STUDY REPORT PART 1. *IN VIVO* EVALUATION OF THE EFFECTS OF THS2.2 MRTP (DEL/C3/FR1/RDO) ON THE RESPIRATORY AND CARDIOVASCULAR SYSTEMS OF APOLIPOPROTEIN E-DEFICIENT MICE (STUDY NUMBER 15015)

Program Name	Systems Toxicology (Sys tox)
Project Name	Animal Clinic
PUC Number	J-1582RDA0140/0001/0001 Animal Clinic
Work Package Name	WP ZRH ApoE switching study
DUC Number	15015
Study Director	PHILLIPS, Blaine
Author(s)	PHILLIPS, Blaine
Date of Study Report (final version)	31 December 2014
Date of Study Report (amended version)	15 July 2015 (see 15015 study report amendment 1)
Date of Study Report (amended version 2.0)	31 Aug 2016 (minor formatting changes to align with 'Global Submit Publishing Standards')

1 Report Structure

This report consists of the following parts:

Part 1. Summary of results collected at the test site.

Part 2. Summary of results from the insulin resistance groups, '*In vivo* evaluation of the effects of THS2.2 on the respiratory and cardiovascular systems of Apo lipoprotein E-deficient mice. Part 2: Metabolic endpoints – Insulin resistance'.

Part 3. Phase report from delegated phase 1 and delegated phase 2 (PMIRL, Neuchatel), 'Study 15015 report, systems toxicology data from samples harvested in study 15015: ApoE$^{-/-}$ ZRH switching study to characterize the toxicity of THS 2.2 in ApoE$^{-/-}$ mice.

Part 4. Phase report from delegated phase 3 (PMIRL, Neuchatel, and Zora Biosciences), 'Lipidomic characterization of ApoE$^{-/-}$ mouse tissue and plasma samples after smoke exposure'.

Part 5. Phase report from delegated phase 4 (Histovia, GmbH), 'Histopathological and morphometric assessment of lung, nose, and liver of ApoE$^{-/-}$ mice'.

Part 6. Rodent MAP analyte analysis in bronchoalveolar fluid, 'Consolidated results and statistical tables from delegated phase 5'.

Part 7. Test report from delegated phase 6,'Determination of HPMA, total NNAL, SPMA, CEMA, 8-OHdG, MDA, 4-HNE, and nine eicosanoids in mouse urine samples and of Nic/Cot in mouse plasma samples from study 15015'.

Part 8. Phase report from delegated phase 7, 'MicroCT analysis of ApoE$^{-/-}$ mouse aortas'.

Part 9. Summary of oxysterols analysis. 'Determination of oxysterols and cholesterol in plasma from a study investigating the effects of Zurich MRTP (DEL/C3/FR1/RDO) on the respiratory and cardiovascular systems of ApoE−/− mice using LC-HR-MS and LC-CAD'.

Part 10. Pathology report, 'In vivo evaluation of the effects of THS2.2 on the respiratory and cardiovascular systems of Apolipoprotein E-deficient mice, histopathology of the pancreas and kidney'.

Table of Contents

1 Report Structure 2
2 Table of Figures 6
3 Table of Tables 8
4 Review and Approval 11
4.1 Study Director's Statement of Compliance 11
5 Abstract 12
6 Introduction 14
7 Objectives 15
8 Methods 15
8.1 Test and Reference Items 15
8.1.1 Test item: THS2.2 15
8.1.2 Reference item: 3R4F conventional cigarettes 16
8.1.3 Treatment administration 16
8.1.4 Test atmosphere generation and characterisation 17
8.1.5 Exposure levels versus target levels 21
8.2 Test System 24
8.3 Experimental Groups and Dosing 25
8.3.1 Allocation of animals to treatment groups 25
8.3.2 Test groups and exposure 28
8.4 Procedures 30
8.4.1 Parameters used to monitor animal health 30
8.4.2 Parameters for monitoring smoke uptake and exposure 31
8.4.3 Necropsy 35
8.4.4 Lung inflammation 38
8.4.4.1 Collection of BALF 38
8.4.5 Histoprocessing and staining 40
8.4.6 Histopathology 41
8.4.7 Lung function measurements 41
8.4.8 Tissue processing for 'omics' analysis (transcriptomics, genomics, proteomics, and lipidomics) 41
8.4.9 Blood collection 41
8.4.9.1 Whole blood for RNA extraction 41
8.4.9.2 Whole blood for DNA extraction 42
8.4.9.3 Whole blood for haematology 42
8.4.9.4 Serum for proteomics/inflammation 42
8.4.9.5 Partitioning of liver and lungs during dissection 42
8.4.10 Determination of lipids 43

8.4.10.1 Plasma collection for determination of oxysterols 43
8.4.10.2 Plasma collection for lipidomics (delegated phase 3) 43
8.4.10.3 Oil red O staining 43
8.4.10.4 Lipoprotein analysis 43
8.4.11 Determination of plaque size measurement 43
8.4.12 Apoptosis/oxidative stress in the lungs and liver 43
8.4.13 Vascular inflammation 44
8.4.14 Platelet aggregation assay 44
9 Statistical Methods 45
9.1 Descriptive Statistics 45
9.2 Missing Values and Values outside Quantification Range 46
9.3 Treatment Comparisons 46
9.4 Extreme Values 46
9.5 Significance Level 46
10 Administrative Aspects 47
10.1 Study Timelines 47
10.2 Names and Addresses 48
10.3 Multi-Site Arrangements 51
11 Study Plan Amendments and Deviations 53
12 Results 54
12.1 Parameters Used to Monitor the General Condition and Health of the Animals 54
12.1.1 Mortality 54
12.1.2 In-life observations 56
12.1.3 Body weight 57
12.2 Parameters used to Monitor Smoke Uptake and Exposure 58
12.2.1 Carboxyhemoglobin 58
12.2.2 Plasma nicotine and cotinine 59
12.2.3 Representative nicotine metabolites in mouse urine 59
12.2.4 Additional smoke constituent metabolites in mouse urine 64
12.3 Lung Function Measurements 70
12.3.1 Snapshot perturbations (single compartment model) 70
12.3.2 Primewave-3 (constant phase model) 71
12.3.3 Quasi-static pressure volume loop (Salazar–Knowles analysis) 73
12.3.4 Negative pressure forced expiration 77
12.4 Haematology 79

12.4.1 Red blood cells 79
12.4.2 White blood cells 81
12.5 Lung Inflammation 84
12.5.1 Absolute and relative numbers of FLCs 84
12.5.2 Differentiation of lymphocytes 88
12.5.3 Macrophage activity 90
12.5.4 Gelatinolytic activity in BALF supernatant 92
12.5.5 Inflammatory mediators in BALF supernatant 93
12.5.6 Inflammatory mediators in serum 93
12.5.7 Oxysterols in plasma 96
12.6 Atherosclerosis and Clinical Chemistry 98
12.6.1 Plaque area measurements 98
12.6.2 Plaque volume by micro-CT imaging 101
12.6.3 Clinical chemistry 104
12.7 Histopathology and Morphometric Analysis 110
12.7.1 Lung volume measurement 110
12.7.2 Histopathology and morphometry of the lung 112
12.7.3 Histopathology of the nose 116
12.7.4 Histopathology of the liver 118
12.7.5 Histopathology of the pancreas and kidney 118
12.8 Gene Expression Profiling and Omics 119
12.9 Lipidomics Profiling 119
12.10 Insulin Resistance (IR) 119
13 Discussion 120
13.1 Exposure and In-life Observations and Results 120
13.2 Emphysema and Pulmonary Inflammation 121
13.3 Cardiovascular Endpoints 123
14 Conclusions 123
15 Archiving 124
16 References 125
17 Abbreviations 127
18 Tables and figures 129
19 Appendix 1. Amendments and deviations 231

2 Table of Figures

Figure 1. Smoking machine layout for 3R4F groups. 19
Figure 2. Smoking machine layout for THS2.2 groups. 19
Figure 3. Nicotine concentrations in the exposure chamber breathing zone. 22
Figure 4. Schematic of the study set-up. 26
Figure 5. Exposure regime. 28
Figure 6. Liver and lung necropsy for 'omics' analysis. 42
Figure 7. Body weight for all exposure groups during the exposure period. 57
Figure 8. Blood carboxyhaemoglobin concentration 58
Figure 9. Plasma nicotine and cotinine concentrations 59
Figure 10. Nicotine metabolites in urine. 60
Figure 11. Nicotine and its metabolites in the urine (individual metabolites). 63
Figure 12. Urinary biomarkers of exposure (non-nicotine exposure metabolites). 65
Figure 13. Urinary biomarkers of exposure (markers of oxidative stress). 66
Figure 14. Urinary biomarkers of inflammation and eicosanoids. 69
Figure 15. Lung function (snapshot perturbations). 71
Figure 16. Primewave-3 perturbations 73
Figure 17. Pressure volume loops. 75
Figure 18. Salazar–Knowles analysis 76
Figure 19. Negative pressure forced exhalation perturbations 78
Figure 20. Haematology, red blood cell parameters 80
Figure 21. Total number of white blood cells 81
Figure 22. Differential cell counts (absolute) of white blood cells and platelets. 82
Figure 23. Differential cell counts (relative) of white blood cells. 83
Figure 24. Reticulocyte counts (absolute and relative amounts). 84
Figure 25. Total FLCs in BALF 85
Figure 26. BALF FLC composition: absolute counts of principal inflammatory cells 86
Figure 27. BALF FLC composition: relative levels of inflammatory cells 87
Figure 28. Lymphocyte differentiation 89
Figure 29. Markers of macrophage activation 91
Figure 30. MMP-9 and Timp-1 protein levels and gelatinolytic activity in lavage fluid. 92
Figure 31. RodentMAP (Myriad/Rules Based Medicine) analyte analysis from cell-free BALF fluid. 94
Figure 32. RodentMAP (Myriad/Rules Based Medicine) analyte analysis from serum. 95
Figure 33. Oxysterol levels, month 8 post-exposure. 97
Figure 34. Aortic arch plaque area measurements 99
Figure 35. Plaque area measurements in aortic arches. 100
Figure 36. Micro-CT images of representative aorta samples. 101
Figure 37. Quantification of aorta (and primary branching vessels) plaque burden by microCT. 103

Figure 38. Clinical chemistry parameters 105
Figure 39. Vertical auto profiling; total cholesterol and HDL/non-HDL fractions 106
Figure 40. Vertical auto profiling; IDL and LDL cholesterol sub-fractions 108
Figure 41. Vertical auto profiling; VLDL cholesterol sub-fractions....109
Figure 42. Lung volume measurements 111
Figure 43. Morphometric assessment of the lung 113
Figure 44. Lung morphology, month 8 timepoint. 114
Figure 45. Infiltration into the alveolar lumen....115
Figure 46. Histopathological assessment of the nose, level 1, respiratory epithelium....117
Figure 47. Morphology of the nose, level 1, respiratory epithelium. 8-month time point. 118

3 Table of Tables

Table 1. Deviations from ISO standards 3308 (2000) for THS2.2. 18
Table 2. Aerosol analysis 20
Table 3. Analysis of test atmospheres 21
Table 4. Analysis of test atmospheres: aldehydes 23
Table 5. Particle size distribution 23
Table 6. Exposure groups, exposure concentration targets and durations. 27
Table 7. Exposure chambers and numbering 29
Table 8. General conditions and health parameters 30
Table 9. Biomarkers of exposure 31
Table 10. Carboxyhaemoglobin sample collection 32
Table 11. Urine sample collection 34
Table 12. Number of animals from each exposure group, and per necropsy phase 35
Table 13. Lung inflammation 39
Table 14. FLC antibody mixes 40
Table 15. Study plan amendments and deviations 53
Table 16. Mortality and moribund state 55
Table 17. Moribund or dead animals by exposure group 56
Table 18. Body weight (g) 129
Table 19. HPMA (absolute, ng) 139
Table 20. NNAL (absolute, pg) 140
Table 21. SPMA (absolute, ng) 141
Table 22. CEMA (absolute, ng) 142
Table 23. 8-OHdG (absolute, ng) 143
Table 24. MDA (absolute, pmol) 144
Table 25. 4-HNE (absolute, pmol) 145
Table 26. t-PGDM (absolute, ng) 146
Table 27. t-PGEM (absolute, ng) 147
Table 28. 2,3-d-8-i-PGF2a (absolute, ng) 148
Table 29. 2,3-d-TXB2 (absolute, ng) 149
Table 30. 11-dh-TXB2 (absolute, ng) 150
Table 31. PGF2a (absolute, ng) 151
Table 32. 8i-PGF2a (absolute, ng) 152
Table 33. LTE4 (absolute, ng) 153
Table 34. 12-HETE (absolute, ng) 154
Table 35. Lung function, Resistance (cmH2O.s/ml) 155
Table 36. Lung function, elastance (cmH2O/ml) 156
Table 37. Lung function, compliance (ml/cmH2O) 157

Table 38. Lung function, Newtonian resistance (cmH2O.s/ml) 158
Table 39. Lung function, tissue resistance (cmH2O/ml/s) 159
Table 40. Lung function, tissue elastance (cmH2O/ml/s) 160
Table 41. Lung function, tissue hypersensitivity (G/H) 161
Table 42. Lung function, A (ml) 162
Table 43. Lung function, Salazar-Knowles equation (/cmH2O) 163
Table 44. Lung function, quasi-static compliance (ml/cmH2O) 164
Table 45. Lung function, quasi-static elastance (cmH2O/ml). 165
Table 46. Area in PV loops (hysteresis) (cmH2O.ml). 166
Table 47. Lung function, FEV0.1 (ml). 167
Table 48. Lung function, FEV0.2 (ml) 168
Table 49. Lung function, FEV0.1/FVC (%) 169
Table 50. Lung function, FEV0.2/FVC (%) 170
Table 51. Lung function, FVC (ml). 171
Table 52. Lung function, Vtr end (ml) 172
Table 53. Erythrocyte count (10^6 cells/ul) 173
Table 54. Mean erythrocyte volume (fL) 174
Table 55. Hemoglobin (g/l) 175
Table 56. Mean hemoglobin content (g/dL) 176
Table 57. Mean hemoglobin volume (pg) 177
Table 58. Hematocrit (L/L) 178
Table 59. Leucocyte count (10^3 cells/ul) 179
Table 60. Lymphocyte count (10^3 cells/ul) 180
Table 61. Neutrophil count (10^3 cells/ul) 181
Table 62. Monocyte count (10^3 cells/ul) 182
Table 63. Basophil count (10^3 cells/ul) 183
Table 64. Eosinophil count (10^3 cells/ul) 184
Table 65. Platelet count (10^3 cells/ul) 185
Table 66. Lymphocytes, relative (%) 186
Table 67. Neutrophils, relative (%) 187
Table 68. Monocytes, relative (%) 188
Table 69. Basophils, relative (%) 189
Table 70. Eosinophils, relative (%) 190
Table 71. Reticulocyte counts (10^3 cells/ul) 191
Table 72. Reticulocytes, relative (%) 192
Table 73.BALF free lung cells, total cell count (10^5 cells) 193
Table 74. BALF alveolar macrophages, absolute (10^5 cells) 194
Table 75. Alveolar dendritic cells, absolute (10^5 cells) 195
Table 76. BALF neutrophils, absolute (10^5 cells) 196

