-h urine in the 2 rat studies (male rats only, urine collected during week 5 for study 15006 and during week 10 for study 15025) as well as blood COHb (male rats only). Levels of BoExp were all strikingly lower in animals exposed to THS aerosol (menthol or regular) compared with those exposed to the smoke of reference cigarettes.

Table 3 shows the reductions for these BoExp when rats are exposed to THS (THS Regular or THS Menthol) relative to cigarette smoke (3R4F, or L or High menthol versions of mentholated reference cigarettes, i.e., MRCL and MRCH). Levels of BoExp were reduced by 56% to 98%, and from 74% to 99% when the values of the Sham-expsoed animals were subtracted.

Table 4 shows the BoExp data obtained from the 18-h urine and blood of female Apoe$^{-/-}$ mice at months 2 and 8. The data shows that switching mice to fresh air (cessation) following 2 months of exposure to 3R4F smoke leads to BoExp levels that are similar to those observed in the air-exposed mice (sham). Similarly, switching mice from 3R4F smoke to THS aerosol after 2 months leads to BoExp levels that are similar to continuous THS exposure, and approach the levels found in the cessation arm of the study. Exposure reductions are also indicated. Levels of BoExp were reduced by 48% to 100% in mice exposed to THS compared with those of animals exposed to the reference cigarettes. These reductions ranged from 86% to 100% when mice were switched from reference cigarettes to fresh air (cessation) and from 82% to 96% when they were switched to THS.

Table 5 shows the BoExp data in 24-h urine and blood of A/J mice for the urine collection time points at months 1-2 and 8-9, as well as the COHb data for blood collected at months 3 and 7. The reductions in BoExp are also shown. Levels of BoExp were reduced by 57% to 99% in mice exposed to fresh air compared with those exposed to the reference cigarettes, and by 61% to 99% in those exposed to THS.

In these non-clinical studies, consistent reductions of the urinary and blood BoExp is found in THS-exposed animals when compared with 3R4F-exposed animals. In a "switching" study, these levels of reductions were found to be comparable in animals being switched from the reference cigarettes to THS and those undergoing "smoking cessation (i.e. animals switched from the reference cigarettes to fresh air). These reductions reflect the reduced levels of HPHCs in THS aerosol compared with those found in cigarette smoke (-section

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6.1.1 Aerosol Chemistry), and are consistent with what has been observed in the clinical studies (section 6.1.3.2 Summary of BoExp Assessment).

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Table 2 Concentrations of BoExp to Selected HPHCs in Non-Clinical Studies – 90-Day Inhalation Studies (15006, 15025)

BoExp	HPHC	Study	Concentrations (mean ± SEM)				
			Sham	THS	3R4F	MRCL	MRCH
3-HPMA (ng/ml)	Acrolein	15006 (THS Reg)	2610.6 ± 276.8	4035.8 ± 355.3	13213.8 ± 1199.8	-	-
		15025 (THS Ment)	2932.3 ± 307.2	5235.5 ± 794.5	12098.7 ± 1911.9	13058.7 ± 1486.1	13297.7 ± 2557.8
S-PMA (ng/ml)	Benzene	15006 (THS Reg)	3.07 ± 0.31	5.02 ± 0.37	309.90 ± 38.73	-	-
		15025 (THS Ment)	3.81 ± 0.46	7.10 ± 0.56	478.13 ± 53.94	505.35 ± 62.06	610.83 ± 89.85
CEMA (ng/ml)	Acrylonitrile	15006 (THS Reg)	4.06 ± 0.47	8.69 ± 0.71	494.19 ± 38.32	-	-
		15025 (THS Ment)	5.49 ± 0.56	12.15 ± 1.07	802.28 ± 114.60	715.62 ± 112.70	765.21 ± 111.39
NNAL (pg/ml)	NNK	15006 (THS Reg)	38.35 ± 3.41	46.61 ± 6.10	412.00 ± 36.10	-	-
		15025 (THS Ment)	0.85 ± 0.00	35.05 ±8.35	715.45 ±95.44	509.21 ± 89.30	610.39 ± 93.20
COHb (%)	CO	15006 (THS Reg)	3.5 ± 0.2	4.0 ± 0.2	26.5 ± 1.6	-	-
		15025 (THS Ment)	3.7 ± 0.3	3.7 ± 0.1	27.0 ± 0.8	22.3 ± 0.8	26.5 ± 0.7

Data shown for sham and for exposure at 23 µg nicotine / L test atmosphere, 24-h urine or blood (COHb only), male rats. Urinary BoExp levels were assessed at week 5 in study 15006 and week 10 in study 15025, while COHb was assessed at week 7 for both studies.

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Table 3 Reduction of BoExp to Selected HPHCs in Non-Clinical Studies – 90-Day Inhalation Studies (15006, 15025)

BoExp	HPHC	Study	Ratios	Ratio (%)	Ratio (%) – Sham exposure value subtracted
3-HPMA	Acrolein	15006 (THS Reg)	THS:3R4F	30.5	13.4
		15025 (THS Ment)	THS:3R4F	43.3	25.1
			THS:MRCL	40.1	22.7
			THS:MRCH	39.4	22.2
S-PMA	Benzene	15006 (THS Reg)	THS:3R4F	1.6	0.6
		15025 (THS Ment)	THS:3R4F	1.5	0.7
			THS:MRCL	1.4	0.7
			THS:MRCH	1.2	0.5
CEMA	Acrylonitrile	15006 (THS Reg)	THS:3R4F	1.8	0.9
		15025 (THS Ment)	THS:3R4F	1.5	0.8
			THS:MRCL	1.7	0.9
			THS:MRCH	1.6	0.9
NNAL	NNK	15006 (THS Reg)	THS:3R4F	11.3	2.2
		15025 (THS Ment)	THS:3R4F	4.9	4.8
			THS:MRCL	6.9	6.7
			THS:MRCH	5.7	5.6
COHb	CO	15006 (THS Reg)	THS:3R4F	15.1	2.2
		15025 (THS Ment)	THS:3R4F	13.7	0.2
			THS:MRCL	16.5	0.2
			THS:MRCH	13.9	0.2

Data shown for 23 µg nicotine / L test atmosphere, 24-h urine or blood (COHb only), male rats

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Table 4 Concentrations and Reductions (Ratios, in %) of BoExp to Selected HPHCs in Non-Clinical Studies – Apoe$^{-/-}$ Study (15015)

BoExp (Units)	HPHC	Months of exposure	Ratio	Concentrations (mean ± SEM)				
				Sham	THS	3R4F	Cessation	Switching
3-HPMA (ng/ml)	Acrolein	2	Concentration	1289.3 ± 171.53	1175.8 ± 165.4	2513.3 ± 180.7	-	-
			*X:3R4F**	*51.3 %*	*46.8 %*	-	-	-
		8	Concentration	1823.0 ± 272.5	2860.7 ± 427.2	4339.4 ± 445.7	606.8 ± 214.5	765.9 ± 270.8
			*X:3R4F**	*4.2 %*	*65.9 %*	-	*14.0 %*	*17.6 %*
S-PMA (ng/ml)	Benzene	2	Concentration	0.88 ± 0.13	0.98 ± 0.30	35.02 ± 5.3	-	-
			*X:3R4F**	*2.5 %*	*2.8 %*	-	-	-
		8	Concentration	1.59 ± 0.26	3.46 ± 0.46	71.12 ± 12.80	1.94 ± 0.15	3.21 ± 0.36
			*X:3R4F**	*1.4 %*	*4.9 %*	-	*2.7 %*	*4.5 %*
CEMA (ng/ml)	Acrylonitrile	2	Concentration	0.34 ± 0.10	1.61 ± 0.45	133.11 ± 20.83	-	-
			*X:3R4F**	*0.2 %*	*1.2 %*	-	-	-
		8	Concentration	0.02 ± 0.00	6.94 ± 1.14	215.71 ± 39.04	0.79 ± 0.51	6.67 ± 1.30
			*X:3R4F**	*0.0 %*	*3.2 %*	-	*0.0 %*	*3.1 %*
Total NNAL (pg/ml)	NNK	2	Concentration	0.27 ± 0.02	10.21 ± 6.50	119.34 ± 12.41	-	-
			*X:3R4F**	*0.2*	*8.6*	-	-	-
		8	Concentration	0.66 ± 0.07	3.42 ± 2.71	154.75 ± 14.05	0.51 ± 0.04	20.67 ± 14.05
			*X:3R4F**	*0.4 %*	*2.2 %*	-	*0.3 %*	*13.4 %*
COHb (%)	CO	2	Concentration	4.9 ± 0.0	5.8 ± 0.1	27.8 ± 2.7	-	-
			*X:3R4F**	*17.6 (0) %*	*20.9 (3.9) %*	-	-	-
		8	Concentration	5.4 ± 0.1	6.0 ± 0.1	33.5 ± 2.0	5.15 ± 0.1	6.58 ± 0.1
			X:3R4F (#)*	*16.1 (0) %*	*17.9 (2.1) %*	-	*15.4 (0) %*	*19.6 (4.2) %*

Exposure to air (sham, cessation) or 29.9 µg nicotine / l test atmosphere (THS, 3R4F, switch), 18-h urine (post-exposure) or blood (COHb only), female mice.

* Ratios of amounts in the respective columns and the 3R4F as indicated in the respective columns; #values between brackets represent ratios, with subtraction of the sham value (only done for COHb – in urine the variance is high due to the use of 18-h urine)

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Table 5 Concentrations and Reductions (Ratios, in %) of BoExp to Selected HPHCs in Non-Clinical Studies – A/J study (15020)

BoExp (Units)	HPHC	Months of exposure	Ratio	Concentrations (mean ± SEM)		
				Sham	THS	3R4F
3-HPMA (ng/ml)	Acrolein	1-2	Concentration	4558 ± 571	4135 ± 590	10668 ± 1703
			*X:3R4F**	*42.7 %*	*38.8 %*	-
		8-9	Concentration	4343 ± 483	2862 ± 547	9350 ± 1582
			*X:3R4F**	*46.4 %*	*30.6 %*	-
S-PMA (ng/ml)	Benzene	1-2	Concentration	2.53 ± 0.30	7.16 ± 0.96	400.90 ± 62.02
			*X:3R4F**	*0.6 %*	*1.8 %*	-
		8-9	Concentration	5.25 ± 0.94	6.11 ± 0.40	271.90 ± 42.83
			*X:3R4F**	*1.9 %*	*2.2 %*	-
CEMA (ng/ml)	Acrylonitrile	1-2	Concentration	5.03 ± 0.42	13.9 ± 1.81	998.6 ± 150.8
			*X:3R4F**	*0.5 %*	*1.4 %*	-
		8-9	Concentration	6.81 ± 0.81	10.58 ± 0.98	1061.0 ± 190.2
			*X:3R4F**	*0.6 %*	*1.0 %*	-
NNAL (pg/ml)	NNK	1-2	Concentration	13.34 ± 4.68	79.04 ± 8.16	2674.0 ± 335.5
			*X:3R4F**	*0.5*	*3.0*	-
		8-9	Concentration	9.76 ± 0.09	67.78 ± 17.6	1686.0 ± 302.5
			*X:3R4F**	*0.6 %*	*4.0 %*	-
COHb (%)	CO	3	Concentration	3.4 ± 0.0	4.2 ± 0.1	32.3 ± 0.9
			*X:3R4F**	*10.5 (0)*	*13.0 (2.7)*	-
		7	Concentration	3.5 ± 0.0	3.9 ± 0.1	29.0 ± 1.3
			X:3R4F (#)*	*12.1 (0) %*	*13.4 (1.6) %*	-

Exposure to air (sham, cessation) or 13 µg nicotine / l test atmosphere (THS, 3R4F), 24-h urine or blood (COHb only), female mice.

* Ratios of amounts in the respective columns and the 3R4F as indicated in the respective columns; #values between brackets represent ratios, with subtraction of the sham value (only done for COHb – in urine the variance is higher due to difficult sample collection).

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6.1.3.26.3 Detailed description of BoExp in the non-clinical studies

6.1.3.26.3.1 Nicotine metabolites

6.1.3.26.3.1.1 Nicotine Metabolites in Rat Studies

Nicotine metabolites were quantified in the 24h urine collected from rats from two 90-day *in vivo* inhalation studies (THS Regular, i.e., non-menthol; THSR), study 15006 and THS Menthol (THSM), study 15025). The urine was collected during the 6h-exposure period and during 16h after completion of the 6h-exposure period. Urine samples collected during exposure and post-exposure were combined per rat to create the 24-h samples. In study 15006 urine collection occurred three times at week 2, week 4-5 and week 12-13 (only week 4-5 shown here) whereas in study 15025 urine were collected only once during the study (week 12).