Table 77. BALF lymphocytes, absolute (10^5 cells) 197
Table 78. Lymphocyte differentiation assay, CD4+ relative to total lymphocytes (%) 198
Table 79. Lymphocyte differentiation assay, CD8+ relative to total lymphocytes (%) 199
Table 80. Lymphocyte differentiation assay, B cells relative to total lymphocytes (%) 200
Table 81. Lymphocyte differentiation assay, lymphocytes, absolute (10^5 cells) 201
Table 82. Lymphocyte differentiation assay, CD4+ cells, absolute (10^5 cells) 202
Table 83. Lymphocyte differentiation assay, CD8+ cells, absolute (10^5 cells) 203
Table 84. Lymphocyte differentiation assay, B cells, absolute (10^5 cells) 204
Table 85. Macrophage activation assay, CD54 positive (median fluorescent intensity) 205
Table 86. Macrophage activity assay, CD86 positive (median fluorescent intensity) 206
Table 87. Macrophage activity assay, CD11b positive (median fluorescent intensity) 207
Table 88. BALF MMP activity (mU/ml) 208
Table 89. BALF MMP-9 protein levels (ng/ml, MAP analysis) 209
Table 90. BALF Timp-1 protein levels (ng/ml, MAP analysis) 210
Table 91. Plasma 4β-hydroxycholesterol (ng/ml) 211
Table 92. Plasma 7β-hydroxycholesterol (ng/ml) 212
Table 93. Plasma 25-hydroxycholesterol (ng/ml) 213
Table 94. Aortic arch plaque area relative to total measured area (%) 214
Table 95. Clinical chemistry, cholesterol (mmol/l) 215
Table 96. Clinical chemistry, glucose (mmol/l) 216
Table 97. Clinical chemistry, high density lipoprotein (HDL, mmol/l) 217
Table 98. Total cholesterol (VAP test, Atherotech) 218
Table 99. Lipoprotein – HDL (VAP test, Atherotech) 219
Table 100. Lipoprotein – Non-HDL (VAP test, Atherotech) 220
Table 101. Lipoprotein – HDL2 (VAP test, Atherotech) 221
Table 102. Lipoprotein – HDL3 (VAP test, Atherotech) 222
Table 103. Lipoprotein – LDL (VAP test, Atherotech) 223
Table 104. Lipoprotein – IDL (VAP test, Atherotech) 224
Table 105. Lipoprotein – VLDL (VAP test, Atherotech) 225
Table 106. Lipoprotein – VLDL3 (VAP test, Atherotech) 226
Table 107. Lipoprotein – LDL-4 (VAP test, Atherotech) 227
Table 108. Lipoprotein – LDL3 (VAP test, Atherotech) 228
Table 109. Lipoprotein – LDL2 (VAP test, Atherotech) 229
Table 110. Lipoprotein - LDL1 (VAP test, Atherotech) 230

4 Review and Approval

This document has been approved or reviewed by:

Name	Justification	Date / Signature
Chua, Joanne	Approved by Test Facility Management	31 Aug 16
Kevin Fong	Formatting and editorial revisions reviewed by QA	31 Aug 16

Note, this version of the report has been modified from the original study report in order to address minor formatting changes required to align with the 'Global Submit Publishing Standards'. There are no changes to the study report contents. This version will be filed with the original study report in the archive.

4.1 Study Director's Statement of Compliance

The study director acknowledges responsibility for the validity of the data.

Name	Justification	Date / Signature
Phillips, Blaine	Signed as Study Director	31Aug16

5 Abstract

The development and progression of chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and metabolic disease (insulin resistance, IR) were evaluated in Apolipoprotein E-deficient ($ApoE^{-/-}$) mice following chronic exposure to mainstream cigarette smoke (CS), or to an aerosol from the Tobacco Heating System 2.2 (THS2.2, a prototypic modified risk tobacco product). Additionally, the biological effects of smoking cessation or switching from conventional CS to the THS2.2 aerosol were examined.

$ApoE^{-/-}$ mice were exposed to diluted CS from conventional cigarettes (3R4F), to an aerosol from THS2.2, or to filtered fresh air for 3 h per day, 5 days per week, for up to 8 months. After 2 months of exposure to CS, designated groups of animals were switched to THS2.2 aerosol exposure (switching group), or exposed to filtered air (cessation group) for up to 6 months following the same daily exposure regimen. Exposure concentrations of 3R4F or THS2.2 aerosols were matched for nicotine content, with a target nicotine concentration of 29.9 µg/l (equivalent to approximately 600 µg TPM/l in CS). Aerosol uptake was confirmed by monitoring levels of carboxyhaemoglobin in the blood and the amounts of secreted urinary metabolites of nicotine and other smoke constituents. At designated time points (months 1, 2, 3, 6, and 8 after the beginning of the exposure), the animals were examined for multiple parameters to comprehensively assess the development and progression of COPD or CVD indicators. These included pulmonary inflammation measurements, assessment of pulmonary function, histopathology, atherosclerotic plaque formation in the aortic arch and descending aorta, blood clinical chemistry, and the examination of molecular changes in various tissues throughout the exposure period by transcriptomics, proteomics, and lipidomics.

Exposure of the mice to CS induced acute inflammatory changes in the lungs and airways as demonstrated by the appearance of increased numbers of inflammatory cells and mediators in the bronchoalveolar lavage fluid (BALF) as early as 1 month following the start of exposure, and persisting until the end of the study. Physiological changes to the lungs (pulmonary function parameters) were evident in CS-exposed animals as of month 1 onwards, particularly the upward and leftward shifting of pressure volume loops (PVs-P), which is characteristic of emphysema. These effects on the lung were substantiated by histopathological and histomorphometric changes of the lung tissue, which were interpreted as the onset of emphysema. The development of atherosclerotic plaques (plaque area) increased following CS exposure at each time point. Although animals in the THS2.2 group were exposed to matched aerosol nicotine levels, they demonstrated only minimal differences from sham-exposed animals with respect to most of the parameters assessed. Both switching and cessation groups showed a reversal of the inflammatory response, which in some cases (i.e., BALF inflammatory infiltrate), returned to sham-exposed levels after 3 months of cessation or switching. In all cases, the switching groups showed a reversal compared with the cessation groups. Reversal and/or attenuation of the pathology progression after the switch and cessation was observed in other parameters analysed in this study (pulmonary function, aorta plaque formation and lung tissue damage).

These responses were also observed at the molecular level as significant network perturbations (both differential gene expression and biological impact factor) and changes in protein levels were identified in lung tissue and

nasal tissue following exposure to CS as early as 1 month post exposure, but were considered relatively mild in THS2.2-exposed animals. Smoking cessation and switching both resulted in recovery at the transcriptional level, leading to the amelioration of most of the observed molecular changes relative to exposure to CS. Similarly, the lipid profiles in the lung, liver and plasma showed primarily a CS effect, with few changes associated with THS2.2 exposure, and recovery to near sham-exposed levels following either switching or cessation.

These data demonstrate that exposure of ApoE$^{-/-}$ mice to an aerosol from THS2.2 for up to 8 months resulted in changes at the anatomical, physiological, and molecular level that were largely indistinguishable from those observed in animals exposed to fresh air. Furthermore, following exposure to CS for 2 months, both cessation and switching to THS2.2 aerosol exposure (matched to similar nicotine levels as CS-exposed animals) resulted in the reversal (inflammation and molecular changes) or no further progression/stabilization (pulmonary function and lung tissue morphometry) of all parameters assessed.

6 Introduction

Cigarette smoking is a strong causative element contributing to the development and progression of a series of diseases, including cardiovascular disease (CVD) (Price et al, 1999) and chronic obstructive pulmonary disease (COPD) (Arunachalam et al, 2010; Pauwels & Rabe, 2004). Significant health benefits have been associated with smoking cessation (Dhariwal et al, 2014), so it is of considerable interest to determine whether such benefits are transferrable to the consumption of, or switching to, alternative tobacco products. However, the underlying mechanisms of both disease progression and the biological impact of smoking cessation or switching to another product remain largely unknown. This study examined the development of indicators of both COPD and CVD in Apolipoprotein E-deficient ($ApoE^{-/-}$) mice exposed to either conventional mainstream cigarette smoke (CS), or to the mainstream aerosol from the prototypic modified risk product (pMRTP) Tobacco Heating System 2.2 (THS2.2) over an 8-month period. In addition to chronic exposure regimes, two animal groups were allocated to investigate effects of cessation or switching to THS2.2 aerosol exposure after 2 months of CS exposure using a range of physiological, histological, and molecular evaluations.

Animals were exposed to CS and/or THS2.2 aerosol (600 µg/l total particulate matter (TPM) CS or 29.9 µg/l nicotine equivalent for THS2.2) for 3 h per day, 5 days per week, for up to 8 months. A previous study (Berges, 2011; Lietz et al, 2013) showed that this exposure regime and timeframe resulted in the formation of larger atherosclerotic plaques relative to sham-exposed animals, or to animals exposed to a prototypic pMRTP from the platform 2 line of products (Smar, MD2). Additionally, the increased pulmonary inflammation and histopathological changes to the lung and other target tissues known to occur in $ApoE^{-/-}$ mice, indicative of emphysematous or cardiovascular changes following CS exposure, make them an excellent model to evaluate both COPD and cardiovascular disease markers (Boue et al, 2013; Boue et al, 2012) .

The animals were allocated to the following study groups:

i) Sham: exposed to filtered air

ii) 3R4F: mainstream CS from 3R4F reference cigarettes (29.9 µg/l nicotine target dose)

iii) THS2.2: aerosol from an electrically heated stick from the Zurich development platform (29.9 µg/l nicotine target dose)

iv) Cessation: 2 months 3R4F, followed by filtered air

v) Switching to THS2.2: 2 months 3R4F, followed by THS2.2 aerosol

vi) Insulin resistance (IR): an additional group of mice were allocated to study the effect of chronic CS or THS2.2 aerosol on the development of insulin resistance. Note, the results from the insulin resistance study arm were reported in entirety in a separate study report (Report part 2)

At the end of several intermediary time points (1, 2, 3, 6, and 8 months), organs, tissues, and blood were collected for molecular analysis (proteomics, transcriptomics, genomics, and lipidomics). Pulmonary pathological and functional changes were assessed by standard histopathology or morphometric analysis as well as by lung

function testing, which was performed in selected groups. In addition, the following endpoints were examined: pulmonary inflammation, atherosclerotic plaque development, biomarkers of exposure, and the effects of aerosols on metabolic parameters (glucose and insulin levels, as well as metabolic analyte levels in the plasma).

The original experimental design included an inflammation arm in which anti-inflammatory compounds were to be assessed in the context of CS, or of THS2.2 aerosol exposure. However, based on the results of the preliminary inflammation study, study 15013, it was decided to instead reassign these animals into the insulin resistance arm, and to introduce additional cardiovascular endpoints (micro CT imaging, delegated phase 7, Numira Biosciences, Inc.) (Amendment 8, and Deviation 4).

All experimental procedures were performed according to PMIRL-S QMS procedures, unless otherwise specified.

7 Objectives

The objectives of this study were to:

- Investigate the effects of THS2.2 on atherosclerosis and respiratory-related endpoints (i.e. emphysema) in comparison with CS- and sham-exposed control groups after 1, 2, 3, 6, and 8 months of exposure.
- Assess the effects of switching from CS after 2 months of exposure to THS2.2 pMRTP in comparison with cessation (after 2 months of CS exposure) or sustained CS smoke exposure on the development and progression of atherosclerosis and respiratory-related endpoints at 3, 6, and 8 months.
- Demonstrate the potential of heating (pMRTPs) versus burning (CS) to reduce exposure-related risks for the development of smoke-related diseases.
- Investigate the effects of conventional CS or THS2.2 pMRTP aerosol on the development and progression of IR.

8 Methods

8.1 Test and Reference Items

8.1.1 Test item: THS2.2

The test item was a THS composed of a heating device (CH FDP4.2) and a sensorial media, THS2.2 including flavour mix (Del/C3/FR1/Rdo). The sensorial medium (hereafter referred to as ‘stick’) is a cigarette containing a tobacco plug, transfer section, and mouth piece with an over-wrapping of cigarette paper. The sticks were provided by Philip Morris International (PMI), Neuchâtel, Switzerland. Aerosol specifications (batch quality control and batch release conditions) were stored in the Project Management Folder as batch release certificates.

THS2.2 sticks were produced at PMI (Neuchâtel, Switzerland) in three batches, because stick stability has only been tested up to 6 months (shorter than the 8-month study period). Production volumes and delivery schedules were as follows:

1. Batch 1 B-05771, 60,000 sticks, delivered 04 July 2013
2. Batch 2 B-06132, 290,000 sticks, delivered 14 August, 2013
3. Batch 3 B-08163, 210,000 sticks, delivered 21 November, 2013

The sticks were packaged in mini princess packs (two collations of 10 sticks each) labelled with the product identification (Del/C3/FR1/Rdo), batch number, and production date. Specific colour of the cork tipping paper was used to physically differentiate the sticks from each batch. All sticks were stored in their original packaging in room 2024 at a controlled room temperature (2–10°C), but uncontrolled humidity conditions. The sticks were conditioned in rooms 3101 to 3103 according to ISO standard 3402 (1999), for between 7–21 days at temperature and humidity ranging from 21.3 ± 0.3°C to 21.5 ± 0.2°C and 57.4 ± 1.2% to 57.5 ± 1.4% respectively before being used for aerosol generation.

As part of the smoking system, the sensorial medium was inserted into a cigarette holder that heats the tobacco plug. The cigarette holder included a battery, electronics for controlling, a heating element, and the cigarette extractor. Cigarette holders were provided by PMI.