As shown in previous rat studies (Phillips 2015), a linear correlation between the total amount of the selected nicotine metabolites (cotinine, nicotine-N'-oxide, nornicotine, norcotinine and 3'-hydroxycotinine) excreted and the nicotine concentrations in test atmosphere has been observed (Figure 1). At equal nicotine exposure concentration, the total amount of nicotine metabolites excreted was slightly higher in the THS-exposed rats than in the 3R4F-exposed rats (Figure 1 and Figure 2).

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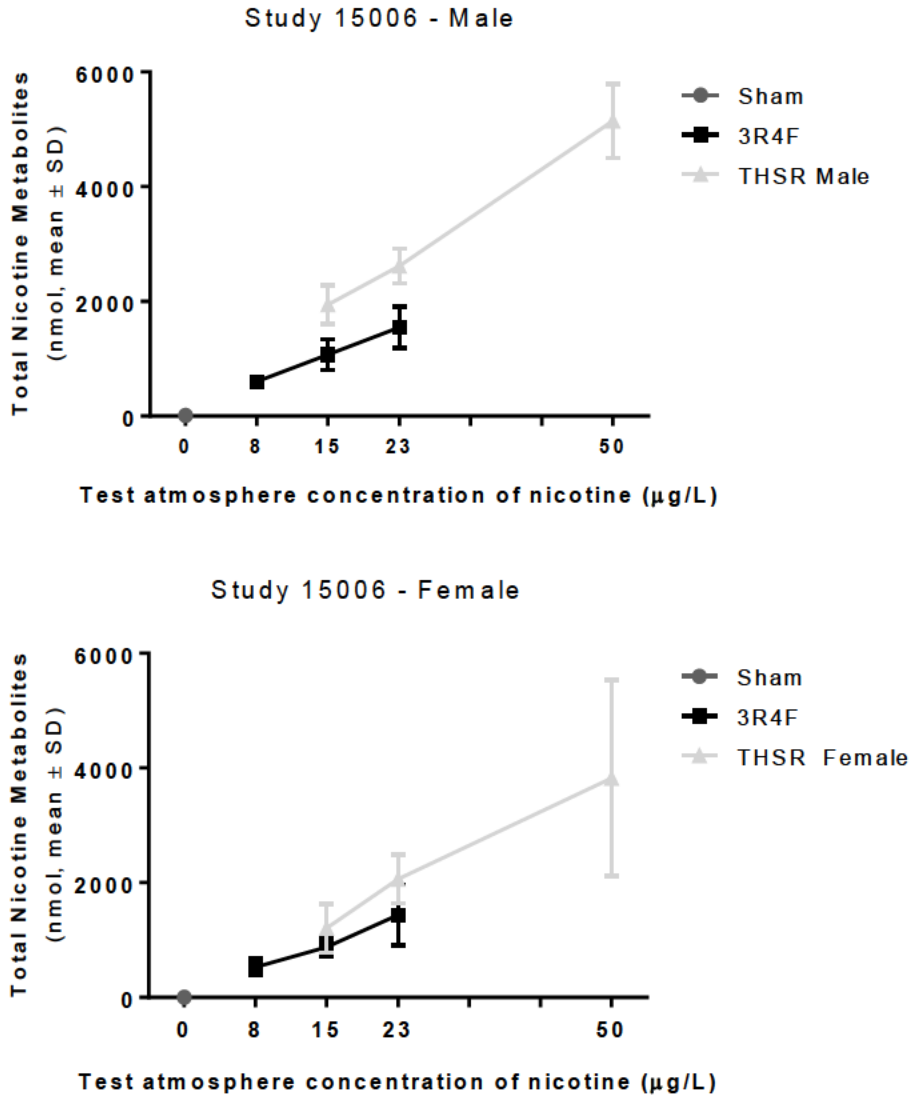


Figure 1 **Total Urine Nicotine Metabolites vs. the Nicotine Concentration in the Test Atmosphere (Week 4-5 of Exposure)**

Results are presented as mean and standard deviation, in male (top panel) and female (bottom panel) rats.

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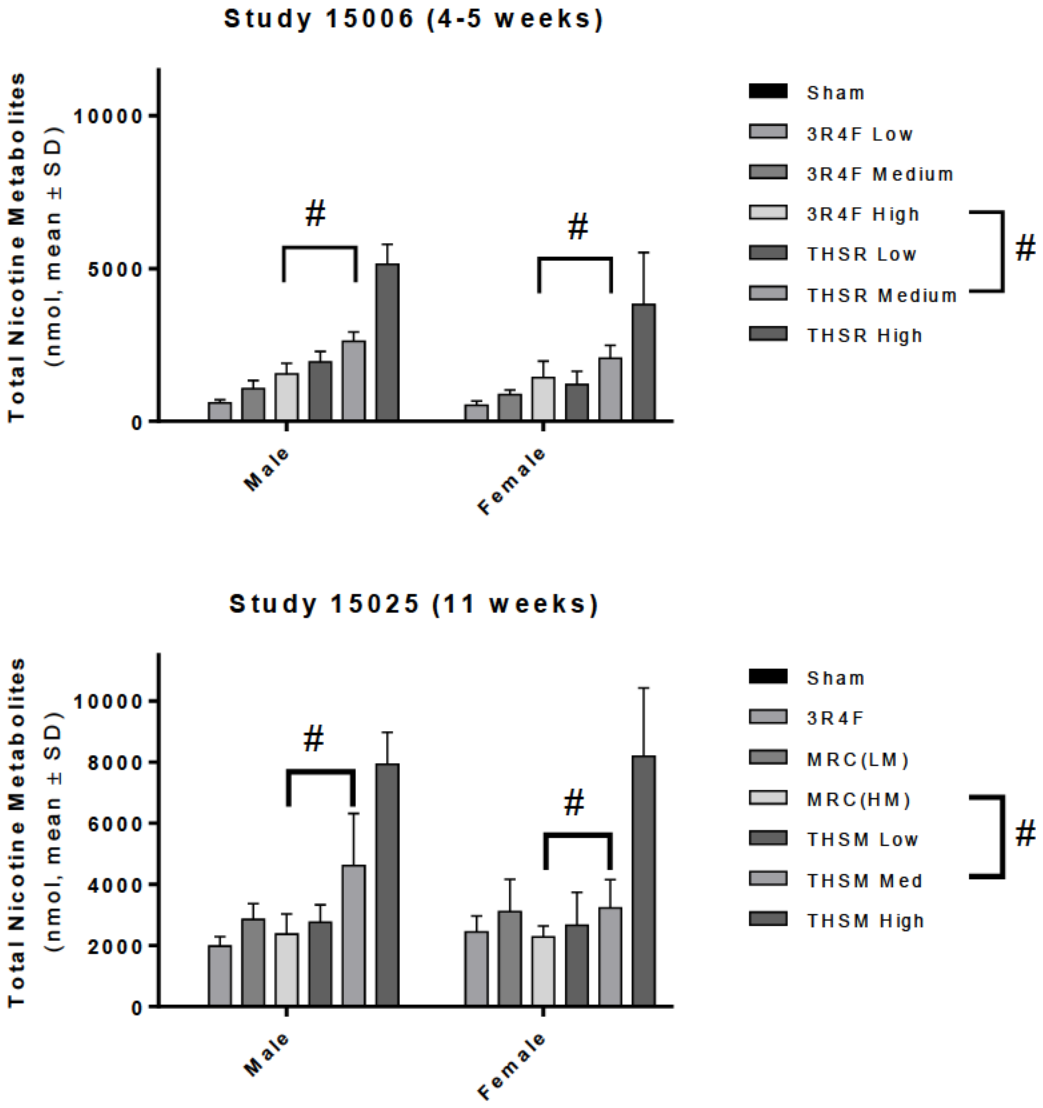


Figure 2 Total Urine Nicotine Metabolites in THSR (15006) and THSM (15025) Studies.

Nicotine metabolites were analyzed from samples collected during week 4-5 in study 15006 study and during week 12 in study 15025. Results are presented as mean and standard deviation. # = Equivalent test atmosphere concentration of nicotine.

The overall higher uptake of nicotine in the THS-exposed rats is most likely related to the lower irritant potential of the THS aerosol than of the mainstream smoke from the reference items (3R4F or Mentholated Reference Cigarette (MRC)). This was consistent with the higher respiratory minute volume observed in THSR-exposed rats (study 15006) or higher respiratory frequency observed in the THSM-exposed rats (study 15025) as compared to 3R4F at similar nicotine test-atmosphere concentrations. Finally, in both studies, the relative distribution of the 5 nicotine metabolites measured was in line with what was

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observed in past studies with nicotine-N'-oxide and cotinine being the major metabolites (data not shown) (Vanscheeuwijck 2002).

In study 15025, the plasma concentrations of nicotine and cotinine were measured. Blood was drawn from the rats after removal from the exposure tubes and under anesthesia. The plasma nicotine concentrations reflected well the amount of nicotine present in the test atmospheres that were delivered to the exposed rats. Plasma nicotine concentrations in the THSM high groups were approximately 2.5-fold higher than in the reference cigarettes smoke-exposed rats and in line with the mean test atmosphere nicotine concentrations of 49 and 22-23 µg nicotine/L respectively. Plasma cotinine concentrations were slightly higher than the plasma nicotine concentrations in exposed rats when compared to the nicotine concentration in the test atmosphere probably due to the longer half-life of cotinine in the exposed animals (Figure 3).

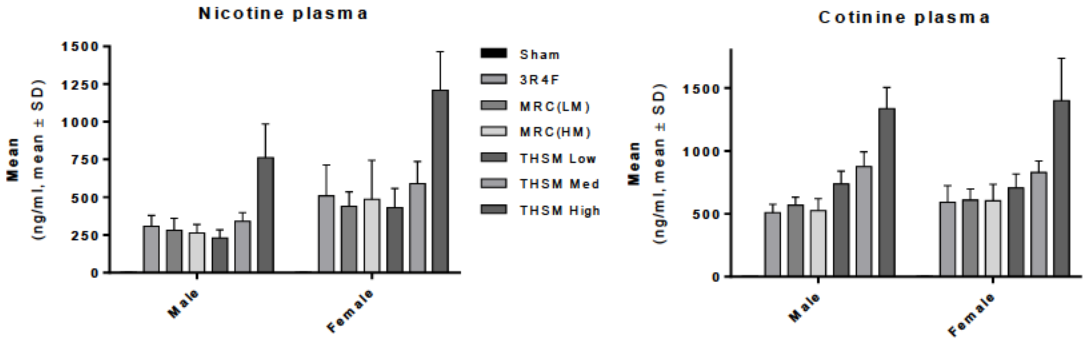


Figure 3 **Mean Concentration of Nicotine and Cotinine Present in the Blood of Male and Female Rats Measured at Week 6.**

Results are presented as mean and standard deviation. # = Equivalent test atmosphere concentration of nicotine.

Altogether, the data presented clearly shows that smoke/aerosol produced in both rat studies (15006 and 15025) was reproducibly taken up by the animals.

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The nicotine exposure concentrations used in the 90-day inhalation studies were representative for or much higher than normal exposure levels of adult smokers. Indeed, based on the formula described by (Alexander 2008), the test atmosphere concentrations of nicotine of 15, 23 and 50 µg/L used in the 90-day inhalation studies, generate a delivered dose (DD) of nicotine aerosol equivalent to 4.2, 6.4 ad 13.6 mg nicotine/kg body weight, calculated as follows:

$$DD = (C \times RMV \times D)/BW$$

Where:
DD = delivered dose (mg/kg);
C = concentration of substance in air (mg/L);
RMV = respiratory minute volume (l/min) for rat (0.194 L/min);
D = duration of exposure (min);
BW = body weight in kg

The human equivalent dose (HED) can be calculated based on the body surface area by dividing the rat dose by a factor of 6.2 (FDA (Food and Drug Administration) 2005) obtaining:

HED_{low} = 4.2 mg/kg/6.2 = 0.7mg/kg

HED_{high} = 6.4 mg/kg/6.2 = 1 mg/kg

HED_{high} = 13.6 mg/kg/6.2 = 2.2 mg/kg

This values translate to a daily nicotine dose of 42, 60 and 132 mg for a 60-kg adult human, equivalent to ≈40 to 130 conventional cigarettes smoked per day. According to that, the lowest dose tested in our rat studies correspond to the highest cigarette consumption estimated in adult smokers (German Federal Office on Statistics 2009, Waingrow 1968).

6.1.3.26.3.1.2 Nicotine Metabolites in Mouse Studies

Nicotine metabolites were quantified at different time points (month 2, 3, 6 and 8) during the 8-month Apoe$^{-/-}$ mouse inhalation study. More specifically, 18-h urine collected post-exposure from female mice were used (THS Regular, study 15015) (Phillips 2016). Urine was not collected during the exposure phase due to technical limitations. In the A/J mouse study (THS Regular, study 15020), nicotine metabolites were determined in 24-h urine collected during the exposure and post-exposure phases from female and male mice at different time points (month 2, 5-6, and 8-9).

In Figure 4 the nicotine metabolite data is shown for the sham, 3R4F and THSR groups only as determined in urine collected at different time points during the study. As observed in the rat studies, at equal nicotine concentrations in test atmosphere the total amount of the selected nicotine metabolites (cotinine, nicotine-N'-oxide, nornicotine, norcotinine and 3'-hydroxycotinine) in the urine of 3R4F- and THSR-exposed groups were similar indicating similar uptake of the aerosols to the mice. The mean study test atmosphere nicotine concentrations were between 28.5 ± 3.2 and 28.7 ± 3.2 µg/L (exposure chambers 1 and 2) for THSR and between 29.2 ± 2.4 and 29.8 ± 2.2 µg/L (exposure chambers 3 and 1) for the 3R4F respectively.

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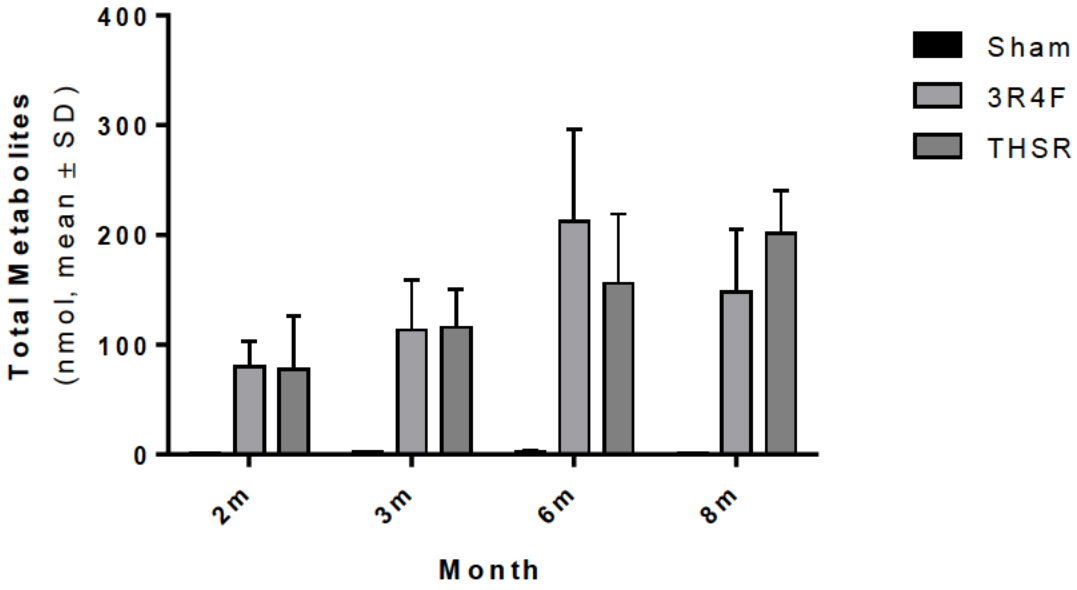


Figure 4 **Total Urine Nicotine Metabolites in Sham, 3R4F, and THSR–Exposed Mice (Female Only, Study 15015).**

Nicotine metabolites were analyzed from samples collected during several time points (month 2, 3, 6 and, 8). Results are presented as mean and standard deviation.

Blood was collected once during the study (at time point 8m) to analyze nicotine and cotinine concentrations in plasma. Although not statistically significant, numerically higher plasma concentrations were observed for both cotinine and nicotine from the THSR group. A similar effect has been observed in the 90-day rat inhalation studies and may be reflective of low irritancy of the THSR aerosol compared to 3R4F, affecting the minute volume of the animals (Figure 5).

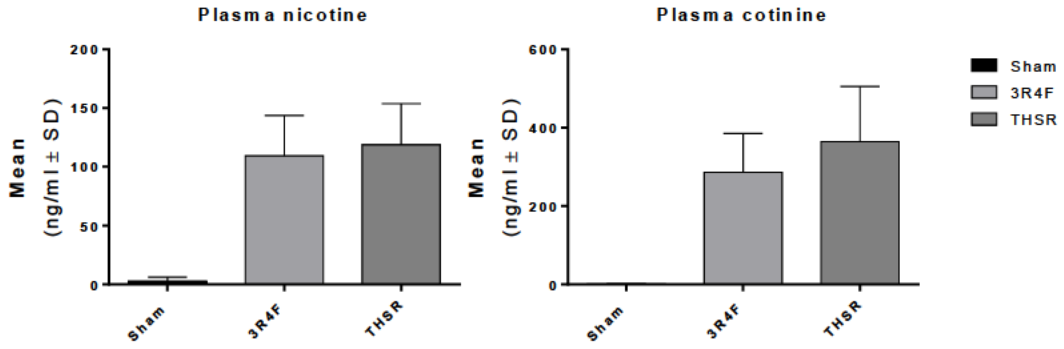


Figure 5 **Nicotine and Cotinine Concentrations in the Plasma of Female Apoe$^{-/-}$ Mice Measured at Month 8.**

Results are presented as means and standard deviation.

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In Figure 4 the total nicotine metabolites are shown for the female A/J mice in the sham, 3R4F and THSR groups (for THSR: low, medium and high exposure concentration), and for the male A/J mice in the sham and THSR groups (for THSR, high exposure concentration) as determined in urine collected at different time points during the study (study 15020). At equal nicotine concentrations in test atmosphere the total amount of the selected nicotine metabolites (cotinine, nicotine-N'-oxide, nornicotine, norcotinine and 3'-hydroxycotinine) in the urine of 3R4F- and THSR-exposed groups was quite similar, but with variations at the different sample collection time points. Despite these variations, mainly driven by the difficulty to accurately and reproducibly sample the small quantities of urine produced by the mice, a similar uptake of the aerosols by the mice can be concluded when exposed to similar nicotine test atmosphere concentrations (THSR medium concentration and 3R4F) at the first collection time point. The amount of urinary nicotine metabolites found at the later time points was higher in THSR-exposed mice than in 3R4F-exposed mice. The mean study test atmosphere nicotine concentrations were 13.1 ± 1.6 µg/L for the 3R4F (female mice only) and 6.6 ± 0.9; 13.1 ± 1.6 and 26.9 ± 2.3 µg/L for THSR, low, medium and high exposure concentration, female mice and 26.1 ± 3.2 µg/L for THSR, high exposure concentration, male mice respectively.

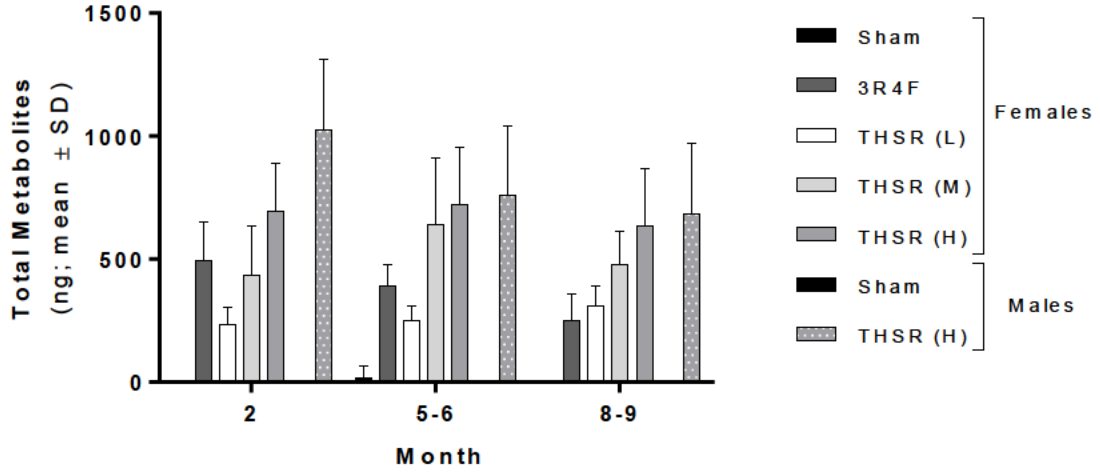


Figure 6 **Total Urine Nicotine Metabolites in A/J mice exposed to air (Sham), THSR and 3R4F- (Female and Male mice, Study 15020).**

Results are presented as means and standard deviation. (L)= low, (M) = medium, (H) = High.

Data on blood nicotine and cotinine concentrations for the A/J mouse study (study 15020) are not shown because of the large variation in the concentrations measured, and the inconsistent test atmosphere nicotine concentrations relationship. This is mainly due to the time required to collect blood from the mice under anesthesia, which was variable and often exceeding 10 minutes, toghether with the short half-lives of nicotine (6-9 min) in mouse blood.

The nicotine exposure concentrations used in the chronic mouse inhalation studies were representative for or higher than normal exposure levels of adult smokers.

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Indeed, based on the formula described in (Alexander 2008), the test atmosphere concentrations of nicotine of approx. 29 µg/L used in the 8 months Apoe$^{-/-}$ study generate a delivered dose (DD) of nicotine aerosol equivalent to 4.5 mg nicotine/kg body weight, calculated as follows:

$$DD = (C \times RMV \times D)/BW$$

Where:
DD = delivered dose (mg/kg);
C = concentration of substance in air (mg/L);
RMV = respiratory minute volume (l/min) for mice (0.02 L/min);
D = duration of exposure (min); 180 min
BW = body weight in kg; 0.023 kg

The human equivalent dose (HED) can be calculated based on the body surface area by dividing the mouse dose by a factor of 12.3 (FDA (Food and Drug Administration) 2005) obtaining:

HED = 4.5 mg/kg/12.3 = 0.37 mg/kg

This value translates to a daily nicotine dose of 22 mg for a 60-kg adult human, equivalent to 22 conventional cigarettes smoked per day, or one pack per day.

In the A/J mouse study (with a daily exposure duration of 360 min), the nicotine exposure concentrations of 6.6, 13.1 and 26.2 µg/L in test atmosphere coorespond to DD of: 2.1, 4.1 and 8.2 mg nicotine/kg body weight for the female mice and 8.3 mg nicotine/kg body weight for the male mice. The HED are then:

HED_{low} = 2.1 mg/kg/12.3 = 0.17 mg/kg

HED_{med} = 4.1 mg/kg/12.3 = 0.33 mg/kg

HED_{high} = 8.2 mg/kg/12.3 = 0.67 mg/kg

These values translate to a daily nicotine dose of 10, 20 and 40 mg for a 60-kg adult human, equivalent to approx.10, 20 or 40 conventional cigarettes smoked per day.

6.1.3.26.3.2 S-PMA

6.1.3.26.3.2.1 S-PMA in Rat Studies

S-PMA was quantified in 24-h urine collected from rats in two 90-day inhalation studies (study 15006 on THSR (urine from male rats only) and study 15025 on THSM (urine from male and female rats). As shown in Figure 7 (only male rats are shown for direct comparison between the two studies) the concentration of S-PMA measured in THS-exposed rats in the two independent studies, and irrespective of the presence of menthol, was very low and not different from Sham-exposed rats. At equal nicotine exposure concentrations the concentrations of S-PMA measured was 62-fold lower in the THSR-exposed groups than in the reference cigarette-exposed groups. Similarly, the quantity of S-PMA measured was 86-fold lower in the THSM-exposed groups than in the reference cigarette-exposed groups (Figure 5). Benzene concentrations were not measured in the test

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atmospheres and consequently a direct correlation between benzene and the urinary levels of S-PMA was not possible. However, the magnitude of reduction in S-PMA levels observed in THS-exposed rats mirrors analytical data performed on THS aerosol (see CMC section) where, on a per nicotine basis, benzene levels were reduced approximately by 99% compared to 3R4F.