The daily use of THS2.2 cigarette holders was documented by the aerosol team. A total of 586 cigarette holders delivered in three batches (B-06143, B-07250, and B-09492) were used in this study. The product description was FPD 4.2, and the product code DV-000067.RD.

8.1.2 Reference item: 3R4F conventional cigarettes

3R4F cigarettes were purchased from the University of Kentucky (http://www.ca.uky.edu/refcig) as soft packs of 20 cigarettes (10 soft packs/carton, 25 cartons/box). All cigarettes were stored in their original packaging in room 2025 at a controlled room temperature (2–10°C) but uncontrolled humidity conditions. Prior to smoking, the cigarettes were conditioned in room 3102 for 7–21 days at a temperature of 21.3 ± 0.3°C to 21.5 ± 0.2°C with a relative humidity ranging from 56.6 ± 0.6% to 56.9 ± 1.5%.

8.1.3 Treatment administration

Whole-body exposure chambers (type WBEC85) were used as they provide the most practical and reproducible means of exposing mice to CS or THS2.2 aerosols. The target concentration of CS from 3R4F conventional cigarettes was 600 µg/l TPM. This concentration provided 29.9 µg/l of nicotine within the test atmosphere, as assessed during the test smoking period which was performed prior to the study start. THS2.2 aerosol exposures were concentration-matched to nicotine levels in the CS.

For conventional cigarettes, the aerosol generated from one smoking machine was diluted with filtered, conditioned air to the target concentration of 29.9 µg/l nicotine (600 µg/l TPM) for delivery to one exposure

chamber (Figure 1). For THS2.2 exposure groups, it was necessary to combine the aerosols from two smoking machines to achieve the target 29.9 µg/l nicotine concentration in the exposure chamber (Figure 2). The mainstream smoke or aerosol from the test or reference items was conveyed via glass tubing from the smoking machines to the exposure chambers. For the sham group, mice were exposed to filtered fresh air. The total flow rate through the whole-body exposure chambers was adjusted as a means to control the aerosol concentrations where required, and to ensure adequate airflow to the animals residing in the exposure chamber. The mean flow through the chambers ranged from 80.1 ± 1.2 L/min to 80.2 ± 1.8 L/min for the sham chambers, from 94.1 ± 5.5 to 105.7 ± 15.9 L/min in the 3R4F chamber, and 104.4 ± 7.9 to 108.4 ± 11.8 L/min for the THS2.2 chambers.

8.1.4 Test atmosphere generation and characterisation

3R4F cigarettes and THS2.2 sticks were smoked according to the Health Canada Intensive Smoking Protocol (1999) based on ISO standards 3308 (revised in 2000), with the exception of the puff volume (55 ml), puff frequency (one puff every 30 s) and ventilation block. Several minor deviations from ISO standards 3308 (2000) were necessary for technical reasons (Table 1).

Table 1. Deviations from ISO standards 3308 (2000) for THS2.2.

Deviation for puff count, relative humidity, and temperature was also applicable for 3R4F.

Parameter	ISO 3308 (2000)	Recommended condition for 3R4F	Recommended condition for THS2.2
Puff count	Rounded to nearest one-tenth of a puff	Only whole puffs	Only whole puffs
Restricted smoking	Restricted smoking	No deviation	Free/open end smoking because of smoking machine configuration
Cigarette holder	Labyrinth seal and perforated disc (washer)	No deviation	Single seal and no washer
Puff termination & counting	Butt length sensor/0.1 puff counting resolution	Butt length sensor/only whole puffs counted	Fixed number of puffs, device configured to 12 puffs/only whole puffs counted
Relative humidity during smoking	60 ± 5%	Air supplied to smoking machine meets requirements. Conditions inside smoking machines unmonitored	Air supplied to smoking machine meets requirements. Conditions inside smoking machines unmonitored
Temperature during smoking	22 ± 2°C	Air supplied to smoking machine meets requirements. Conditions inside smoking machines unmonitored	Conditions inside smoking machine unmonitored
Air velocity around sticks	170–230 mm/s	No deviation	THS stick enclosed within cigarette holder. Airflow around stick controlled by device

CS from the 3R4Fsticks was generated on 30-port rotary smoking machines with active side stream exhausts (type PMRL-G, SM2000) and equipped with programmable dual-port syringe pumps (PDSPs). Aerosols from THS2.2 sticks were generated using 30-port carousel “Smoking Machines Smoking Device Docking Station Zurich (SM-SDDS-ZRH)” equipped with cigarette holders and PDSPs. SM-SDDS-ZRH were developed by PMI R&D. The SDDS served as an interface to ensure synchronization between the cigarette holder and the smoking machine. In previous studies using THS2.2 mainstream aerosols, condensation was noted in the glass tubing, and therefore SM-SDDS-ZRH also included a temperature-controlled insulation kit (tube warming system) in the undiluted aerosol pathway,which has been shown to reduce the overall condensation effect. The smoking machine and chamber layout for both 3R4F (SM-2000) and THS2.2 (SM-SDDS-ZRH) aerosol generation are depicted in Figure 1 and Figure 2, respectively. The sham layout was similar to that of the 3R4F, except that the machines were loaded with sham sticks with no ignition step (i.e., no aerosol generated, and only filtered air delivered to the chambers)

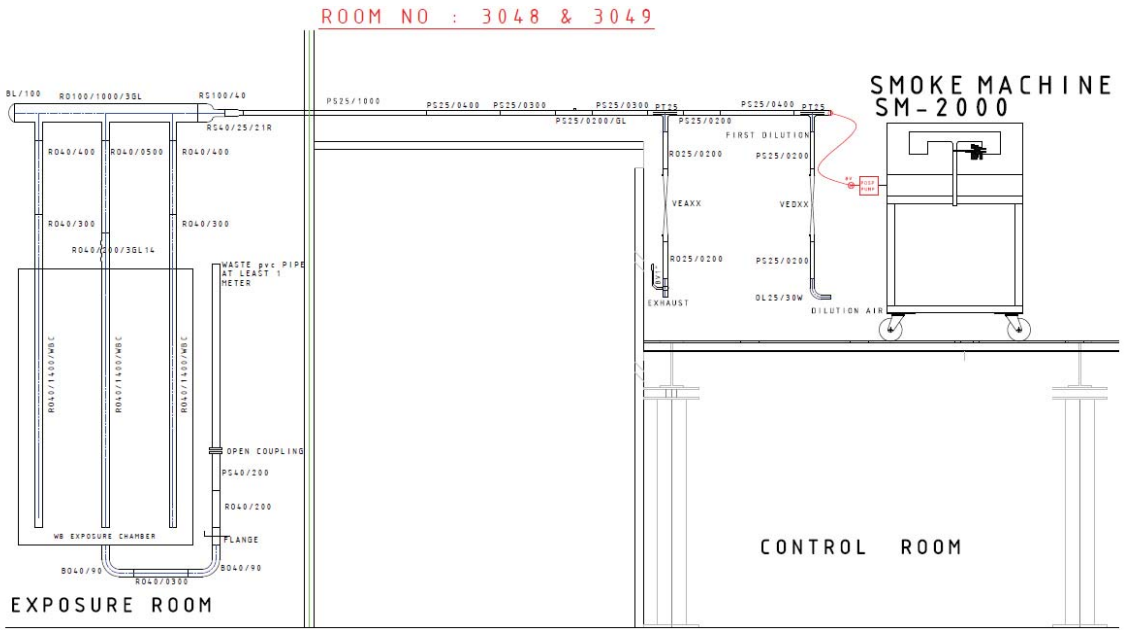


Figure 1. Smoking machine layout for 3R4F groups.

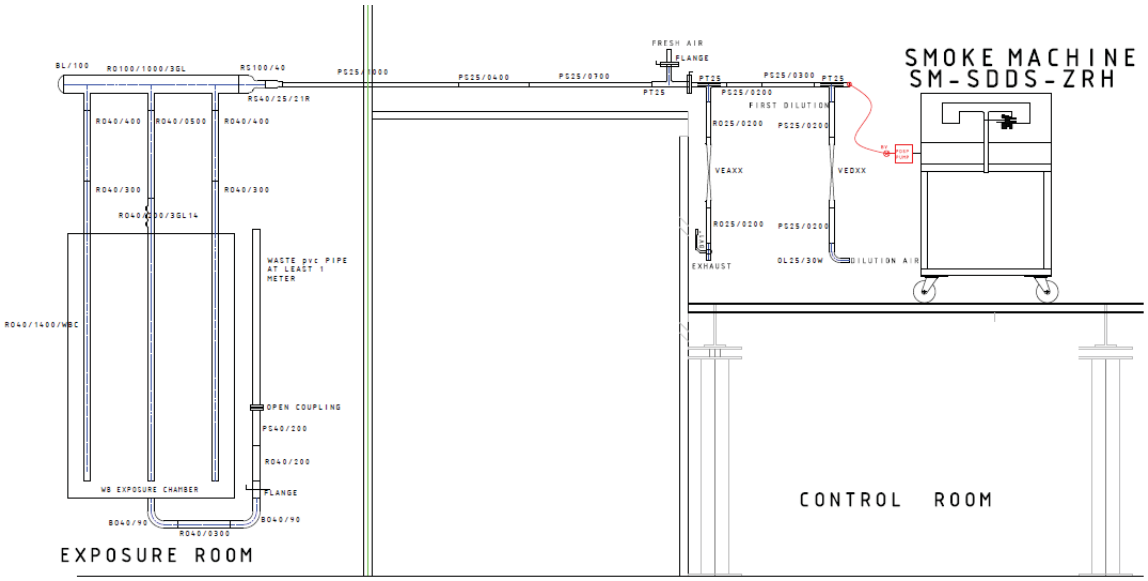


Figure 2. Smoking machine layout for THS2.2 groups.

Note that THS2.2 aerosol delivery requires the use of two smoke machines (SM-SDDS-ZRH) to achieve target aerosol concentrations.

During the exposure period, the chamber inlet temperature of the test atmosphere in the exposure chamber was maintained between 21.7 ± 0.4°C and 23.4 ± 0.6°C and the relative humidity between 58.4 ± 1.7% and 59.8 ± 2.2%. Air flow and relative humidity through the exposure chamber were monitored continuously and recorded during the entire exposure period.

To characterize the test atmosphere and to check the reproducibility of aerosol generation and dilution, several analytical parameters were determined around the animal breathing area (Table 2). Particle size distribution was performed using the PIXE impactor. Several measurements were also performed in the Sham exposure chamber.

Table 2. Aerosol analysis

Parameter	Assay principle	Determination schedule SHAM	Determination schedule 3R4F	Determination schedule THS2.2
TPM	Gravimetry after trapping on Cambridge filters	≥ 1/week	During every exposure block	During every exposure block
Carbon monoxide	Non-dispersive infrared photometry of gas/vapour phase	Continuously	Continuously	Continuously
Nicotine	Capillary gas chromatography after trapping on sulfuric acid-impregnated silica gel	≥ 1/week	During every exposure block	During every exposure block
Formaldehyde, acetaldehyde, and acrolein	Reversed phase HPLC of DNPH derivatives after trapping in DNPH solution	N/A	≥ 1/week	≥ 1/week
Relative humidity	Capacitive	Continuously	N/A	N/A
Temperature	Thermistor probe Pt100	Continuously	Continuously	Continuously
(Static) puff volume	Soap bubble flow meter	Before and after daily smoke generation	Before and after daily smoke generation	Before and after daily smoke generation
Flow rate through exposure chamber	Pressure difference over a Venturi tube	Continuously	Continuously	Continuously
Particle size distribution	Impactor	≥1/month	≥1/month	≥1/month

HPLC, high-performance liquid chromatography; DNPH, 2,4-dinitrophenylhydrazine

The puff count for THS2.2 sticks was set to 12 puffs because of the configuration of the smoking machine, while the 3R4F puff count ranged from 10–12 puffs because they were smoked to a specified butt length (average, 10.5 puffs per stick across each of the smoking machines). The static burning rate for the 3R4F cigarettes ranged from 414.9 +/- 26.0 s/40mm to 452.6 +/- 35.3 s/40mm. The 3R4F cigarettes were smoked to a butt length of 34.1 ± 0.7

mm to 35.4 ± 1.2 mm; the butt length and puff count for 3R4F were determined monthly while static burning rate was determined at least once during the inhalation period. Due to the nature of the THS2.2 product, butt length and static burning rate are not applicable, and were therefore not assessed.

8.1.5 Exposure levels versus target levels

Following a 2-week adaptation period, aerosol target concentrations in the exposure chambers were set at 29.9 µg nicotine/l (equivalent to 600 µg/l TPM from conventional 3R4F CS) for both 3R4F and THS2.2 groups. Nicotine concentrations were determined three times daily for both THS2.2 and 3R4F exposure chambers. In the animal breathing zone of the exposure chambers, mean daily nicotine concentrations showed a fluctuation (Figure 3), occasionally falling outside of the ±20% concentration range (described in deviation 2). High maintenance smoking machines, particularly for THS2.2, were subject to blockages or technical faults resulting in occasional smoking machine downtime. During these periods, the chamber aerosol concentration dropped, thereby affecting average daily concentrations. Nonetheless, the resulting average nicotine concentrations across all chambers ranged from 29.2–29.8 µg/l for 3R4F chambers, and 28.5–28.7 µg/l for THS2.2 chambers, which were within the specified study target range of ± 10% of target concentrations (Table 3).

Table 3. Analysis of test atmospheres

Test atmosphere	Exposure chamber	TPM (µg/l) ± SD	Carbon monoxide (ppm)	Nicotine (µg/l)
Sham	Sham 1	0 (173)	0.1 ± 0.1 (173)	<LLOQ (41)
	Sham 2	0 (77)	0.2 ± 0.2 (77)	<LLOQ (19)
3R4F	3R4F 1	578.8 ± 26.9 (40)	613.9 ± 26.0 (40)	29.8 ± 2.2 (40)
	3R4F 2	603.2 ± 26.1 (176)	667.1 ± 36.9 (176)	29.3 ± 2.2 (176)
	3R4F 3	597.8 ± 31.2 (40)	616.8 ± 28.5 (40)	29.2 ± 2.4 (40)
THS2.2	THS2.2_1[1]	391.2± 62.2 (173)	14.9 ± 2.6 (173)	28.5 ± 3.2 (173)
	THS2.2_2[1]	318.8 ± 29.8 (77)	13.6 ± 0.7 (77)	28.7 ± 3.2 (77)

Mean ± SD, number in brackets represents sample number (n). Values exclude the first 7 days (dose adaptation).