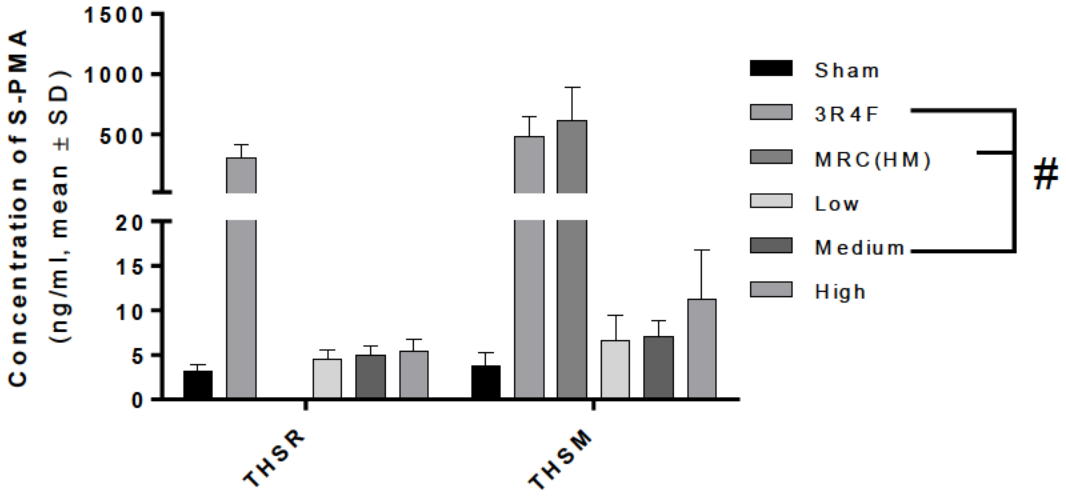


Figure 7 **Concentration of S-PMA (male only) in the 90-Day Inhalation Study on THSR (15006) and THSM (15025).**

S-PMA levels were analyzed at week 4-5 in 15006 study and at week 12 in 15025 study. Results are presented as mean and standard deviation. # = Equivalent test atmosphere concentration of nicotine.

6.1.3.26.3.2.2 S-PMA in Mouse Studies

S-PMA was quantified at different time points (month 2, 3, 6 and, 8) during an 8-month Apoe$^{-/-}$ mouse inhalation study. More specifically, 18-h urine collected post-exposure from female mice were used (THS Regular, study 15015) (Phillips 2016). Urine was not collected during the exposure phase due to technical limitations. In the A/J mouse study (THS Regular, study 15020), S-PMA was determined in 24-h urine collected during the exposure and post-exposure phases from female mice at different time points (month 1-2, 5-6, and 8-9).

As shown in Figure 8 the S-PMA data is shown for the sham, 3R4F and THSR groups only as determined in urine collected at different time points during the study (for cessation and switching data see 6.1.3.26.2). The concentration of S-PMA measured in THSR-exposed mice was very low and not different from Sham-exposed mice. At equal test atmosphere nicotine exposure concentrations and, depending on the time point of sample collection, the quantity of S-PMA was between 21 and 36-fold lower in the THSR-exposed groups than in the reference cigarette-exposed groups.

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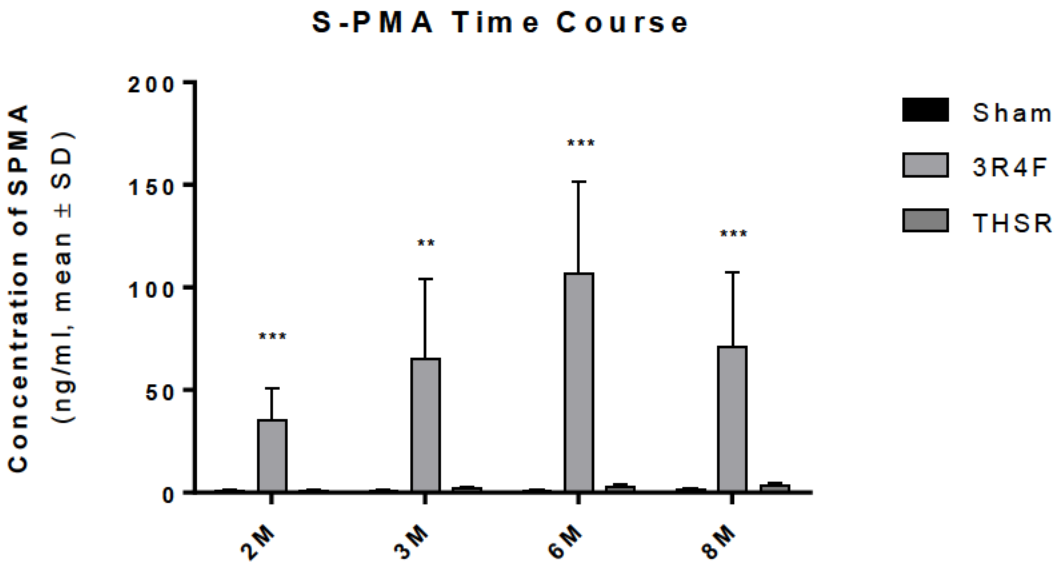


Figure 8 **Concentration of Urine S-PMA at Four Different Time Points in Sham, 3R4F, and THSR-Exposed Female Apoe$^{-/-}$ Mice (Study 15015).**

Results are presented as mean and standard deviation. P-values against Sham obtained by t-test accounting for variance heterogeneity. *** $p < 0.001$; ** $p < 0.01$.

In Figure 9 The urinary S-PMA concentrations are shown as determined in the A/J mouse study in femal moice at different time points. The S-PMA concentrations in the smoke-exposed female mice was 45-56 fold higher than those in THSR aerosol-exposed mice at equal test atmosphere nicotine concentrations (3R4F vs. THSR (M)), reflecting the differences in benzene concentrations in THSR aerosol and 3R4F smoke. Statistically significantly higher S-PMA concentrations were found in all THSR groups relative to Sham, except at the sample collection time point 8-9 months.

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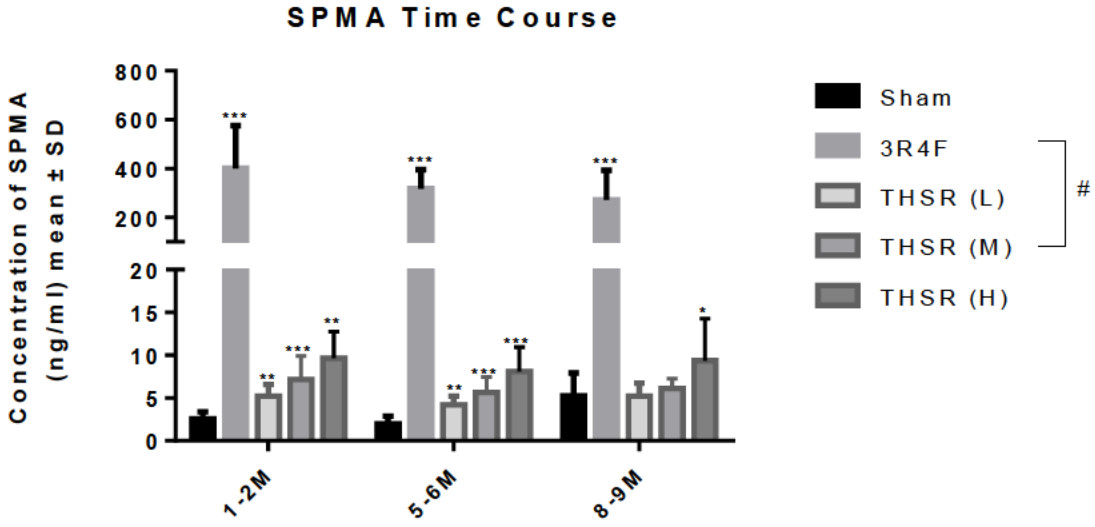


Figure 9 **Concentration of Urine S-PMA at three Different Time Points in Sham, 3R4F, and THSR-Exposed Female A/J Mice (Study 15020).**

Results are presented as mean and standard deviation. # Equivalent test atmosphere concentration of nicotine. P-values against Sham obtained by t-test accounting for variance heterogeneity. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. (L)= low, (M) = medium, (H) = High

6.1.3.26.3.3 3-HPMA

6.1.3.26.3.3.1 3-HPMA in Rat Studies

3-HPMA was quantified in 24-h urine collected from rats in two 90-day inhalation studies (study 15006 on THSR (urine from male rats only) and study 15025 on THSM (urine from male and female rats). Endogenous production of acrolein in normal untreated rats (Stevens 2008) was responsible of the background levels of 3-HPMA measured in Sham-exposed rats and served as baselines for comparison with THS-exposed rats. As shown in Figure 10 (only male rats are shown for direct comparison between the two studies), at equal nicotine exposure concentration the concentration of 3-HPMA measured in the THSR-exposed groups was 3.2-fold lower than the concentrations measured in the reference cigarette-exposed groups. Similarly, the quantity of 3-HPMA measured was 2.5-fold lower in the THSM-exposed groups than in the reference cigarette-exposed groups. Moreover, the recovered concentrations of 3-HPMA were related to acrolein test atmosphere concentrations in the 3R4F-groups (Figure 11).

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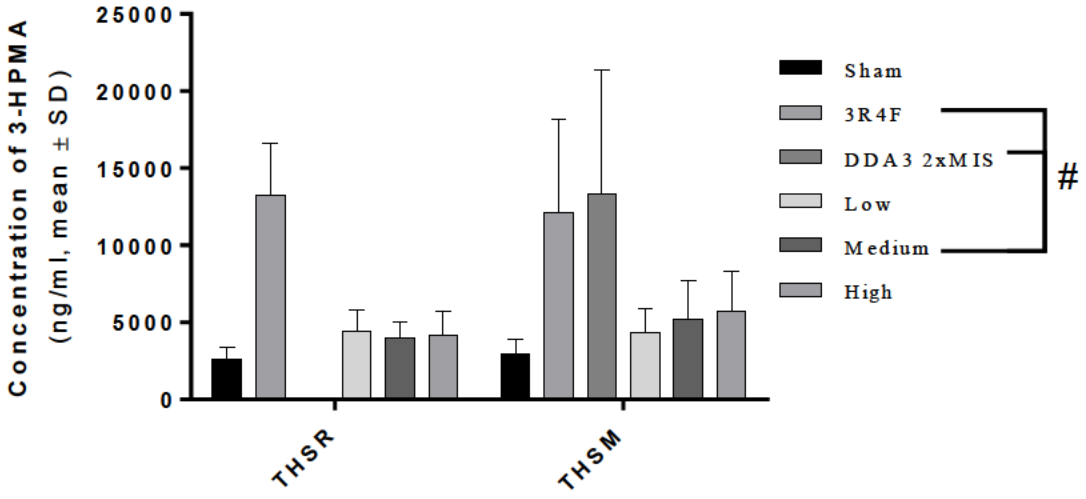


Figure 10 **Concentration of 3-HPMA (Male Only) in the 90-Day Inhalation Study on THSR (15006) and THSM (15025).**

HPMA levels were analyzed at week 4-5 in 15006 study and at week 12 in 15025 study. Results are presented as mean and standard deviation. # = Equivalent test atmosphere concentration of nicotine.

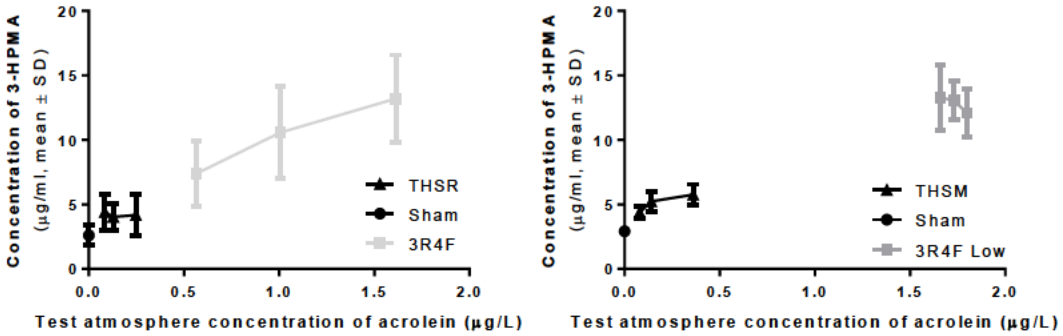


Figure 11 **Acrolein Test Atmosphere Concentrations and Concentration of 3-HPMA Excreted in Urine in THSR-Exposed Rats (Left Panel) and THSM-Exposed Rats (Right Panel).**

Results are presented as means and standard deviation.

6.1.3.26.3.3.2 3-HPMA in Mouse Studies

3-HPMA was quantified at different time points (month 2, 3, 6 and, 8) during an 8-month Apoe$^{-/-}$ mouse inhalation study. More specifically, 18-h urine collected post-exposure from female mice were used (THS Regular, study 15015) (Phillips 2016). Urine was not collected during the exposure phase due to technical limitations.

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In the A/J mouse study (THS Regular, study 15020), 3-HPMA was determined in 24-h urine collected during the exposure and post-exposure phases from female mice at different time points (month 1-2, 5-6, and 8-9).