[1] In some forms and documents, THS2.2_1 and THS2.2_2 exposure chambers are described as ZRH 1 and ZRH 2, respectively, according to a former naming convention.

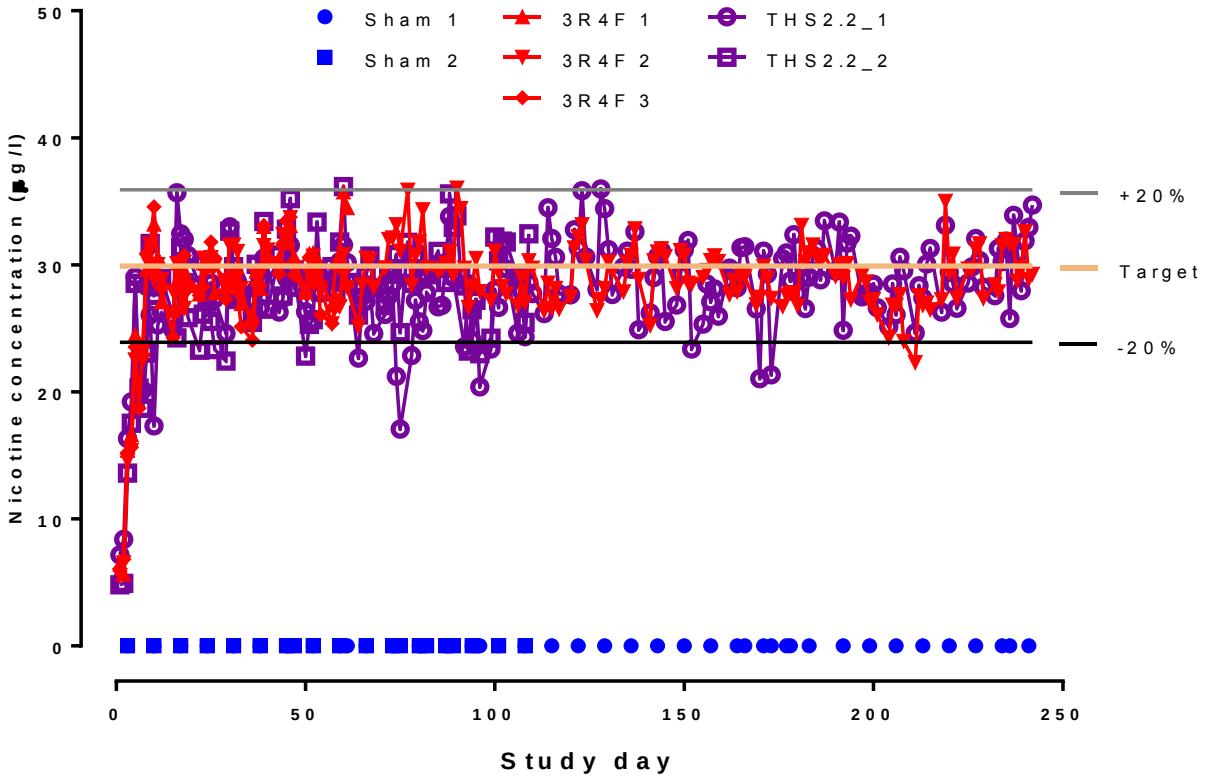


Figure 3. Nicotine concentrations in the exposure chamber breathing zone.
The number next to the exposure group indicate the chamber
Daily mean values (n = 3 samples per day)

The additional sample collection of aldehydes was performed on a weekly basis for each exposure chamber. The results showed an aldehyde profile typical for CS, in which higher levels of formaldehyde, acetaldehyde, and acrolein compared with the THS2.2 aerosol were observed (Table 4). These values were within expected ranges observed in historical studies using the same or related product (Wong, 2014) (Study 15006).

Table 4. Analysis of test atmospheres: aldehydes

Test atmosphere	Exposure chamber	Formaldehyde (µg/l)	Acetaldehyde (µg/l)	Acrolein (µg/l)
3R4F	3R4F1	0.59 ± 0.06 (8)	31.79 ± 1.48 (8)	3.06 ± 0.11 (8)
	3R4F2	0.66 ± 0.10 (34)	33.41 ± 2.47 (34)	3.14 ± 0.24 (34)
	3R4F3	0.60 ± 0.08 (8)	32.13 ± 2.00 (8)	3.11 ± 0.17 (8)
THS2.2	THS2.2_1[1]	0.11 ± 0.01 (34)	7.67 ± 0.85 (34)	0.29 ± 0.03 (34)
	THS2.2_2[1]	0.11 ± 0.01 (15)	7.07 ± 0.33 (15)	0.28 ± 0.02 (15)

Mean ± SD, number in brackets represents number of samples (n)

[1] In some forms and documents, THS2.2_1 and THS2.2_2 chambers are described as ZRH 1 and ZRH 2, respectively, according to a former naming convention.

The particle size distribution was measured per chamber on a monthly basis during the study (Table 5), and the resulting mass median aerodynamic diameter and geometric standard deviation values for both 3R4F and THS2.2 chambers were found to be consistent with historical data sets, i.e. study 14364, COPD I (Yi, 2012), and study 15006 (Wong, 2014). The MMAD and GSD ranged from 0.79 to 0.87 µm, and 1.34 to 1.38 respectively for the 3R4F chambers, and 0.69 to 0.70 µm and 1.43 to 1.50 for the THS2.2 chambers.

Table 5. Particle size distribution

Test Atmosphere	Exposure chamber	MMAD (µm)	GSD
3R4F	3	0.87 (2)	1.34 (2)
3R4F	4	0.81 (8)	1.38 (8)
3R4F	5	0.79 (2)	1.36 (2)
THS2.2[1]	6	0.69 (9)	1.43 (9)
THS2.2[1]	7	0.70 (4)	1.50 (4)

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation. Values in brackets represent the number of samples (n)

[1] In some forms and documents, THS2.2_1 and THS2.2_2 chambers are described as ZRH 1 and ZRH 2, respectively, according to a former naming convention.

8.2 Test System

It was previously demonstrated that ApoE$^{-/-}$ mice were a suitable murine model system to study atherosclerosis (Boue et al, 2012; Lietz et al, 2013; Veniant et al, 2001), as well as emphysema development and mainstream CS-induced physiological effects at exposure concentrations of 600 µg/l TPM (Arunachalam et al, 2010) (PMRL Study 13882). This model system was therefore used in this study to evaluate the effects of exposure to smoke from 3R4F, aerosol from pMRTP THS2.2, fresh air, cessation, and switching from 3R4F smoke to aerosol from THS2.2 on the development of atherosclerotic and emphysema markers. Female ApoE$^{-/-}$ mice (TC-ApoE-F) bred under specific pathogen-free conditions were obtained from Taconic Biosciences (Germantown, NY, USA). The mice were approximately 6–8 weeks old on arrival (8–10 weeks old at the start of the exposure) and their health status on arrival was verified using the health check certificate provided by the breeder. Additional health checks were performed at Harlan Laboratories (Leicestershire, U.K.). Health checks were performed according to the following schedule:

Month 0: Six live animals

Month 4: Serum (from six reserve animals)

Month 8: Serum (from six reserve animals); six live animals

The results of the health screening, which did not indicate any unusual or unexpected findings, were reviewed by the attending veterinarian and the study director.

The study was conducted in an Agri-Food & Veterinary Authority of Singapore-licensed facility, where the animals were cared for and used in accordance with the guidelines of the National Advisory Committee for Laboratory Animal Research (NACLAR, 2004).

Individual mice were identified using transponders subcutaneously implanted approximately 2 weeks prior to the start of the exposure. They were kept and exposed in the restricted access animal laboratory (rooms 3046 to 3051) under specific pathogen-free conditions. The laboratory air was filtered, conditioned fresh air. Positive pressure was maintained inside the laboratory. The temperature and humidity of the animal housing rooms were maintained at 22.0 ± 0.5°C and 56.7 ± 1.8%, respectively, for room 3050, and 22.4 ± 0.5°C and 53.1 ± 1.7%, respectively, for room 3051. The light/dark cycle was 12 h/12 h.

A maximum of eight mice were housed per cage. Cage-enrichment (Igloo®, Biosy, Malaysia, and Nylabone®, New Jersey, U.S.A.) was provided in each cage during the non-exposure period. The bedding material (Lignocel® BK 8-15, J. Rettenmaier & Sohne, GmbH & Co KG., Rosenberg, Germany) was composed of autoclaved softwood granulate and was analysed for aflatoxins, selected pesticides, heavy metals, and polychlorinated biphenyls by the supplier. A gamma-irradiated pellet diet (T2914C rodent diet (standard chow), or TD.88137 for high-fat diet (HFD) groups of the IR arm, both from Harlan) was provided on top of the cage lids. The diet was subject to analysis by the supplier, with guidance from the panel of dietary components and contaminants outlined by the National Toxicology Program (NTP, 2000), which describes the analysis for levels of aflatoxins,

nitrosamines, heavy metals, chlorinated hydrocarbons, organophosphates, polychlorinated biphenyls, and bacterial contaminants. Filtered tap water from water bottles with steam-sterilised sipper tubes was supplied *ad libitum* for each cage. Food was unavailable during the exposure period, but the animals had constant access to drinking water. Unexposed reserve mice (see Figure 4) had free access to food and water throughout the experimental period.

To check the hygiene conditions within the animal holding and exposure rooms, microbial tests were performed at the test facility. Samples from specific areas were collected according to the schedule described below throughout the entire period of animal occupancy (i.e., from animal arrival until dissection of the last study animal):

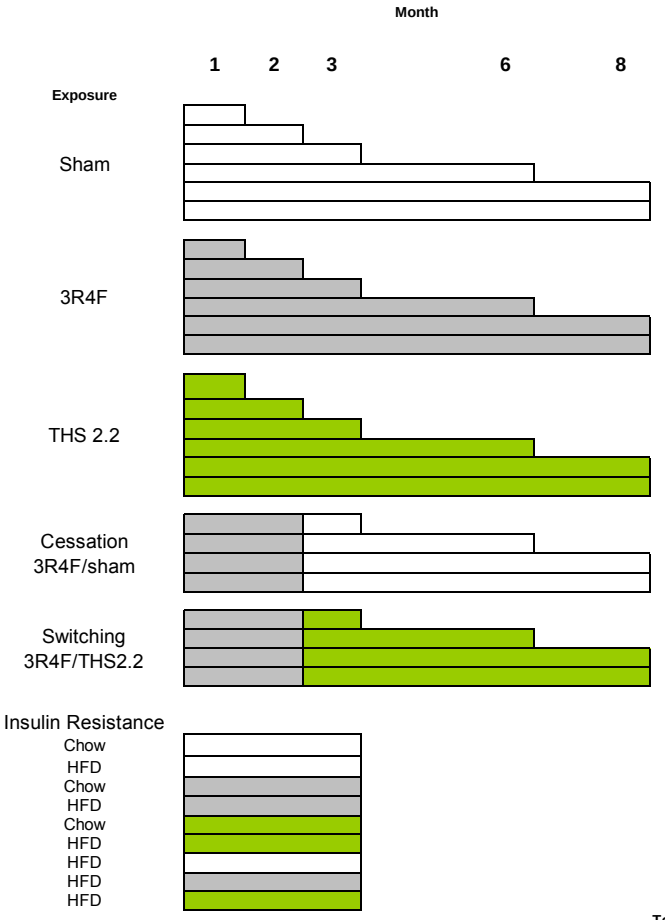
i) Drinking water: screened every 2 weeks
ii) Food: screened monthly
iii) Bedding: screened monthly
iv) Air: screened every 2 weeks
v) Floor and tabletop surfaces: screened weekly

The results of these screening procedures were all within predetermined specification ranges.

8.3 Experimental Groups and Dosing

8.3.1 Allocation of animals to treatment groups

A total of 1,043 mice were allocated to the exposure groups (Figure 4, Table 6, Table 12) using a Provantis-generated randomisation sequence stratified by body weight. Group allocation was completed 4 days prior to the exposure start to obtain similar mean body weights for all groups at first exposure day. The group sizes have been used routinely at PMIRL-S (Phillips, 2013) (COPD II, study 15001) to deliver acceptable statistical interpretation of the data while keeping the animal numbers in line with the expectations of our animal welfare policy.



Animal dissection group									
A BALF	B (Histo-pathology)	C Lung Function	D Omics	E Vascular inflammation	F Numira	G IR/T2DM	H Exposed Reserves	I IR/T2DM (Exposure biomarker)	Total mice number
10	10	10	8	8					46
10	10	10	8						38
10	10	10	8	3	3		3		47
10	10	10	8		6		3		47
10	10	10	8						38
							8		8
10	10	10	8	8					46
10	10	10	8						38
10	10	10	8	6	3				47
10	10	10	8		6		1		45
10	10	10	8						38
							8		8
10	10	10	8	8					46
10	10	10	8						38
10	10	10	8	6	3				47
10	10	10	8		6		1		45
10	10	10	8						38
							8		8
10	10	10	8	8					46
10	10	10	8		6		2		46
10	10	10	8						38
							8		8
10	10	10	8	8					46
10	10	10	8		6		2		46
10	10	10	8						38
							8		8
						10			10
						15			15
						10			10
						15			15
						10			10
						15			15
								8	8
								8	8
								8	8
Tota 210	210	210	168	55	39	75	52	24	1043

Figure 4. Schematic of the study set-up.

Depicted are all exposure groups, including IR arm, dissection timepoints, distribution of mice to each dissection point (animal dissection group), and total mice per group.

Table 6. Exposure groups, exposure concentration targets and durations.

Short name	Group code	Nicotine target concentration (µg/l)	Daily exposure duration (h)	Exposure duration (months)
Sham_1m	101	0	3	1
Sham_2m	102			2
Sham_3m	103			3
Sham_6m	106			6
Sham_8m	108			8
3R4F_1m	201	29.9 µg/l	3	1
3R4F_2m	202			2
3R4F_3m	203			3
3R4F_6m	206			6
3R4F_8m	208			8
THS2.2_1m[1]	301	29.9 µg/l	3	1
THS2.2_2m[1]	302			2
THS2.2_3m[1]	303			3
THS2.2_6m[1]	306			6
THS2.2_8m[1]	308			8
Cessation_3m	403	29.9 µg/l nicotine, 3R4F then fresh air		3
Cessation_6m	406			6
Cessation_8m	408			8
Switching_3m	503	29.9 µg/l 3R4F then THS2.2		3
Switching_6m	506			6
Switching_8m	508			8
Sham_chow	601	fresh air or 29.9 µg/l cigarette smoke/THS2.2		3
Sham_HFD	611			3
3R4F_chow	602			3
3R4F_HFD	612			3
THS2.2[1]	603			3
THS2.2_HFD[1]	613			3

[1]In some forms and documents, these groups are described as ‘ZRH’ according to a former naming convention.

8.3.2 Test groups and exposure

Following arrival at the test facility, animals were acclimatised for a period of 18 days before beginning exposure. After allocation to groups, they underwent whole-body exposure to fresh, conditioned air or to the aerosol generated from either the test or reference item: CS from 3R4F reference cigarettes at a target nicotine concentration of 29.9 µg/l (equivalent to 600 µg TPM/l), or to mainstream aerosol from THS2.2 at matched nicotine levels (29.9 µg/l), 3 h per day, 5 days per week, for up to 8 months (Figure 4). Prior to aerosol exposure to the target aerosol concentrations, both the 3R4F and THS2.2 exposure groups were subjected to an increasing concentration adaptation period of 7 days as follows:

Day 1–2: 150 µg/l TPM or nicotine equivalent

Day 3–4: 300 µg/l TPM or nicotine equivalent

Day 5–7: 450 µg/l TPM or nicotine equivalent

Note that the concentration adaptation timing was adjusted from the 6-day adaptation period originally planned (amendment 2).

The study included a cessation group (3R4F exposure for 2 months followed by fresh air for 1, 4, or 6 months) and a switch group (3R4F exposure for 2 months followed by THS2.2 for 1, 4, or 6 months), as shown in Figure 4. Mice scheduled for dissection were exposed for at least 2 consecutive days before necropsy.

During the daily aerosol administration, animals were exposed to filtered fresh air for 30 min after completing the first hour of mainstream smoke exposure, and for 60 min after completing the second exposure hour (Figure 5). This was to limit the accumulation of carboxyhaemoglobin to potentially toxic levels, particularly in the CS exposure groups. Animals exposed to THS2.2 aerosols followed the same regime although test atmosphere CO-concentrations were much lower than in 3R4F aerosols.

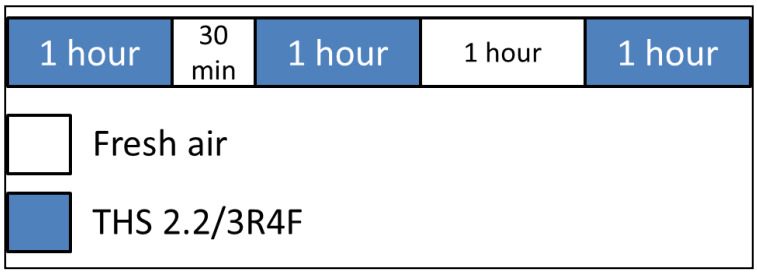


Figure 5. Exposure regime.

Mice were exposed for a total of 3 h per day, but with fresh air breaks as shown.

Food and cage enrichment were removed during exposure, but drinking water bottles were retained to give the animals free access to water during exposure. The position of the cages within the exposure chamber was rotated once a week according to an insertion schedule. The cages were removed from the exposure chambers at the completion of each daily exposure (i.e., all 3 blocks) and put back on the racks.

During the first month of the study, a total of eight exposure chambers were used for all animal groups. However, as the animal numbers decreased, cages were systematically removed. The chamber numbering and naming conventions are shown in Table 7.

Table 7. Exposure chambers and numbering

Short name	Chamber #
SHAM1	1
SHAM2	2
3R4F1	3
3R4F2	4
3R4F3	5
THS2.2 1[1]	6
THS2.2 2[1]	7

[1] In some forms and documents, THS2.2 1 and THS2.2 2 chambers are described as ZRH 1 and ZRH 2, respectively, according to a former naming convention.

8.4 Procedures

8.4.1 Parameters used to monitor animal health

The general condition and health of the mice following smoke uptake/exposure were monitored throughout the study (Table 8). This included body weight measurements, performed weekly, as well as group and individual observations performed daily after exposure. Animals with identified ailments or injuries were tracked daily on a special observations list until they were deemed fit by the study director in consultation with the attending veterinarian.

Table 8. General conditions and health parameters

Parameter	Method	Frequency (THS2.2-ApoE switching)	Frequency (insulin resistance)	Number of mice	Remarks
Body weight[1]	Balance	1/week	1/week	All mice	Individually
In-life observations[1]	Group observations	Daily	Daily	All mice	After exposure
	Individual observations according to checklist	Daily	Daily	2 cages/ group/day	
Food consumption	Gravimetry	At discretion of SD	Weekly	All groups	N/A
Mortality	Observation	Daily	Daily	All mice	-

[1]Animals scheduled for urine collection or blood collection were excluded from post-exposure observation schedules. SD, study director.

8.4.2 Parameters for monitoring smoke uptake and exposure

In addition to the aforementioned analysis of aerosols in animal breathing zones, the aerosol uptake was measured by determination of metabolites of smoke constituents in biological fluids. This was achieved by measuring COHb levels in the bloodstream immediately after the third hour of exposure (Figure 5), by quantifying the five main nicotine metabolites in the urine (samples for 18 hours after the exposure period). The COHb concentration in the blood and the nicotine metabolites in the urine were tested three times throughout the study (Table 9, Table 10). Blood nicotine and cotinine were measured once during month 8 in plasma (delegated phase 6; amendment 9).

Table 9. Biomarkers of exposure

Parameter	Method	Frequency		Number of mice per group	Remarks
		THS2.2- ApoE switching	IR		
Blood HbCO	Spectrophotometric measurement	3 times/study (months 2, 4, 7)	Once (month 2). HFD animals only from exposed reserve mice	12 (THS2.2 ApoE Switching) 8 (IR)	Individually
Urine nicotine + major metabolites	HPLC	4 times/study (months 2, 3, 6, 8)	N/A	8	Not including exposure period Month 2: Groups of 4 mice per metabolic cage. Remaining months individual urine samples (Amendment 6, Deviation 5)
Urine smoke metabolites	Performed at delegated phase 6	4 times/study (months 2, 3, 6, 8)	N/A	8	Note, same urine sample as nicotine metabolites
Plasma nicotine and cotinine	Performed at delegated phase 6	Once (month 8)	N/A	8	Described in amendment 9

HPLC, high-performance liquid chromatography; HFD, high-fat diet; IR, insulin resistance.

Table 10. Carboxyhaemoglobin sample collection

Short Name	Group code	Month							
		1	2	3	4	5	6	7	8
Sham_1m	101								
Sham_2m	102		12						
Sham_3m	103								
Sham_6m	106				12				
Sham_8m	108							12	
3R4F_1m	201								
3R4F_2m	202		12						
3R4F_3m	203								
3R4F_6m	206				12				
3R4F_8m	208							12	
THS2.2_1m[1]	301								
THS2.2_2m[1]	302		12						
THS2.2_3m[1]	303								
THS2.2_6m[1]	306				12				
THS2.2_8m[1]	308							12	
Cessation_3m	403								
Cessation_6m	406				12				
Cessation_8m	408							12	
Switching_3m	503								
Switching_6m	506				12				
Switching_8m	508							12	
Sham_chow	601								
Sham_HFD	611		8						
3R4F_chow	602								
3R4F_HFD	612		8						
THS2.2_chow[1]	603								
THS2.2_HFD[1]	613		8						

[1]In some forms and documents, these groups are described as ZRH, according to a former naming convention.

Urine samples were collected overnight after exposure for approximately 16 hours (Table 11), split into two aliquots and stored at ≤ −70°C. Collection at the month 2 time point was performed using a group metabolic cage (four animals per cage); after this, metabolic cages containing single animals were used (Amendment 6, Deviation 5). The four sample collection time points (months 2, 3, 6, and 8) were selected to demonstrate consistency in test aerosol uptake at different points of the study. One of the aliquots was used to analyse urine nicotine metabolites with standard high-performance liquid chromatography methods after 1,3 diethyl-2-thiobarbituric acid derivatisation at PMIRL-S. An additional aliquot was collected for the analysis of additional biomarkers of aerosol exposure determined at delegated phase 6, as follows:

i) hydroxypropylmercuric acid (HPMA)

ii) total (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)

iii) S-phenylmercapturic acid (S-PMA)

iv) 2-cyanoethylmercapturic acid (CEMA)

v) Oxidative stress and inflammation biomarkers:
   - 4-hydroxynonenal (4-HNE)
   - malondialdehyde (MDA)
   - 8-hydroxy-2′-deoxyguanosine (8-OH-dG)

vi) Eicosanoids:
   - tetranor PGE-M
   - 2,3-dinor-8-iso-PGF2a
   - 8-iso-PGF2a
   - 2,3-dinor-TBX2
   - 11-dehydro-TBX2
   - 12-hydroxyeicosatetraenoic acid (12-HETE)

Table 11. Urine sample collection

Short name	Group code	Month							
		1	2	3	4	5	6	7	8
Sham_1m	101								
Sham_2m	102		8 × 4[2]						
Sham_3m	103								
Sham_6m	106						8		
Sham_8m	108								8
3R4F_1m	201								
3R4F_2m	202		8 × 4[2]						
3R4F_3m	203			8					
3R4F_6m	206						8		
3R4F_8m	208								8
THS2.2_1m[1]	301								
THS2.2_2m[1]	302		8 × 4[2]						
THS2.2_3m[1]	303			8					
THS2.2_6m[1]	306						8		
THS2.2_8m[1]	308								8
Cessation_1m	403			8					
Cessation_4m	406						8		
Cessation_6m	408								8
Switching_1m	503			8					
Switching_4m	506						8		
Switching_6m	508								8

[1]In some forms and documents, these groups are described as ZRH, according to a former naming convention. [2]8 × 4 refers to eight metabolic cages containing four mice per cage, as collected in month 1. Individual metabolic cages were used for urine collection from month 3 onwards (Amendment 6, Deviation 5).

8.4.3 Necropsy

In accordance with the study objectives, terminal endpoints were oriented towards the examination of pulmonary inflammation, and the development and progression of emphysema/COPD, atherosclerosis, and IR. This was logistically performed by splitting the animals into seven dissection streams (A through G), as shown in Table 12.

Table 12. Number of animals from each exposure group, and per necropsy phase

Short name	Group code	A BALF	B Histopathology	C Lung function	D Omics	E Vascular inflammation/ platelet	F microCT	G IR	Total mice
Sham_1m	1	10	10	10	8	8			46
Sham_2m	2	10	10	10	8				38
Sham_3m	3	10	10	10	8	3	3		44
Sham_6m	6	10	10	10	8				38
Sham_8m	8	10	10	10	8		6		44
3R4F_1m	101	10	10	10	8	8			46
3R4F_2m	102	10	10	10	8				38
3R4F_3m	103	10	10	10	8	6	3		47
3R4F_6m	106	10	10	10	8				38
3R4F_8m	108	10	10	10	8		6		44
THS2.2_1m[1]	201	10	10	10	8	8			46
THS2.2_2m[1]	202	10	10	10	8				38
THS2.2_3m[1]	203	10	10	10	8	6	3		47
THS2.2_6m[1]	206	10	10	10	8				38
THS2.2_8m[1]	208	10	10	10	8		6		44
Cessation_1m	303	10	10	10	8	8			46
Cessation_4m	306	10	10	10	8				38
Cessation_6m	308	10	10	10	8		6		44
Switching_1m	403G	10	10	10	8	8			46
Switching_4m	406G	10	10	10	8				38
Switching_6m	408G	10	10	10	8		6		44
Sham_chow	601							10	10
Sham_HFD	611							15	15
3R4F_chow	602							10	10
3R4F_HFD	612							15	15
ZRH_chow	603							10	10
ZRH_HFD	613							15	15
Total		210	210	210	168	55	39	75	967[2]

[1]In some forms and documents, these groups are described as ZRH, according to a former naming convention.

[2]This total number of 967 dissected mice differs from the total in Figure 4 (n=1043) because it does not include the 76 exposed reserve mice.

Each subgroup (Table 12) provided samples for several endpoints during dissection, as follows:

A) Bronchoalveolar lavage fluid (BALF) (10 mice)

- Free lung cells (FLCs): Quantification and differential cell count
- BALF: Inflammatory mediators (delegated phase 5, Myriad/Rules-based Medicine, RodentMAP analysis)
- BALF: Matrix metalloproteinase (MMP) activity
- Aortic arch: Plaque size determination
- Thoracic aorta: Histopathology (paraffin embedding)
- Blood: White blood cell counts (Sysmex)
- Serum: Serum inflammatory mediators (delegated phase 5, Myriad/Rules-based Medicine, RodentMAP analysis)

B) Histopathology (10 mice)

- Left lung: Histopathology/morphometric assessment/immunohistochemistry
- Right lung (all lobes): Histopathology/morphometry back-up
- Liver (left lobe, Figure 6): Histopathology (haematoxylin and eosin (H&E), Periodic Acid Schiff (PAS), PAS diastase (D), immunohistochemistry (IHC)
- Liver (left lobe, Figure 6): Histopathology (cryosections; Oil Red O)
- Nose (three levels): Histopathology (H&E, AB-PAS)
- Blood: Glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) (Singapore)
- Plasma: Oxysterols

C) Lung function (10 mice)

- Lung function analysis
- Serum: Proteomics
- Serum: Cholesterol measurements (delegated phase 8)

D) ‘Omics’ (8 mice)

- Aortic arch: Plaque size
- Nasal epithelium: Transcriptomics (left and right nostrils)
- Lung (left): Transcriptomics and genomics
- Lung (right lung c): Lipidomics

- Lung (right lung a and b): Proteomics
- Liver (left lobe): Transcriptomics
- Liver: Genomics
- Liver: Proteomics
- Liver (left lobe): Lipidomics
- Liver (left lobe): Histopathology – (cryosection Oil red O)
- Liver (left lobe): Histopathology (H&E, PAS, PASD, IHC)
- Liver (remaining lobes): back-up tissue (frozen)
- Heart (left ventricle, apex): Storage (frozen)
- Thoracic aorta: Transcriptomics,
- Abdominal aorta: Lipidomics (delegated phase 3)
- Blood: Transcriptomics
- Blood: Genomics
- Plasma: Lipidomics (delegated phase 3)
- Serum: Proteomics

E) Vascular inflammation and platelet aggregation

- Aortic arch and descending aorta: Cell dissociation/fluorescence-activated cell sorting (FACS)
- Blood: Platelet aggregation/FACS

This entire dissection group was redistributed to accommodate Numira micro-computed (micro-CT) tomography imaging (Amendment 8, Deviation 4)

F) Micro-CT imaging of aorta

- Whole carcasses were fixed and sent to delegated phase 7 for imaging analysis and plaque volume measurements

G) IR endpoints

- Mesenteric fat: Transcriptomics
- Liver (left lobe): Histopathology (H&E, PAS, PASD)
- Liver (left lobe): Histopathology (cryosections, oil red O)
- Liver (left lobe): Transcriptomics
- Liver (left lobe): Protein (western blotting for validation of biomarkers)
- Liver (left lobe): Lipidomics
- Pancreas: Histopathology (H&E)

- Blood: Glucose, HDL, LDL, VLDL (Singapore)

- Plasma: Lipidomics (delegated phase 3)

- Plasma: Metabolic MAP and ELISA (insulin and GLP-1) (delegated phase 5, Myriad/Rules-based Medicine, Metabolic MAP analysis)

- Muscle (soleus and gastrocnemius as a single tissue piece): Transcriptomics

- Aortic arch: Morphometric evaluation of total plaque area

- Thoracic aorta: Transcriptomics

- Heart (left ventricle, apex): Transcriptomics

8.4.4 Lung inflammation

The experimental methods listed in Table 13 were used to assess lung inflammation. These methods included analysis of the BALF cellular composition (FLC), as well as the levels of specific proteins (multi-analyte profile (RodentMAP® v 3.0) – delegated phase 5 or MMP activity.