For the Apoe$^{-/-}$ mouse study, 3-HPMA data is shown for the sham, 3R4F and THSR groups only (for cessation and switching data see 6.1.3.26.2) as determined in urine collected at different time points during the study. As discussed for the rat 90-day inhalation studies and, according to the available literature, endogenous production of acrolein in normal mice (Carmella 2009) was responsible of the background levels of 3-HPMA measured in Sham-exposed mice and served as baseline for comparison with THSR-exposed mice. In particular, Sham-exposed animals had an average urine basal 3-HPMA level of 2000 ng/ml (range 1829–2500 ng/ml depending on the month), and showed no significant differences from THSR-exposed animals. However, at equal nicotine exposure concentration (i.e. 29.9 µg nicotine / l test atmosphere), the quantity of 3-HPMA was about 2-fold lower in the THSR-exposed groups than in the reference cigarette-exposed groups (Figure 12). The study mean acrolein concentrations in the test atmospheres were between 3.06 ± 0.11 and 3.14 ± 0.24 µg/L for 3R4F (exposure chamber 1 and 2) and between 0.28 ± 0.02 and 0.29 ± 0.03 µg/L for the THSR (exposure chamber 1 and 2).

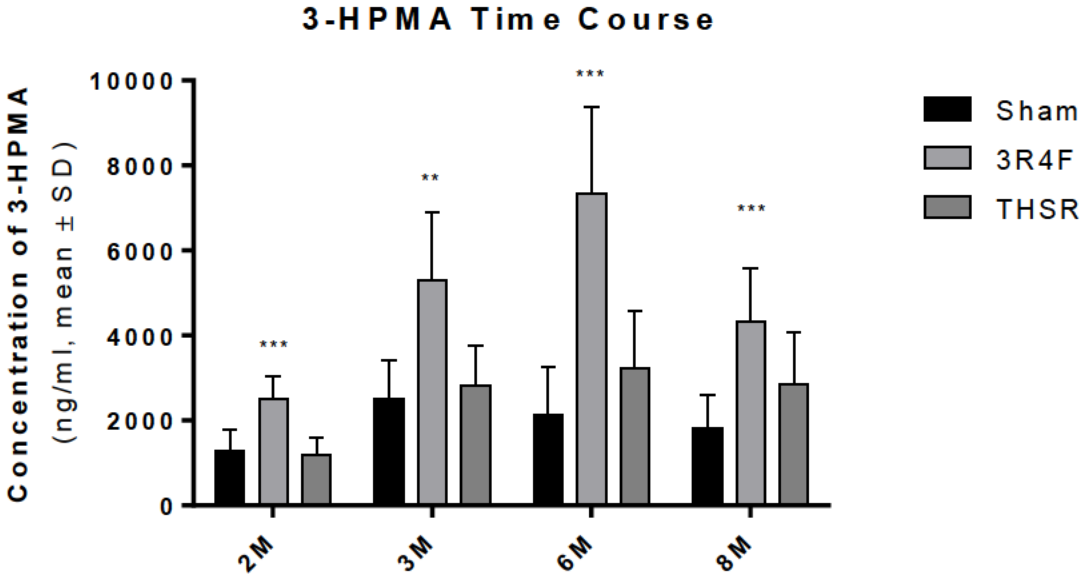


Figure 12 **Concentration of Urine HPMA at Four Different Time Points in Sham, 3R4F, and THSR-Exposed Female Apoe$^{-/-}$ Mice (Study 15015).**

Results are presented as mean and standard deviation. P-values against Sham obtained by t-test accounting for variance heterogeneity. *** $p < 0.001$; ** $p < 0.01$.

Similarly, in the A/J mouse study, urinary 3-HPMA concentrations in THSR-exposed mice were at least two-fold lower than in 3R4F-exposed mice at a similar nicotine test atmosphere concentrations (Figure 13). Although acrolein is measurable in the THSR test

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atmospheres , 3-HPMA concentrations were similar in the the THSR-exposed as compared with sham-exposed mice. Study mean acrolein concentrations were 0.06 ± 0.01 µg/L for THSR Low, 0.12 ± 0.01 µg/L for THSR Med and 0.22 ± 0.02 µg/L for THSR High; for 3R4F the acrolein test atmosphere concentration was 1.80 ± 0.21 µg/L. The lack of linearity between test atmosphere concentrations of acrolein and urinary 3-HPMA is due to the endogenous production of 3-HPMA in mice masking additional low concentrations of 3-HPMA produced as metabolite from external (acrolein) exposure sources (Carmella 2009).

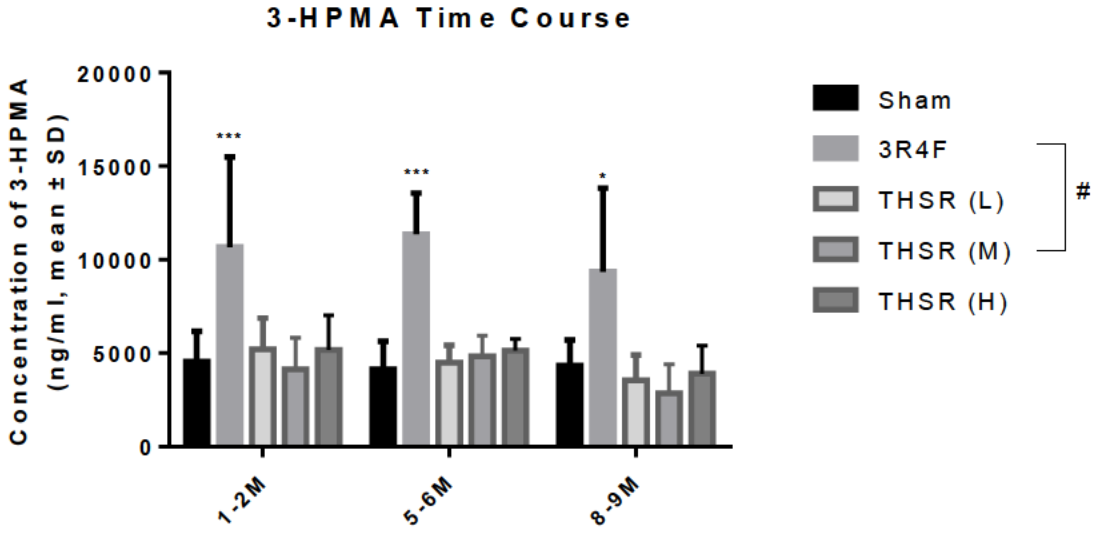


Figure 13 **Concentration of Urine 3-HPMA at Three Different Time Points in Sham, 3R4F, and THSR-Exposed Female A/J Mice (Study 15020).**

Results are presented as mean and standard deviation. P-values against Sham obtained by t-test accounting for variance heterogeneity. *** $p < 0.001$; * $p < 0.05$. (L)= low, (M) = medium, (H) = High

6.1.3.26.3.4 COHb

6.1.3.26.3.4.1 COHb in Rat Studies

Measurement of carboxyhemoglobin (COHb) in blood was performed to assess the exposure and uptake of the gas vapour phase component CO by the rats. As shown in Figure 14, the levels of COHb measured in Sham-exposed rats in two independent studies and irrespective of the presence of menthol was very low, but not below 2 % for individual rats. It has been described that the method used is not very sensitive at low proportions of COHb (Mahoney 1993). Nevertheless, the proportion of COHb in the Sham animals, exposed to fresh conditioned air only, has been used as baseline for the THS-exposed rats, and was in average 3.4% in both the studies.

At equal nicotine exposure concentration (i.e. 23 µg nicotine / l test atmosphere), the COHb plasma levels were between 25% and 26.5% in 3R4F-exposed rats and only between 3.7% and 4.0% in THSR-exposed rats (female and male rats, study 15006). The study mean CO concentrations in the test atmospheres were 333.4 ppm for 3R4F and 6.4 ppm for the THSR (at equal nicotine exposure concentration). Similarly, the COHb plasma levels of THSM-

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exposed rats were between 3.6% and 3.7% whereas the levels of COHb measured with 3R4F or mentholated reference cigarettes were between 22.3% and 27% (male and female, study 15025). The study mean CO concentrations in the test atmospheres were 362.6 ppm for 3R4F and 6.8 ppm for the THSM. In both studies there was a good correlation between COHb levels in the exposed animals and CO concentrations in the test atmospheres, indicating proper uptake of the gas phase of the aerosols by the animals (Figure 15). For both 90-day inhalation studies, measurements were repeated at the end of the recovery period and showed that all COHb values were at basal levels (data not shown).

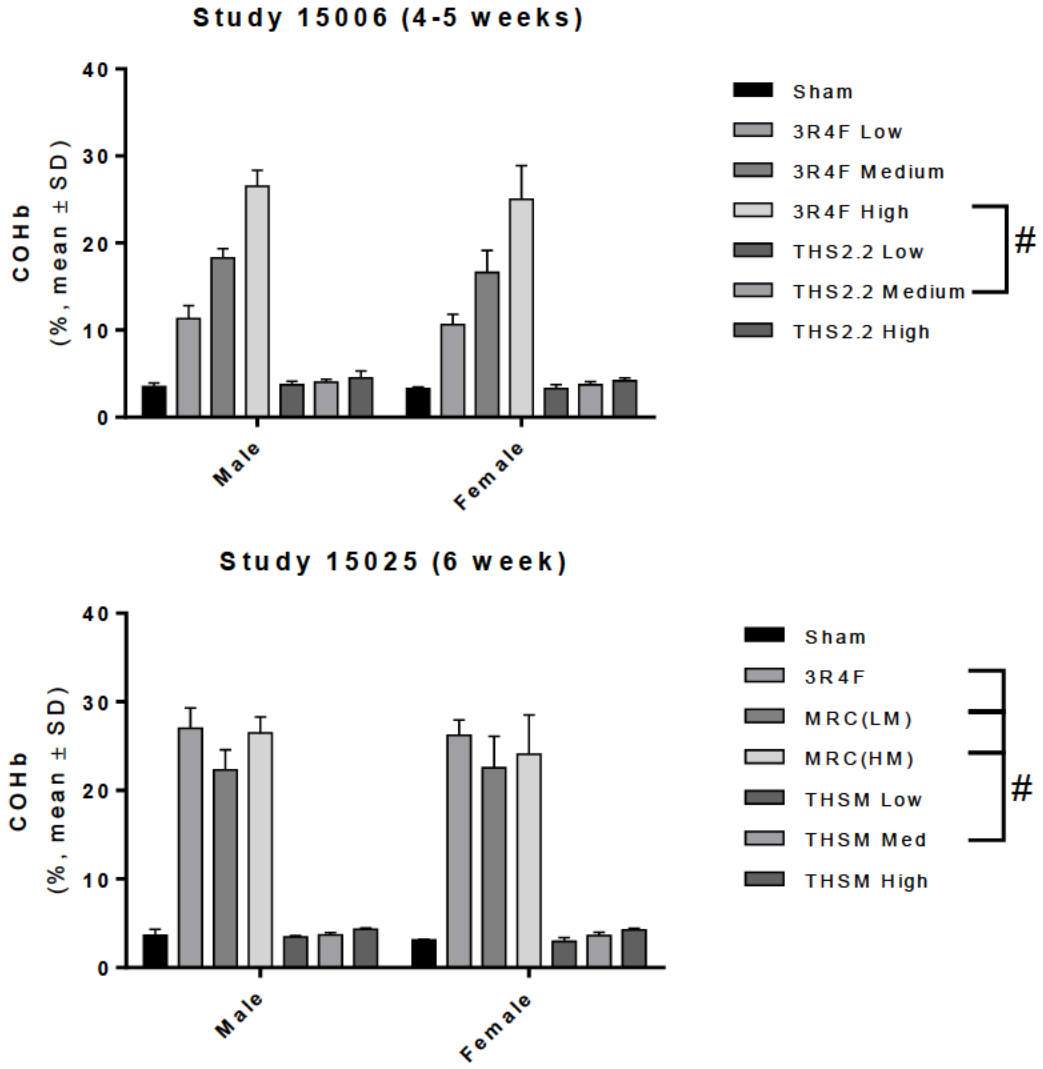


Figure 14 **Mean COHb Proportion in the Blood of Sham, Reference Cigarettes and THS-Exposed Animals.**

COHb levels were analyzed at week 4-5 in the study 15006 (THSR, let panel) and at week 6 in the study 15025 (THSM, right panel). Results are presented as mean and standard deviation. # = Equivalent test atmosphere concentration of nicotine