8.4.4.1 Collection of BALF

BALF collection was performed according to standard working instructions (PMI_RD_WKI_000319, 'Prepare lavage samples: Lavage of mouse lung – syringe method'). BALF was divided as follows to assess different downstream processes: The cell free BALF from cycle 1 was collected in phosphate buffered saline (PBS), and used for protein and enzyme analysis (gelatinolytic activity). Cycles 2–5 were collected in PBS + bovine serum albumin, combined with the cell pellet from cycle 1, and processed using flow cytometry.

BALF supernatant (first lavage cycle) was aliquoted into separate tubes for the following applications, in the following order of priority:

i) RodentMAP 3.0 (300 µl)

ii) MMP activity determination (optimal 150 µl, minimum 120 µl)

iii) Remaining supernatant

The fluid was stored at ≤ −70°C until further use (for shipment on dry ice to delegated phase 5). Any residual supernatant was stored in a separate tube under the same conditions for future potential analysis.

Table 13. Lung inflammation

Parameter	Biological material	Method	WKI/SOP #
Collection of FLCs	BALF (cells)	Bronchoalveolar lavage of the lung	'Prepare lavage samples: Lavage of mouse lung – syringe method', PMI_RD_WKI_000319
Determination of cellular viability	BALF (cycles 2–5 plus FLCs from cycle 1)	Flow cytometric analysis after staining with fluorescein diacetate and propidium iodide	'Perform analysis: Flow cytometric measurement of cell viability with simultaneous determination of cell count by means of BD TruCount beads', PMI_RD_WKI_000419
FLC count	BALF (cycles 2–5 plus FLCs from cycle 1)	Flow cytometrical counting using Trucount® tubes	'Perform analysis: Flow cytometric measurement of cell viability with simultaneous determination of cell count by means of BD TruCount beads', PMI_RD_ WKI_000419
FLC differentiation	BALF (cycles 2–5 plus FLCs from cycle 1)	Flow cytometric analysis after staining with cell-type specific antibodies conjugated to fluorochromes	Perform analysis, Flow cytometric differential counting of mouse free lung cells by labelling with CD11c, Ly6G, CD3e, B220 and MHCII. See also Table 14 for revised antibody mixture.
FLC lymphocyte differentiation	BALF (cycles 2–5 plus FLCs from cycle 1)	Flow cytometric analysis after staining with cell-type specific antibodies conjugated to fluorochromes	'Flow cytometric determination of lymphocyte differentiation in murine bronchoalveolar lavage fluid', PMI_RD_WKI_000647
FLC macrophage activation	BALF (cycles 2–5 plus FLCs from cycle 1)	Flow cytometric analysis after staining with cell-type specific antibodies conjugated to fluorochromes	'Flow cytometric quantification of activated marker expression in murine alveolar macrophages', PMI_RD_WKI_000648
Inflammatory mediators (multi-analyte profile)	BALF (cell-free supernatant of cycle 1)	Multiplexed bead array according to the RodentMAP® v 3.0 program	Rules-based medicine (Delegated phase 5)
MMP activity	BALF (cell-free supernatant of cycle 1)	Fluorescence method using fluorescein-conjugated gelatin	'Quantitative determination of matrix metalloproteinase (MMP) activity in rodent bronchoalveolar lavage (BALF)' PMI_RD_WKI_000620.

FLC, free lung cells; MMP, matrix metalloproteinase

BAL cells (FLC, lavage cycles 1, and 2 through 5) were processed as follows: cells from cycle 1 were combined with those from cycles 2–5 for viability and cell quantification. Cells were then fixed, and partitioned for different analytical endpoints (differential count, lymphocyte differentiation, and macrophage activation). The antibody mixes are described in Table 14:

Table 14. FLC antibody mixes

Assay	Name	Company	Cat#
Mouse DiffCount	CD11c-APC	Becton Dickinson	550261
	Ly6G (Gr1) - FITC	Becton Dickinson	551460
	CD3 - PE	Becton Dickinson	553064
	B220 - APC - Cy7	Becton Dickinson	552094
	MHCII - PerCP-eFluo710	e-Bioscience	46-4031-82
Lymphocyte differentiation	Ly6G-PE	BD biosciences	553141
	CD4-PerCP	BD biosciences	553052
	CD8a-PE-Cy7	BD biosciences	552877
	CD45R/B220-APC-Cy7	BD biosciences	552094
Macrophage activation	CD68-Pe-Cy7	BD Biosciences	553141
	CD54/ICAM-1-PE	BD Biosciences	553253
	CD11b-APC-Cy7	BD Biosciences	557657
	MHCII-PerCP-eFluor710	e-Biosciences	46-4031-82
	Cd11c-APC[1]	Becton Dickinson	550261

[1]CD11c included as a macrophage and CD11c and MHCII as dendritic cell markers in addition to the antibody mix described in the working instructions.

The MMP activity of BALF supernatants was determined using a commercially available assay kit based on the cleavage of fluorochrome-labelled gelatin by gelatinolytic metalloproteinases in BALF (EnzChek® Gelatinase/Collagenase Assay Kit; Life Technologies (Invitrogen), Singapore).

8.4.5 Histoprocessing and staining

Histological processing of frozen and/or formalin-fixed tissues was performed at PMIRL-S according to internal work instructions. Briefly, 4-µm thick, formalin-fixed left lung, nose, pancreas, and left liver lobes were routinely processed and stained with H&E. Additional histochemical staining included alcian blue (AB) and PAS for the left lung and nose (polysaccharides and glycoproteins, including mucus); resorcin fuchsin (RF) for the lung (elastic fibres); PAS and PASD for the liver (glycogen); and Oil Red O for cryosections of the liver (lipids).

Formalin-fixed thoracic aortic tissue was processed and embedded in paraffin blocks.

The left lung lobe was serially sectioned (4-µm thick) in 20 step-sections (150-µm apart) for an overall assessment of the entire lung lobe.

8.4.6 Histopathology

Histopathological evaluation of the left lung (serial sections), nose (three representative levels), pancreas, and liver was performed at PMIRL-S according to internal work instructions.

Digitalised (Aperio digital imaging), 4-µm serial sections of the lung were also evaluated for pulmonary emphysema. This measurement was performed at delegated phase 4, and a design-based stereological approach was used to obtain quantitative data (newCAST, VisioPharm) describing the morphometry of the lungs. Morphometric parameters investigated were mean cord length, bronchiolar attachments, and destructive index.

8.4.7 Lung function measurements

Lung function measurements were made using the flexivent system for measurement of respiratory mechanics (Scireq, Canada), performed according to our working instructions, PMI_RD_WKI_000420, 'Perform terminal measurement: Lung function measurement'.

8.4.8 Tissue processing for 'omics' analysis (transcriptomics, genomics, proteomics, and lipidomics)

Tissues for 'omics' analysis (dissection block 'D') were analysed 16–24 h post exposure. Mice were anaesthetised using sodium pentobarbital, then blood was collected through the retro-orbital plexus (whole blood for RNA and DNA extraction, plasma for lipids, and serum for proteomics). Tissues were perfused using 0.9% NaCl. To minimise RNA degradation during the removal process, all surfaces and instruments were wiped with RNAse-impregnated tissues (RNAse AWAY surface decontaminant, Thermo Scientific, Singapore) between animals. Tissues were extracted and frozen as quickly as possible by immersion of the tube in liquid nitrogen (N2), or by pouring liquid N2 into the containers.

Note, collection and processing of the tissues was prioritised according to specific degradation sensitivity. The target was to remove the nasal epithelium within 15 min post-mortem (perfusion time). To control for the risk of RNA degradation, all tissues were isolated and processed/frozen within 20 min post-mortem.

Samples were stored at ≤ −70°C until completion of the study, after which they were processed or shipped.

8.4.9 Blood collection

Multiple downstream applications required blood collection at the necropsy phase, each requiring specific methods of collection and processing.

8.4.9.1 Whole blood for RNA extraction

Blood samples were collected from the retro-orbital plexus using an uncoated (i.e., no heparin) capillary or PE tube. The blood was transferred directly into an RNA collection tube of appropriate size (~100 µl; RNAprotect Animal Blood Tubes, Qiagen, Singapore), avoiding overloading. The tube was mixed by inversion 10 times, rested overnight (minimum 2 h) at 15–25°C, then stored at ≤ −70°C before further processing or shipment.

8.4.9.2 Whole blood for DNA extraction

Blood samples were collected from the retro-orbital plexus using an uncoated (i.e., no heparin) capillary or PE tube. The blood was transferred directly into a 4 ml ethylenediaminetetraacetic acid (EDTA)-coated tube, then inverted eight times to mix.

8.4.9.3 Whole blood for haematology

Blood samples were collected from the retro-orbital plexus using an uncoated (i.e. no heparin) PE tube. The blood was collected into EDTA tubes, mixed well, and then placed on a tube rotator until analysis.

8.4.9.4 Serum for proteomics/inflammation

Blood was collected from the retro-orbital plexus using non-heparinised capillary tubes. The blood was transferred directly to a serum separator tube and processed according to our work instructions. Briefly, after centrifugation, the gel formed an interface between the serum and the supernatant. The serum was then transferred into an Eppendorf tube, and frozen at ≤ −70°C for storage or shipment. Importantly, there was no heat inactivation, which would denature or inactivate the heat labile components in the serum, thereby disrupting the proteomic and lipidomic analysis.

8.4.9.5 Partitioning of liver and lungs during dissection

The liver and lungs were partitioned as described in Figure 6 for multiple uses.

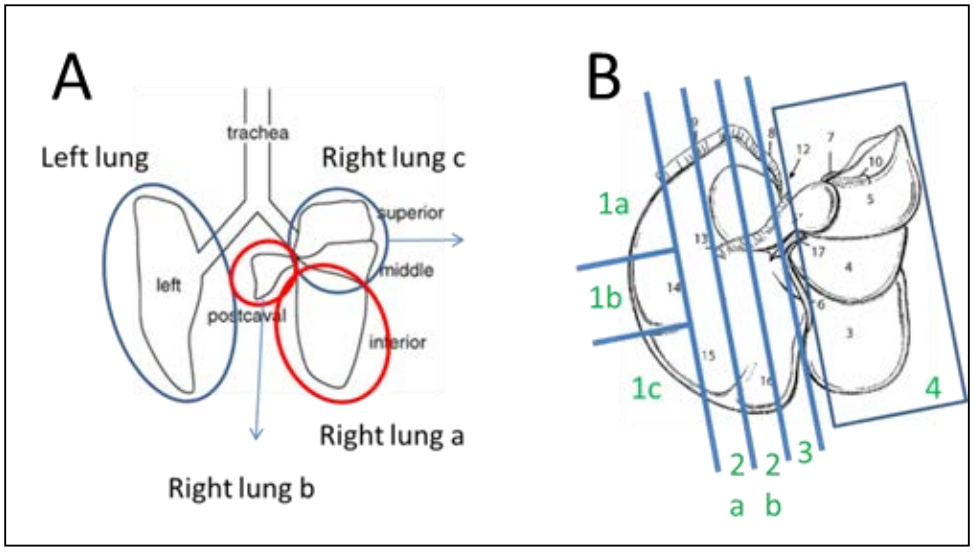


Figure 6. Liver and lung necropsy for ‘omics’ analysis.

(A) Lungs were partitioned into four parts: the entire left lung, and three right lung partitions (a, interior; b, postcaval; c, superior and middle). (B) Livers were partitioned into multiple pieces as shown by the numbering system.

8.4.10 Determination of lipids

8.4.10.1 Plasma collection for determination of oxysterols

Plasma was collected using standard methods (PMI_RD_WKI_000625, 'Exsanguinate: Serum and plasma preparation'. Immediately after isolation, plasma was stabilised with 0.4 µmol/l Probucol and butyl hydroxy-toluol (BHT). The stabilising solution was prepared by making 4 mM stock solutions of BHT and Probucol (individually) in 100% ethanol. These were then combined 1:100 in ethanol as a 100× stock solution (40 µmol/l), which is stable for 1 week at 4−C. The 100× stock solution was then added to the plasma at a 1:100 dilution and samples were frozen on dry ice and stored at ≤ −70°C until the time of shipment.

8.4.10.2 Plasma collection for lipidomics (delegated phase 3)

Blood was collected into EDTA-plasma tubes, separated as soon as possible after collection, and snap-frozen at −80°C. Samples were stored until shipment to delegated phase 3.

8.4.10.3 Oil red O staining

Portions of the liver were frozen using cryopreservation freezing media, followed by embedding into a Cryomold block. Approximately 10-µm thick cryosections were stained with Oil Red O.

8.4.10.4 Lipoprotein analysis

Serum samples were prepared according to our standard methodology (see section 8.4.9.4), and stored frozen until shipment to delegated phase 8.

8.4.11 Determination of plaque size measurement

The aortic arch was microdissected and cut longitudinally then pinned onto a rubber surface. The arch was then imaged, stained with oil red O (according to our standard working instruction, 'Oil red O stain'), and re-imaged to generate both an unstained and an oil red O-stained image.

Image analysis to determine the plaque area was performed using Visiopharm image analysis software (version 4.6). Using software macros, the perimeter of the aortic arch was autodetected and selected. This 'area of interest' was then evaluated using a separate macro to localize and quantify the oil red O-stained regions. The remaining area was quantified as the aortic area (total area of evaluation was defined as area of interest + aortic area). The final relative plaque area (%) was calculated by dividing the plaque area by the total area of evaluation.

8.4.12 Apoptosis/oxidative stress in the lungs and liver

The occurrence of oxidative stress in left lung and liver sections will be assessed by measuring the level of the oxidative stress marker 8-OH-dG by IHC. To detect apoptosis in lung cells, left lung sections from mice exposed

to fresh air or CS will be subjected to the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling assay. These exploratory studies will be performed at delegated phase 2 and will be reported separately.

8.4.13 Vascular inflammation

Flow cytometry was used to analyse the aorta immune cell composition from mice exposed to fresh air, 3R4F, and THS2.2 aerosols. In brief, animals were perfused with PBS containing heparin and the aortic arch and thoracic aorta isolated and enzymatically digested according to standard protocols, using an enzyme mixture containing elastase and liberase (both at 0.1 mg/ml for 60 minutes). After digestion, single cell suspensions were labelled with antibodies to differentiate macrophages, neutrophils, dendritic cells, and T and B lymphocytes by flow cytometry. Based on preliminary results, this phase of the study was discontinued after the first two analysis periods, and the remaining animals were reallocated to other study endpoints (Deviation 4).

8.4.14 Platelet aggregation assay

Flow cytometry-based analysis was applied to detect platelet aggregates using whole blood isolated from mice exposed to fresh air, 3R4F, or THS2.2 aerosols. In brief, two portions of cells from the same donor mouse were labelled with different fluorochromes of the CD9 antibody. After mixing and appropriate stimulation (PMA, collagen), these were analyzed for the formation of double-colored aggregates by flow cytometry. Based on preliminary results, this phase of the study was discontinued after the first month, and the animals were reallocated to other study endpoints (Deviation 4).

9 Statistical Methods

This study was exploratory and no formal hypothesis was specified *a priori*. The statistical analysis was therefore also exploratory and no confirmatory statistical testing was performed. Nevertheless, 95% confidence intervals and unadjusted *p*-values are presented.

The analyses described below applies to the biological parameters of the following categories (note that 'omics' data analysis is described in a separate study plan):

- Lung function measurements
- Histopathology
- Lung inflammation
- Systemic inflammation
- Plaque size
- Oxysterols
- Biomarkers of exposure/oxidative stress/inflammation
- Hematology
- Clinical chemistry
- Plaque area measurements

Summary statistics were provided for measurements that do not belong to the above list (test atmosphere characterization, general condition and health, and aerosol uptake).

9.1 Descriptive Statistics

- For each treatment group, continuous variables are presented by showing the number of measurements used to compute the statistics. When applicable, the number of values below the limits of detection or quantification is also provided. The median, 25% and 75% percentiles, minimum and maximum are provided for all groups. In addition the mean, standard deviation, relative standard deviation (CV%), and standard error (SE) are provided. Data are shown graphically.

- For each treatment group, ordinal and incidence variables are presented by showing the number of measurements used to compute the statistics, as well as the number (and/or percentage) of mice falling in each level of the variable of interest (contingency tables). For ordinal variables, mean and standard error are also computed. Graphs are also provided.

9.2 Missing Values and Values outside Quantification Range

- Missing values were not substituted.
- Values below the limit of detection (LOD) were substituted for LOD/2.
- Values <LLOQ (but >= LOD) or >ULOQ were not substituted.
- For Myriad RBM data, values were reported as < LLOQ, or values less than LOD were substituted for max (LOD,LLOQ)/2. Values reported as > ULOQ were substituted for 1.1 * ULOQ.

9.3 Treatment Comparisons

- Exploratory pairwise comparisons between treatment groups and within time points were performed.
- For continuous variables, this was done using as follows. For each pairwise comparison, the data normality of the 2 groups being compared was assessed by the Shapiro-Wilk test performed on the studentized residuals of the 2 groups altogether. If the normality assumption was not rejected (at the 5% level), a pairwise t-test accounting for variance heterogeneity was performed. If the normality assumption was rejected, a Mann-Whitney-Wilcoxon was used (exact Monte-Carlo version).
- For discrete variables:
  - Independent binary data were analyzed using Fisher's exact test.
  - Ordinal data were analyzed using the Cochran–Mantel–Haenszel test on row mean score (modified ridit scores).

9.4 Extreme Values

- If extreme values were identified, they were discussed with the experimenters. These values were used in the analysis unless they were obvious measurement or manipulation mistakes. In that case, they were excluded from the analysis.

9.5 Significance Level

- Because the study was exploratory, no specific significance level was required. Therefore, *p*-values were reported as such without considering any adjustments.

10 Administrative Aspects

10.1 Study Timelines

Arrival of animals:	16 Aug 2013
Starting date of exposure:	02 Sep 2013
End date of exposure:	02 May 2014
Experimental completion date:	25 Oct 2014
Start of delegated phase 1 at PMIRL, Neuchatel (transcriptomics/genomics)	01 Jun 2014
Start of delegated phase 2 at PMIRL, Neuchatel (proteomics)	01 Jun 2014
Start of delegated phase 3 at PMIRL, Neuchatel (lipidomics)	01 Jun 2014
Start of delegated phase 4 at Histovia (histopathology)	01 Nov 2013
Start of delegated phase 5 at Myriad/RBM (inflammatory mediators)	01 Dec 2013
Start of delegated phase 6 at ABF (biomarkers of exposure)	19 Nov 2013
Start of delegated phase 7 at Numira, Inc (micro CT imaging)	16 Dec 2013
Start of delegated phase 8 at PRISM (Maldi imaging)	30 Apr 2014
End of delegated phase 1 at PMIRL, Neuchatel (transcriptomics/genomics)	31 Dec 2014
End of delegated phase 2 at PMIRL, Neuchatel (proteomics)	31 Dec 2014
End of delegated phase 3 at PMIRL, Neuchatel (lipidomics)	31 Dec 2014
End of delegated phase 4 at Histovia (histopathology)	01 July 2014
End of delegated phase 5 at Myriad/RBM (inflammatory mediators)	01 July 2014
End of delegated phase 6 at ABF (biomarkers of exposure)	13 June 2014
End of delegated phase 7 at Numira, Inc (micro CT imaging)	17 Sep 2014
End of delegated phase 8 at PRISM (Maldi imaging)	Reported separately
Date for final study report:	31 December 2014
Date for Amended study report (study report amendment 1):	15 July 2015

RBM, rules-based medicine; TBD, to be determined

10.2 Names and Addresses

Sponsor	Manuel Peitsch VP Biological Systems Research Philip Morris International CH-2000 Neuchâtel, Switzerland
Test Facility	Philip Morris International Research Laboratories Pte Ltd 50 Science Park Road, #02-07 The Kendall Singapore Science Park II Singapore 117406
Test Facility Management	Yin Boll. Manager Support & Technology Philip Morris International Research Laboratories Pte Ltd 50 Science Park Road, #02-07 The Kendall, Singapore Science Park II, Singapore 117406 Tel: +65-66314524 Fax: +65 64130948 Email: Yin.Boll@pmi.com
Study Director	Blaine Phillips Philip Morris International Research Laboratories Pte Ltd 50 Science Park Road, #02-07 The Kendall Singapore Science Park II, Singapore 117406 Tel.: +65-6631-4510 Fax: +65 64130948 Email: Blaine.Phillips@pmi.com
Deputy Study Director	Alberto Oviedo Philip Morris International Research Laboratories Pte Ltd 50 Science Park Road, #02-07 The Kendall Singapore Science Park II, Singapore 117406 Tel.: +65-6631-4591 Fax: +65 64130948 Email: Alberto.Oviedo@pmi.com

Test site for delegated phases 1, 2, and 3	Philip Morris International CH-2000 Neuchâtel, Switzerland
Principal investigator for delegated phase 1 (transcriptomics/genomics)	Marja Talikka Philip Morris International CH-2000 Neuchâtel, Switzerland Tel: +41-582422677 Fax: +41(0)58 242 28 11 E-mail: Marja.Talikka@pmi.com
Principal investigator for delegated phase 2 (proteomics)	Ashraf Elamin Philip Morris International CH-2000 Neuchâtel, Switzerland Tel: +41-582422065 Fax: +41(0)58 242 28 11 E-mail: Ashraf.Elamin@pmi.com
Principal investigator for delegated phase 3 (lipidomics)	Stephanie Boue Philip Morris International CH-2000 Neuchâtel, Switzerland Tel: +41-582422243 Fax: +41(0)58 242 28 11 E-mail: Stephanie.Boue@pmi.com
Delegated phase 4 (histopathology)	Histovia GmbH Schone Aussicht 5, D-51491, Overath, Germany Tel: +49-2206 863774 Fax: +49-2206 864033
Principal investigator for delegated phase 4 (histopathology)	Dr. A. Buettner Histovia GmbH Schone Aussicht 5, D-51491, Overath, Germany. Tel: +49-2206 863774 Fax: +49-2206 864033 E-mail: a.buettner@histovia.com
Delegated phase 5 (inflammatory mediators)	Myriad Rules-Based Medicine, Inc. 3300 Duval Road Austin, Texas 78759 USA Tel: +1-512-835-8-26 (ext 336) Fax: +1-512-835-4687
Principal investigator for delegated phase 5 (inflammatory mediators)	Ashley Garrett Myriad Rules-Based Medicine, Inc. 3300 Duval Road Austin, Texas 78759 USA Tel: +1-512-835-8-26 (ext 336) Fax: +1-512-835-4687 E-mail: ashley.garrett@rulesbasedmedicine.com

Delegated phase 6 (biomarkers of exposure)	Analytisch-Biologisches Forschungslabor (ABF) Goethestrasse 20 80336 Muenchen Germany
Principal investigator for delegated phase 6 (biomarkers of exposure)	Gerhard Scherer Analytisch-Biologisches Forschungslabor (ABF) Goethestrasse 20 80336 Muenchen Germany
Delegated phase 7 (micro CT imaging)	Numira, Inc. 420 E. Temple Street, Suite 520 Salt Lake City, UT 84111 USA
Principal investigator for delegated phase 7 (micro CT imaging)	Debbie Palmon Numira, Inc. 420 E. Temple street, Suite 520 Salt Lake City, UT 84111 USA
Delegated phase 8 (Maldi imaging)	Laboratoire Proteomique, Reponse Inflammatoire et Spectrometrie de Masse (PRISM) Universite Lille 1 Bat SN3, 1er etage F-59655 Villeneuve d'Ascq Cedex France
Principal investigator for delegated phase 8 (Maldi imaging)	Dr. Isabelle Fournier Laboratoire Proteomique, Reponse Inflammatoire et Spectrometrie de Masse (PRISM) Universite Lille 1 Bat SN3, 1er etage F-59655 Villeneuve d'Ascq Cedex France Tel: +33 (0)3 20 43 41 94 Fax: +33 (0)3 20 43 40 54

10.3 Multi-Site Arrangements

This study included analytical endpoints evaluated at both Singapore (PMIRL-S) and Neuchâtel (PMIRL), together with selected phases that were outsourced to external contract research organisations. The separation of activities was as follows:

1) PMIRL-S (Singapore)

- Aerosol generation and dilution
- Animal housing, husbandry, and in-life observations
- Blood COHb measurements
- Urinary nicotine metabolite measurements
- Dissection and necropsy
- Collection of BALF and analysis of different cell types:
  - FLC count, viability, and differential cell count
  - level of macrophage activation
  - lymphocyte differentiation index
  - MMP activity in BALF
- Tissue collection for 'omics' analysis
- Histotechnical processing and generation of tissue sample digital images
- Clinical chemistry
- Plaque area measurements
- Histopathology (kidney and pancreas)
- Fasting glucose

2) PMIRL (Neuchâtel)

- Genomics/transcriptomics analysis (delegated phase 1)
- Proteomics (delegated phase 2)
- Lipidomics (delegated phase 3)

3) Histovia Gmbh (delegated phase 4)

- Pathological assessment
- Lung morphometric measurements

4**) Rules-Based Medicine** (delegated phase 5)

- Multi-analyte profile of BALF and plasma

5) **ABF** (delegated phase 6)

- Urine analysis of exposure biomarkers
- Nicotine and cotinine plasma measurements

6) **Numira Biosciences** (delegated phase 7)

- Micro CT imaging of aorta
- Immunohistochemistry (aorta), exploratory

7) **Laboratoire Proteomique, Reponse Inflammatoire et Spectrometrie de Masse (PRISM)** (delegated phase 8)

- MALDI-TOF analysis of aortic arch cryosections

Storage and shipment of the tissues depended on the nature of the experiments. FLCs were processed within 48 h of collection, while the remaining BALF was frozen and then shipped on dry ice to Myriad Rules-Based Medicine. Tissue samples were frozen until the end of the study, after which they were stored long-term or shipped to test sites on request from principal investigators. Histological samples were typically stored in ethanol after fixation, but were embedded in paraffin within 2 weeks of fixation. Paraffin blocks and all downstream histological specimens are stable indefinitely at room temperature.

11 Study Plan Amendments and Deviations

All amendments and deviations are indicated in Table 15. Note, the full documents can be found in Appendix 1.