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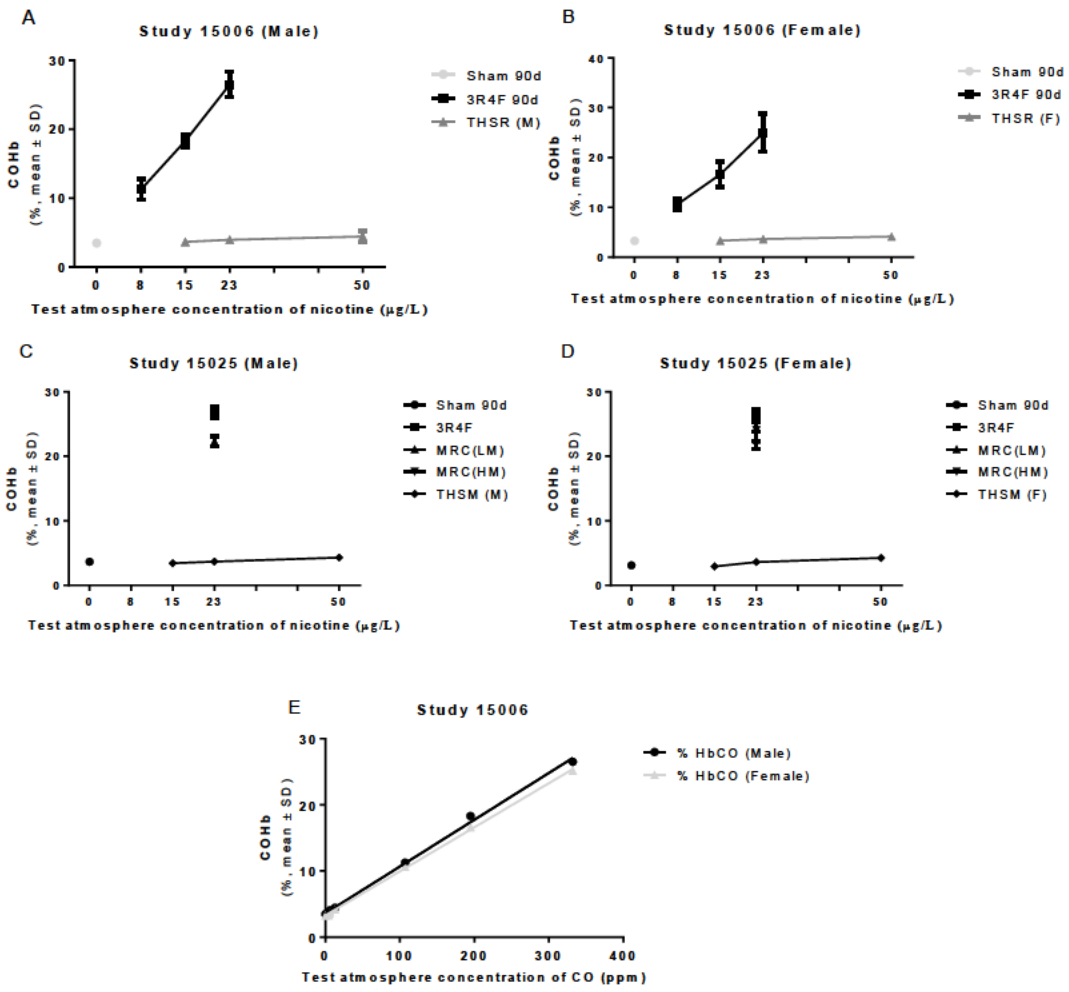


Figure 15 **Mean Carboxyhemoglobin Content in the Blood of Sham, Reference Cigarettes and, THS-Exposed Animals**

Male (A and C) and female (B and D). Correlation between plasma carboxyhemoglobin content and CO concentration in the test atmosphere (E).

6.1.3.26.3.4.2 COHb in Mouse Studies

Measurement of COHb was performed to assess the exposure to and uptake of the gas phase consitutent CO by the mice. Blood was collected towards the end of the daily exposure period. COHb levels were analysed three times (at months 2, 4, and 7) in an 8-month Apoe$^{-/-}$ mouse inhalation study.

In the A/J mouse study, blood was collected twice for the determination of COHb, during months 3 and 7 of the exposure period.

As expected, and in relation to the CO-concentration in the test atmospheres, the proportion of blood COHb was higher in the 3R4F-exposed Apoe$^{-/-}$ than in the sham-exposed mice. The aerosol from THSR at the same nicotine concentration as 3R4F has a considerably

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lower CO concentration (CO-concentration study means are between 613.9 ± 26.0 and 667.1 ± 36.9 ppm in the test atmosphere of 3R4F (exposure chamber 1 and 2) and 13.6± 0.7 and 14.9 ± 2.6 ppm in THSR (exposure chamber 2 and 1), which was reflected in the lower blood COHb proportions of the exposed THSR animals. In particular, at equal nicotine exposure concentration (i.e. 29.9 µg nicotine / L test atmosphere), the COHb plasma levels were between 27.8% and 36.6% in 3R4F-exposed mice and between 4.9% and 6.0% in THSR-exposed mice (female only, study 15015) (Figure 16).

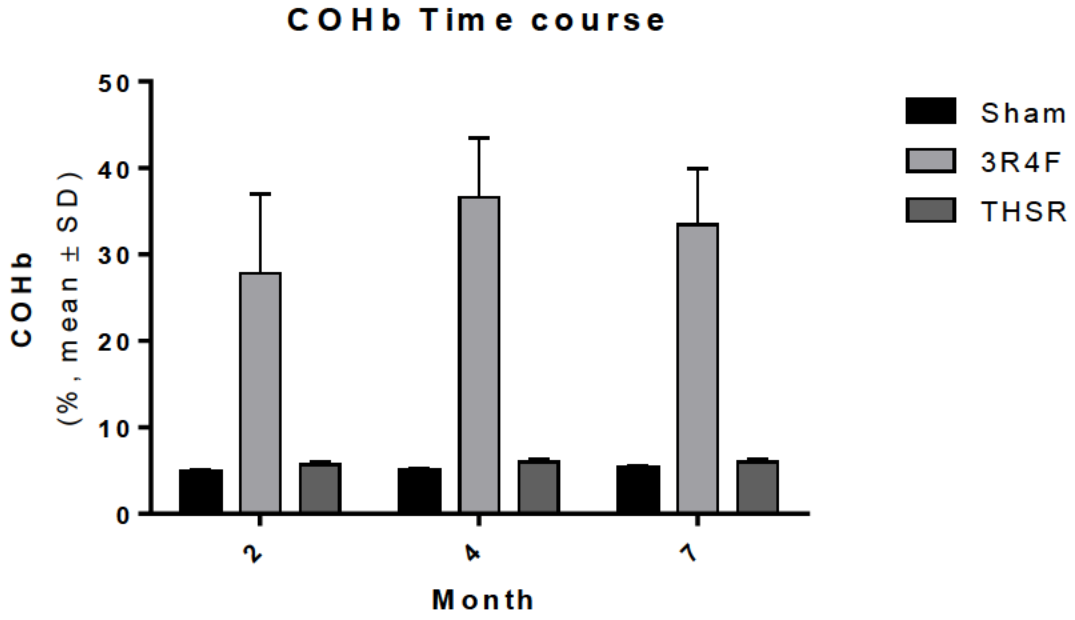


Figure 16 **COHb proportions in the Blood of Sham, 3R4F and Sham, 3R4F, and THSR-Exposed Female Apoe$^{-/-}$ Mice (Study 15015).**

COHb levels were analyzed at three different time points (female only, study 15015). Results are presented as mean and standard deviation.

The proportions of COHb in the exposed A/J mice reflect the aerosol or smoke CO-concentrations (Figure 17). The mean (± SD) CO-concentration in the 3R4F test atmosphere was 386.9 ± 26.6 ppm throughut the exposure period, and 3.9 ± 0.4 ppm, 7.0 ± 1.1 ppm, and 11.6 ± 1.5 ppm in THSR low, medium and high respectively for the female mouse exposure chambers, and 13.4 ± 1.8 ppm for the male THSR High test atmosphere. CO concentrations in the Sham inhalation chambers were 0.2 ± 0.4 ppm and 0.4 ± 0.3 ppm in the female and male exposure chambers respectively.

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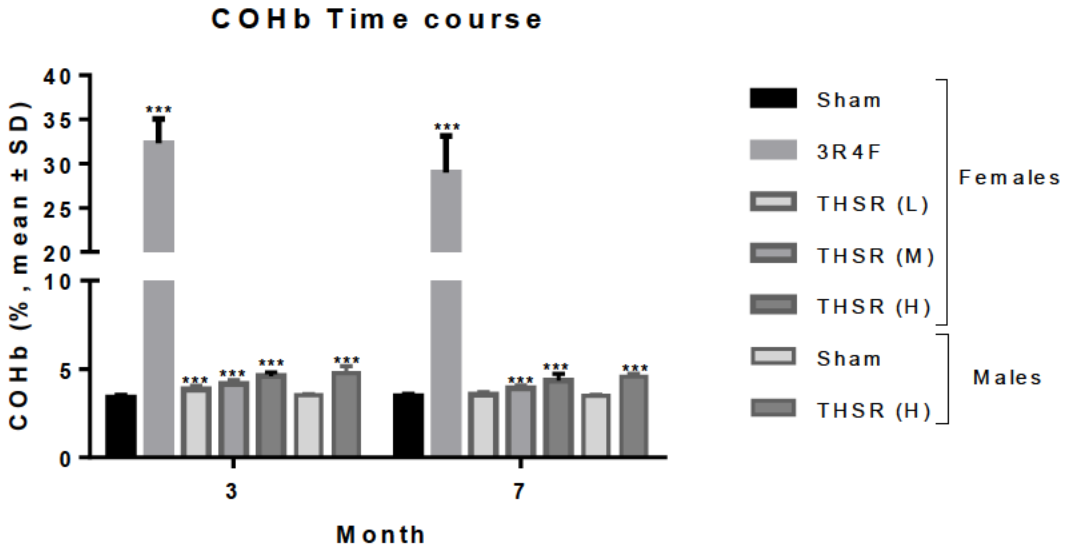


Figure 17 **COHb proportions in the Blood of Sham, 3R4F and THSR-Exposed A/J Mice (Study 15020).**

COHb levels were analyzed at 2 different time points. Results are presented as mean and standard deviation. P-values against Sham: *** $p<0.001$. (L)= low, (M) = medium, (H) = High.

6.1.3.26.3.5 CEMA

6.1.3.26.3.5.1 CEMA in Rat Studies

CEMA was quantified in 24-h urine collected from rats in two 90-day inhalation studies (study 15006 on THSR (urine from male rats only) and study 15025 on THSM (urine from male and female rats)). As shown in Figure 18 (only male rats are shown for direct comparison between the two studies) the concentration of CEMA measured in Sham-exposed rats in two independent studies and irrespective of the presence of menthol was very low and served as baseline for comparison with THS-exposed rats. At equal nicotine exposure concentration the concentration of CEMA measured was 56-fold lower in THSR-exposed animals than in the reference cigarette-exposed groups. Similarly, the concentration of CEMA measured was 63-fold lower in the THSM-exposed groups than in the reference cigarette-exposed groups (Figure 18). Acrylonitrile concentrations were not measured in the test atmospheres and consequently a direct correlation between acrylonitrile and the urinary levels of CEMA was not possible. However, the magnitude of reduction in CEMA levels observed in THS-exposed rats mirrors previous analytical data performed on THS aerosol (see CMC section) where, on a per nicotine basis, acrylonitrile levels were reduced approximately by 99% compared to 3R4F.

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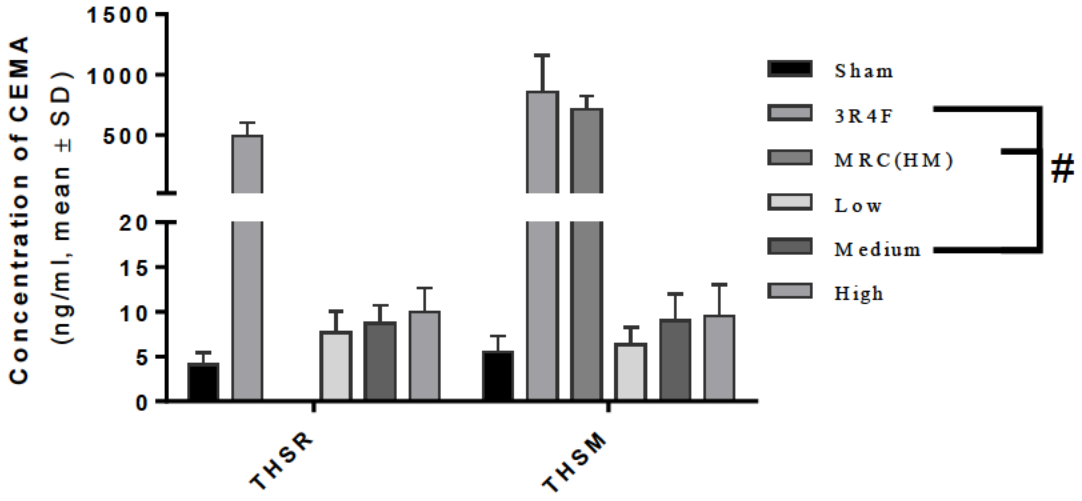


Figure 18 **Concentration of CEMA (Male Only) in the 90-Day Inhalation Study on THSR (15006) and THSM (15025).**

CEMA levels were analyzed at week 4-5 in 15006 study and at week 12 in 15025 study. Results are presented as mean and standard deviation. # = Equivalent test atmosphere concentration of nicotine.

6.1.3.26.3.5.2 CEMA in Mouse Studies

CEMA was quantified at different time points (month 2, 3, 6 and, 8) during an 8-month Apoe$^{-/-}$ mouse inhalation study. More specifically, 18-h urine collected post-exposure from female mice were used (THS Regular, study 15015) (Phillips 2016). Urine was not collected during the exposure phase due to technical limitations. In the A/J mouse study (THS Regular, study 15020), CEMA was determined in 24-h urine collected during the exposure and post-exposure phases from female mice at different time points (month 1-2, 5-6, and 8-9).

For the Apoe$^{-/-}$ mouse inhalation study, CEMA data is shown for the sham, 3R4F and THSR groups only (for cessation and switching data see 6.1.3.26.2) as determined in urine collected at different time points during the study. As shown in Figure 19 the concentrations of CEMA measured in THSR-exposed mice were very low and not different from Sham-exposed mice. At equal test atmosphere nicotine exposure concentrations and, depending on the month of analysis, the concentration of S-PMA was between 31 and 83-fold lower in the THSR-exposed groups than in the reference cigarette-exposed groups.

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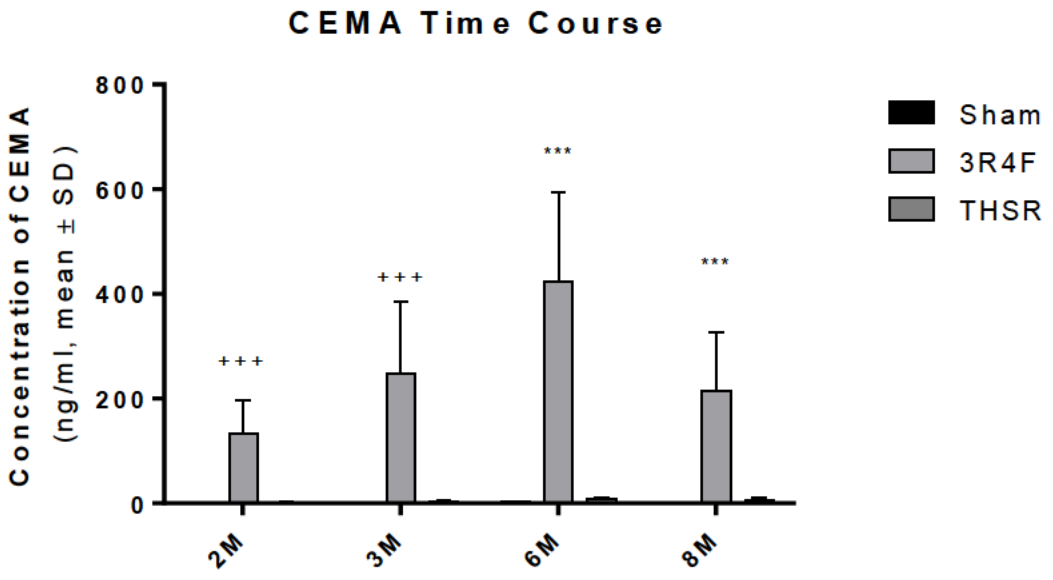


Figure 19 **Concentration of Urine CEMA at Four Different Time Points in Sham, 3R4F, and THSR-Exposed Mice (Female Only, Study 15015).**

Results are presented as mean and standard deviation. P-values against Sham obtained by t-test accounting for variance heterogeneity. *** $p < 0.001$; ** $p < 0.01$.

In the A/J mouse study (study 15020), CEMA was determined three times during the study in the urine of female mice (Figure 20). At all three time points, the concentration of urinary CEMA was approximately 70-fold lower in the THSR aerosol than in the smoke from 3R4F at matched test atmosphere nicotine concentration. Although acrylonitrile was not measured in the test atmospheres in the A/J mouse study, a concentration response for its metabolite CEMA is observed in the THSR groups, further demonstrating the appropriate exposure to the test atmospheres.

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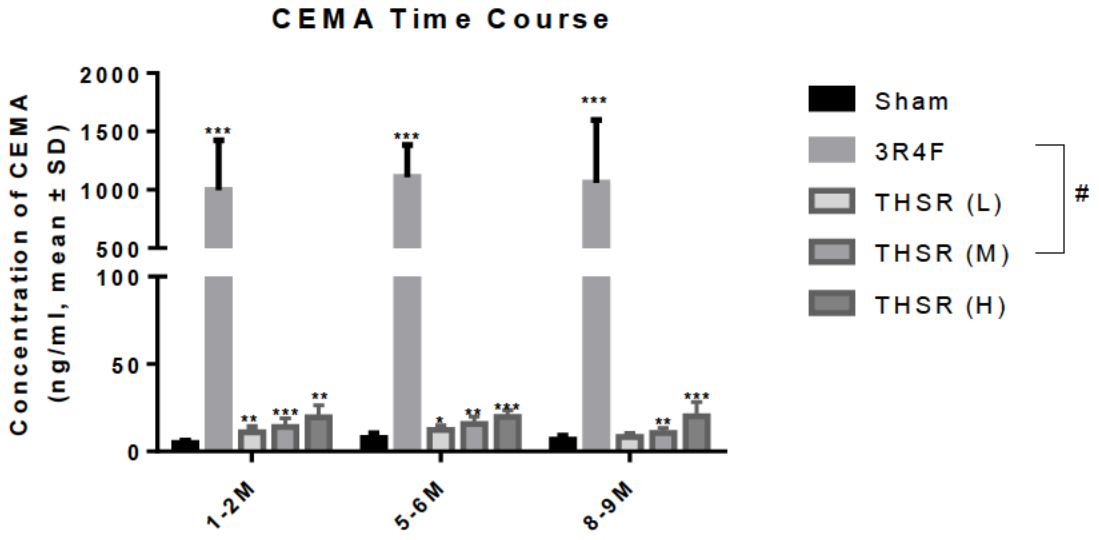


Figure 20 **Concentration of Urine CEMA Three Different Time Points in Sham, 3R4F, and THSR-Exposed Female A/J Mice (Study 15020).**

Results are presented as mean and standard deviation. P-values against Sham *** $p < 0.001$; ** $p < 0.01$; * $p<0.05$. (L)= low, (M) = medium, (H) = High.

6.1.3.26.3.6 Total NNAL

6.1.3.26.3.6.1 Total NNAL in Rat Studies

Total NNAL was quantified in 24-h urine collected from rats in two 90-day inhalation studies (study 15006 on THSR (urine from male rats only) and study 15025 on THSM (urine from male and female rats)). As shown in Figure 21 (only male rats are shown for direct comparison between the two studies) the concentrations of total NNAL measured in Sham-exposed rats in two independent studies and irrespective of the presence of menthol was very low and served as baseline for comparison with THS-exposed rats. At equal nicotine exposure concentration the concentration of total NNAL was 9-fold lower in the THSR-exposed groups than in the reference cigarette exposed groups. Similarly, the concentration of NNAL measured was 17-fold lower in the THSM-exposed groups than in the reference cigarette-exposed groups (Figure 21). 4-(methyl-nitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) concentration was not measured in the test atmosphere and consequently a direct correlation between NNK and total NNAL was not possible. However, the reduction in total NNAL levels observed in THS-exposed rats reflect, at least partially, previous analytical data performed on THS aerosol (see CMC section) where, on a per nicotine basis, acrylonitrile levels were reduced approximately by about 96% compared to 3R4F.

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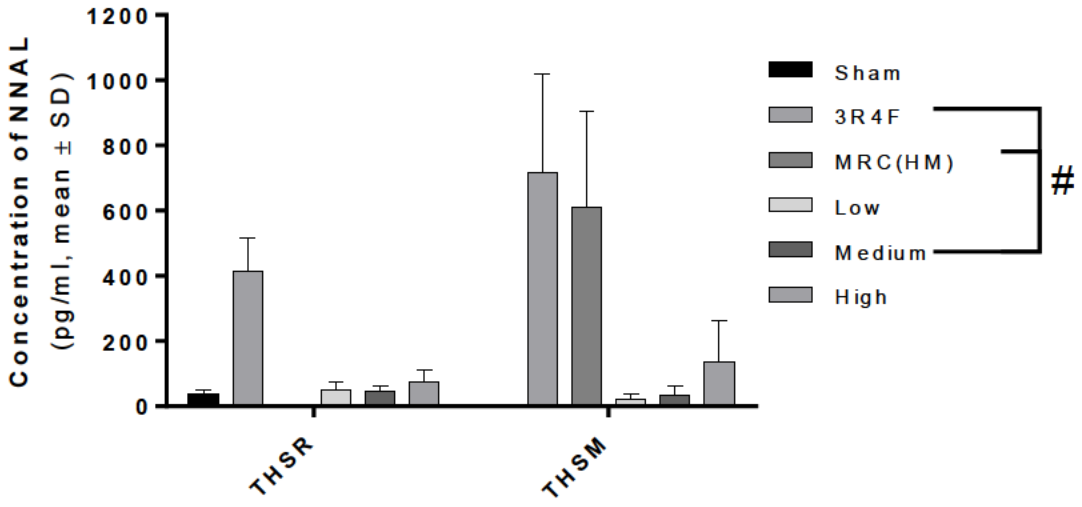


Figure 21 **Concentration of total NNAL (Male Only) in the 90-Day Inhalation Study on THSR (15006) and THSM (15025).**

NNAL levels were analyzed at week 4-5 in 15006 study and at week 12 in 15025 study. Results are presented as mean and standard deviation. #= Equivalent test atmosphere concentration of nicotine.

6.1.3.26.3.6.2 Total NNAL in Mouse Studies

Total NNAL was quantified at different time points (month 2, 3, 6 and, 8) during an 8-month Apoe$^{-/-}$ mouse inhalation study. More specifically, 18-h urine collected post-exposure from female mice were used (THS Regular, study 15015) (Phillips 2016). Urine was not collected during the exposure phase due to technical limitations. In the A/J mouse study (THS Regular, study 15020), total NNAL was determined in 24-h urine collected during the exposure and post-exposure phases from female mice at different time points (month 1-2, 5-6, and 8-9).

Total NNAL data is shown for the sham, 3R4F and THSR groups only (for cessation and switching data see 6.1.3.26.2) as determined in urine collected at different time points during the study. As shown in Figure 22 the concentration of NNAL measured in THSR-exposed mice was very low and not different from Sham-exposed mice. At equal test atmosphere nicotine exposure concentrations and, depending on the month of analysis, the concentration of NNAL was between 11 and 45-fold lower in the THSR-exposed groups than in the reference cigarette-exposed groups.

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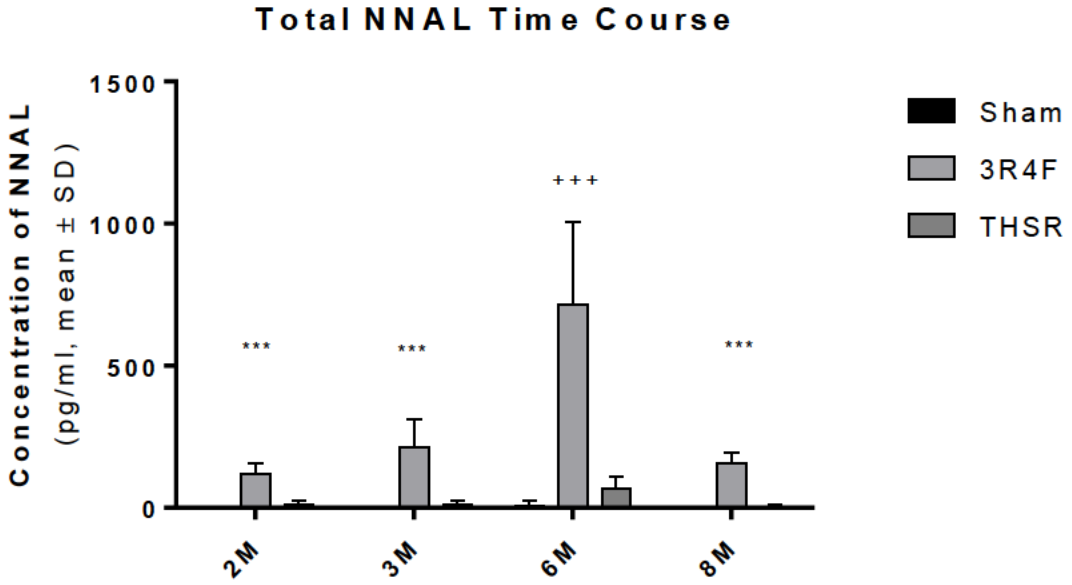


Figure 22 **Concentration of Urine total NNAL at Four Different Time Points in Sham, 3R4F, and THSR-Exposed Female Apoe$^{-/-}$ Mice (Study 15015).**

Results are presented as mean and standard deviation. P-values against Sham obtained by t-test accounting for variance heterogeneity (*) or by Wilcoxon exact MC (+). ***/+++p < 0.001.

In the A/J mouse study (study 15020), total NNAL was determined three times during the study in the urine of female mice (Figure 23). At all three time points, the concentration of urinary total NNAL was approximately 20-fold lower in the THSR aerosol than in the smoke from 3R4F at matched test atmosphere nicotine concentration. Although NNK was not measured in the test atmospheres in the A/J mouse study, a concentration response for its biomarker total NNAL is observed in the THSR groups, further demonstrating the appropriate exposure to the test atmospheres.

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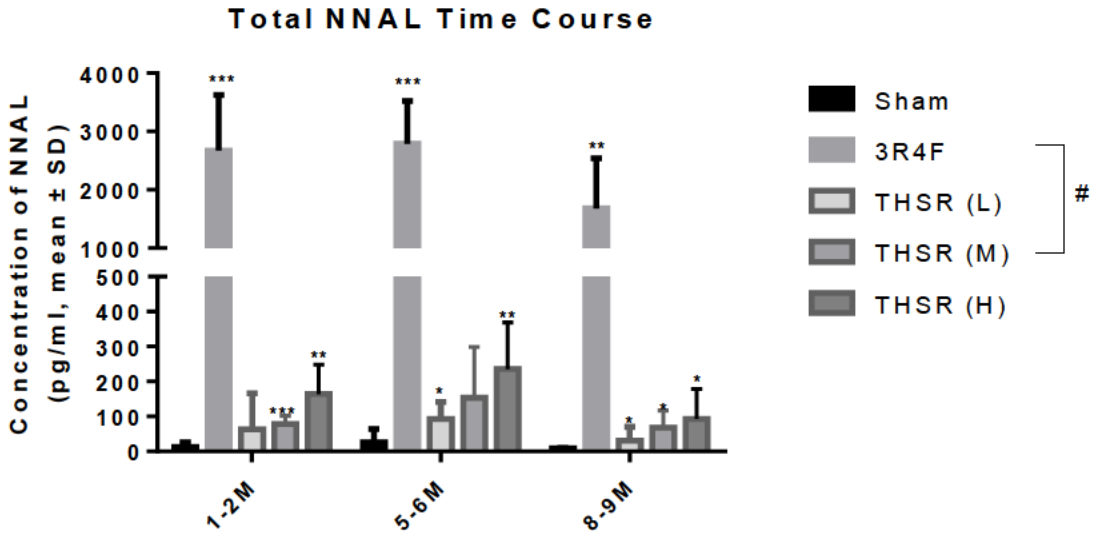


Figure 23 **Concentration of Urine Total NNAL at Three Different Time Points in Sham, 3R4F, and THSR-Exposed Female A/J Mice (Study 15020).**

Results are presented as mean and standard deviation. P-values against Sham *** $p < 0.001$; ** $p < 0.01$; * $p<0.05$. (L)= low, (M) = medium, (H) = High.

6.1.3.26.3.7 Menthol

The uptake of menthol in the 90-day rat inhalation study performed with THSM and mentholated reference cigarettes (study 15025) was verified by quantification of menthol and one of its metabolites p-menthane-3,8-diol (M-I) in plasma. At equal test atmosphere nicotine exposure concentrations, the plasma concentrations of menthol in the THSM-exposed rats were between 2.0 to 2.7-fold lower (male and female) that of reference cigarette-exposed rats, whereas the concentrations of M-I were between 1.1 to 1.2-fold lower (male and female) in the THSM-exposed rats that of reference cigarette-exposed rats (Figure 24). The observed plasma menthol and M-I concentrations showed a concentration-dependent increase with the menthol content measured in the test atmosphere from the THSM exposed and MRC(LM)/MRC(HM)-exposed rats (Figure 25).

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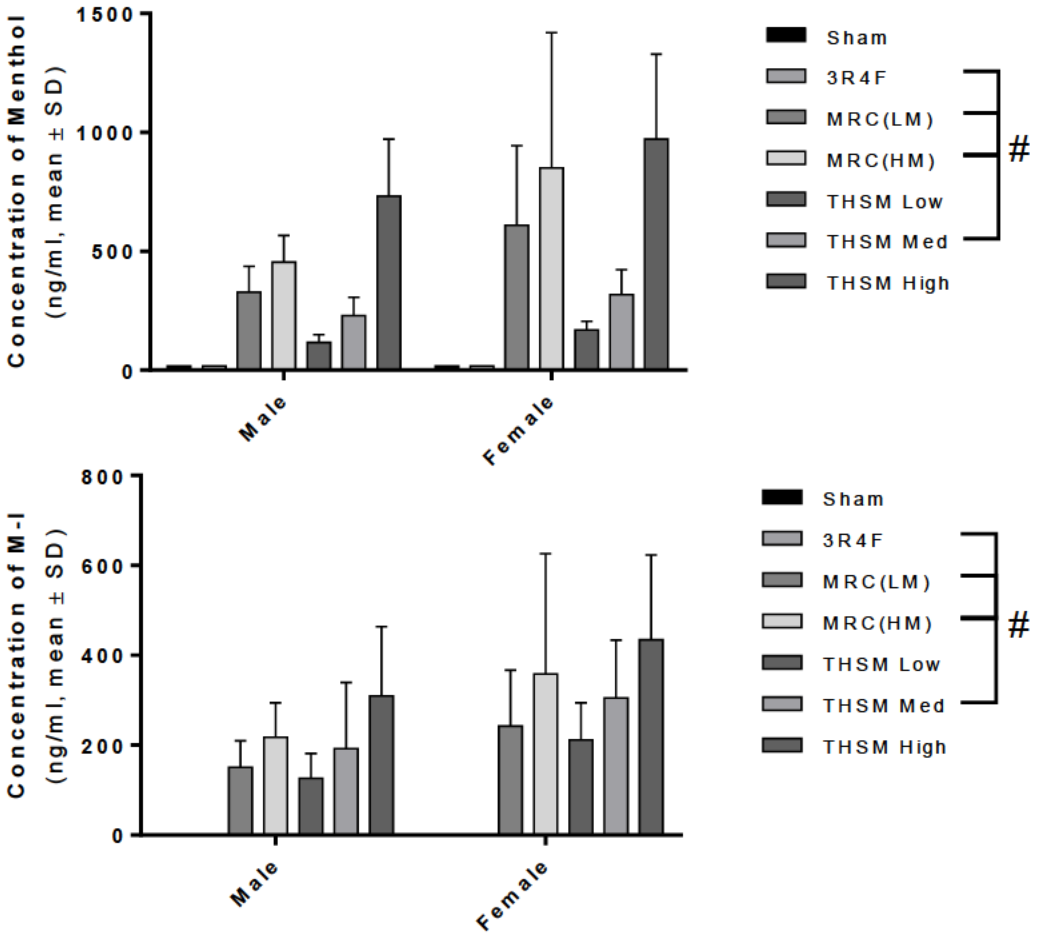


Figure 24 **Concentration of Menthol and M-I in Plasma Collected from THSM-Exposed Animals (15025).**

= Nicotine equivalent concentration. Results are presented as mean and standard deviation. Top panel represents concentrations of menthol and bottom panel concentrations of M-I.

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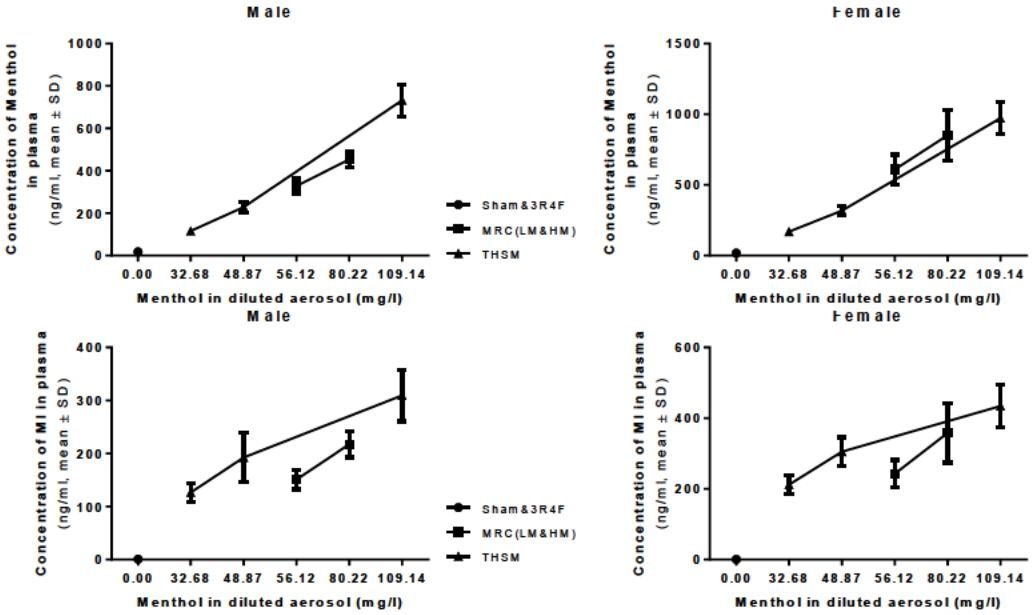


Figure 25 **Concentration of Plasma Menthol and MI Compared with the Menthol Concentration Measured in the Test Item (Diluted Aerosol, Study 15025).**

6.1.3.26.4 Conclusion

In the non-clinical studies, several bio-monitoring parameters have been used to demonstrate that the rats and mice have been adequately exposed and that the animals took up the selected marker substances from the particulate and gas-vapor phase of the different aerosols. The uptake of test atmospheres, respectively particulate and gas vapor phase constituents, by the animals was reflected in the amounts of nicotine metabolites present in the urine and in blood, as well as in the exposure concentration-dependent COHb levels in the blood. For the bio-monitoring parameters for which the chemical (parent) compound was measured in the test atmosphere (e.g. COHb for CO and 3-HPMA for acrolein), a good correlation was shown between test atmosphere concentrations and concentration of bio-monitoring parameters in body fluids. The concentrations of total NNAL, CEMA and S-PMA were very low in all THS-exposed rats and reflective of the aerosol constituent concentrations, based on the data from the analytical smoking i.e. in undiluted aerosol. One exception was observed for 3-HPMA levels which was present in Sham rats and mice due to endogenous production of acrolein (as metabolic product) in normal rats/mice that can contribute to the baseline level of HPMA in urine (Stevens 2008). Plasma samples were also analyzed for nicotine and cotinine levels as well as for menthol and its metabolite M-I showing a correlation between the nicotine present in the aerosol and a concentration-dependent response with the menthol levels present in the undiluted aerosols. The respiratory frequency across THS groups was similar to that of Sham-exposed animals (even with the test atmosphere containing double nicotine concentration) and at equal test atmosphere concentrations of nicotine, THS groups had higher nicotine and nicotine metabolites recovered in blood and/or urine reflecting the lower irritant capacity of the test

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aerosols. The nicotine dose at which the rats and mice were exposed in the non clinical studies were similar to much larger than the doses a normal adult smoker is exposed to.

6.1.3.26.5 List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
BW	Body Weight
CA	Carcinogen
CAS number	Chemical Abstracts Service number
CEMA	2-cyanoethylmercapturic acid
CMC	Chemistry, Manufacturing and Controls
CO	Carbon monoxide
COHb	Carboxyhemoglobin
CT	Cardiovascular toxicant
DD	Delivered Dose
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime
HED	Human equivalent dose
3-HPMA	3-Hydroxypropylmercapturic acid
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime
IUPAC	International Union of Pure and Applied Chemistry
MRC	Mentholated Reference Cigarette
MW	Molecular weight
NA	Not applicable
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
OECD	Organization for Economic Cooperation and Development
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RMV	Respiratory minute volume
RT	Respiratory toxicant
S-PMA	S-phenylmercapturic acid
THS	Tobacco Heating System
THSM	Tobacco Heating SystemMenthol

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THSR	Tobacco Heating System Regular
WHO	World Health Organization

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