Table 15. Study plan amendments and deviations

Type	Change no.	Date of Study Director's approval	Change	Author of change
Amendment	1	30 Aug 2013	Blood collection for IR study arm procedure	D.C. Sharma
Amendment	2	06 Sep 2014	Extension of concentration adaptation	Blaine Phillips
Amendment	3	16 Sep 2013	Blood collection for fasting glucose procedure	D.C. Sharma
Amendment	4	01 Oct 2013	Dissection plan update	Blaine Phillips
Amendment	5	11 Nov 2013	IR dissection update	Blaine Phillips
Amendment	6	14 Nov 2013	Individual urine collection pilot	Blaine Phillips
Amendment	7	18 Nov 2013	Additional dissection endpoints IR arm	D.C. Sharma
Amendment	8	24 Apr 2014	Dissection grouping VI arm	Blaine Phillips
Amendment	9	11 Jun 2014	Plasma for nicotine analysis	Blaine Phillips
Deviation	1	11 Sep 2013	Food consumption in high fat diet group	Blaine Phillips
Deviation	2	11 Jun 2014	Exposure, nicotine out of limits	Blaine Phillips
Deviation	3	17 Jun 2014	Scale bar correction for plaque area images	Blaine Phillips
Deviation	4	22 July 2014	VI group redistribution	Blaine Phillips
Deviation	5	13 Nov 2014	Individual metabolic cages for urine collection	Blaine Phillips

12 Results

12.1 Parameters Used to Monitor the General Condition and Health of the Animals

12.1.1 Mortality

A total of 42 animals were found dead or were declared moribund and euthanized during the exposure period. The clinical observations or post-mortem cause of death for the animals is summarized in Table 16

i) **Technical deaths: 5 animals**

- Physical death from trauma
- Death during blood collection procedures

ii) **Moribund: 20 animals**

- Weak, hunched, or weight loss: six mice
- Visible growth, not healing: six mice
- Persistent wound: six mice
- Internal bleeding: one mouse
- Rectal prolapse: one mouse

iii) **Found dead: 16 animals**

iv) **Dead upon arrival: one animal**

The mice declared moribund or found dead did not appear with any treatment bias, and were generally distributed among the groups (Table 17), though a possible bias was evident in the sham group relative to the others. Four animals were removed from the study prior to the start date (because of technical or transport-related deaths). The animals declared moribund or found dead during the study were replaced with exposed reserve animals. The ‘found dead’ animals underwent a post-mortem examination to identify the cause of death, despite various degrees of cannibalism and/or decomposition.

Table 16. Mortality and moribund state

Incident #	Date	Study day	UAN	CAN	Replacement mouse	Cause of death
1	16 Aug 13	−17	N/A	N/A	N/A	Dead upon arrival
2	21 Aug 13	−12	9992048	N/A	N/A	Technical death
3	29 Aug 13	−4	5062014	920679	9992021	Technical death
4	31 Aug 13	−2	6022009	920581	9992017	Technical death
5	2 Sep 13	1	5062034	920381	9992018	Found dead
6	12 Sep 13	11	6132018	920537	N/A	Moribund: weak, hunched
7	12 Sep 13	11	5082010	920594	5992005	Found dead (possible technical)
8	13 Sep 13	12	1012002	920430	1992003	Technical death
9	17 Sep 13	16	8882016	920272	N/A	Moribund: body weight loss
10	17 Sep 13	16	3022027	920528	3992007	Found dead
11	24 Sep 13	23	4082012	920532	4992002	Moribund: swollen right mandible
12	9 Oct 13	38	2082026	920556	2992001	Moribund: persistent wound
13	11 Oct 13	40	4992006	920492	N/A	Moribund: growth on mandibular region
14	24 Oct 13	54	1032036	920903	1992005	Moribund: growth on mandibular region
15	24 Oct 13	54	1062021	920125	1992002	Moribund: growth under right eye
16	24 Oct 13	54	6132013	920502	6132023	Moribund: growth on mandibular region
17	25 Nov 13	85	2032031	920814	2992004	Found dead
18	25 Nov 13	85	1062024	921011	1992004	Moribund: persistent wound
19	24 Dec 13	114	1062034	920892	1992001	Moribund: weight loss and weak
20	29 Jan 14	151	1082037	921027	1992008	Found dead
21	3 Feb 14	156	1082023	921070	1992006	Moribund: persistent wound
22	11 Feb 14	164	3062021	920651	3992004	Moribund: rectal prolapse and weight loss
23	17 Feb 14	170	1062027	920176	1992007	Found dead
24	17 Feb 14	170	3082012	921024	3992002	Found dead
25	19 Feb 14	172	1032046	920217	N/A	Found dead
26	28 Feb 14	181	2082017	920852	2992008	Moribund: weak, hunched, and weight loss
27	1 Mar 14	182	3062031	920277	3992001	Found dead
28	1 Mar 14	182	1082034	921077	N/A	Moribund: persistent wound
29	11 Mar 14	192	2062043	920767	2992005	Moribund: growth on mandibular region, weak
30	14 Mar 14	194	5082020	920896	5992008	Moribund: persistent wound
31	14 Mar 14	194	1082032	920184	N/A	Moribund: persistent wound
32	21 Mar 14	202	3082008	921100	3992003	Found dead
33	23 Mar 14	204	4082029	920199	4992007	Found dead
34	28 Mar 14	208	1082019	920011	N/A	Found dead
35	28 Mar 14	208	4082018	920662	4992003	Moribund: internal bleeding
36	31 Mar 14	212	4082003	920307	4992004	Moribund: weak, hunched
37	3 Apr 14	214	3082031	920488	3992008	Found dead
38	7 Apr 14	218	1062041	920644	N/A	Found dead
39	10 Apr 14	221	3062045	920717	N/A	Technical death
40	27 Apr 14	239	1082030	920207	1062043	Found dead
41	28 Apr 14	240	3082007	920965	3032046	Found dead
42	29 Apr 14	241	4082038	920499	4992001	Moribund: weight loss

UAN: Uncoded animal number, CAN: Coded animal number

Table 17. Moribund or dead animals by exposure group

Group	Number dead/moribund
Sham	14
3R4F	4
THS2.2	8
Cessation	6
Switch	4

12.1.2 In-life observations

The animals were frequently observed throughout the study. Both scheduled group and individual observations were performed daily shortly after the final exposure hour (see Table 8). Any animal with an unusual observation was placed in a 'special observation' schedule for extended monitoring. The special observations noted in this study were categorised as follows:

i) Growths and swelling: 25 animals

ii) Alopecia: 121 animals

iii) Wounds: 14 animals

iv) Teeth (malocclusion): 18 animals

v) Weakness: 1 animal

vi) Stereotypy: 1 animal

These findings highlight the level of dominant behaviour, presented in the form of barbering (alopecia) and bullying behaviour (wounds). Many cases of malocclusion were observed, and this was controlled by careful observation and trimming of the teeth. Throughout the study, a number of growths (tissue masses) were observed. In most cases, these were not considered life-threatening nor moribund, and the animals were therefore scheduled for the upcoming dissection. However, in five cases (Table 16), the growth was accompanied by weight loss or weakness, and the animals were declared and processed as moribund. The above observations were distributed across all treatment groups, and were therefore unlikely to have affected the results of the study. A pathological assessment indicated that the majority of growths were xanthomas. Xanthomatosis has previously been observed in ApoE$^{-/-}$ mice, though mainly noted late in life (Moghadasian et al, 2001).

12.1.3 Body weight

Mice in all groups gained weight throughout the course of the study. However, the rate of weight gain differed among the groups, with three clear clusters developing early in the study period (Figure 7,Table 18). Conventional CS-exposed animals (3R4F) exhibited the lowest bodyweight, which became significantly different from that of sham-exposed animals from study day 44 onwards (p<0.05). Sham animals had a typical body weight gain profile, reaching a maximum average weight of 28.1 g at the study end. The body weight profile of animals exposed to THS2.2 aerosol was indistinguishable from that of sham-exposed animals. Following cessation or switching to THS2.2, the animals rapidly gained weight, approaching the sham body weight after just 3 weeks post-switch/cessation. This was followed by a slight weight reduction in both the cessation and switching groups and a final settling at a level just below the sham group for the remainder of the study period. This pattern was not related to any experimental procedures (i.e., urine or blood collection), and has also been observed in a previous pMRTP switching study (Phillips, 2013), which used a platform 2 prototype.

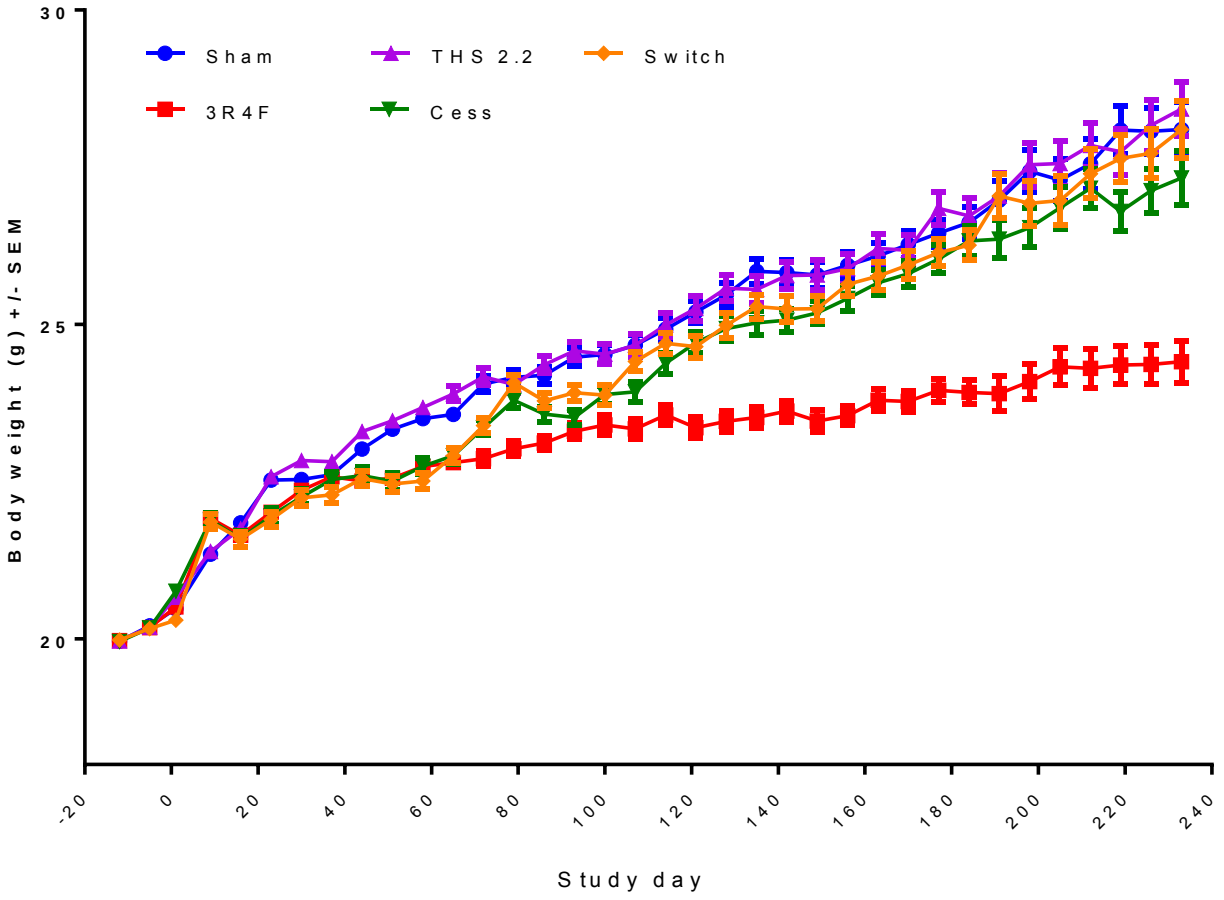


Figure 7. Body weight for all exposure groups during the exposure period.
Mean ± SEM.

12.2 Parameters used to Monitor Smoke Uptake and Exposure

12.2.1 Carboxyhemoglobin

Blood COHb levels were analysed three times in the study, at months 2, 4, and 7 (Figure 8). As expected, the percentage of blood CoHb was consistently higher in the 3R4F-exposed group compared with the fresh air-exposed group. The aerosol from THS2.2 nicotine-matched to 3R4F is known to have a considerably lower CO content, which was reflected in the lower blood COHb levels (Table 3) of the chronically exposed THS2.2 and switch groups of animals. The cessation group had COHb levels similar to those measured in sham group animals.

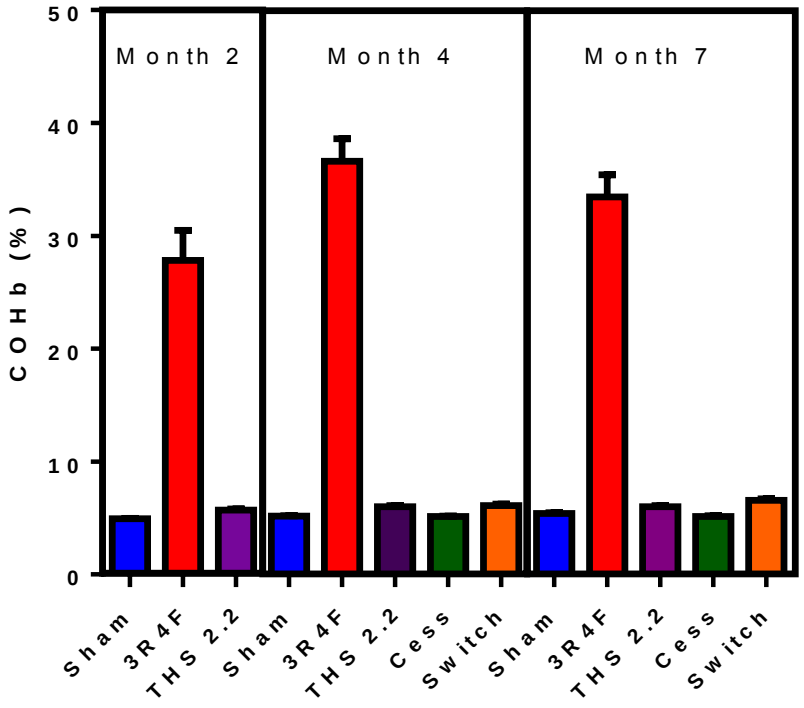


Figure 8. Blood carboxyhaemoglobin concentration

Mean ± SEM, n = 9–12.

12.2.2 Plasma nicotine and cotinine

Plasma was collected once during the study (in month 8) to analyse nicotine and cotinine levels (Amendment 9, analysis performed at test site 6). Both nicotine and cotinine were present in substantial (>LLOQ) levels (p>0.05) in the CS- and THS2.2-exposed groups only (Figure 9). Although not significant, a slightly higher trend was observed for both cotinine and nicotine in plasma from the THS2.2 group, which has been observed in a previous rat study (Wong, 2014), and may be reflective of reduced irritant to the respiratory tract from the THS2.2 aerosol compared to CS, affecting the minute volume of the animals.

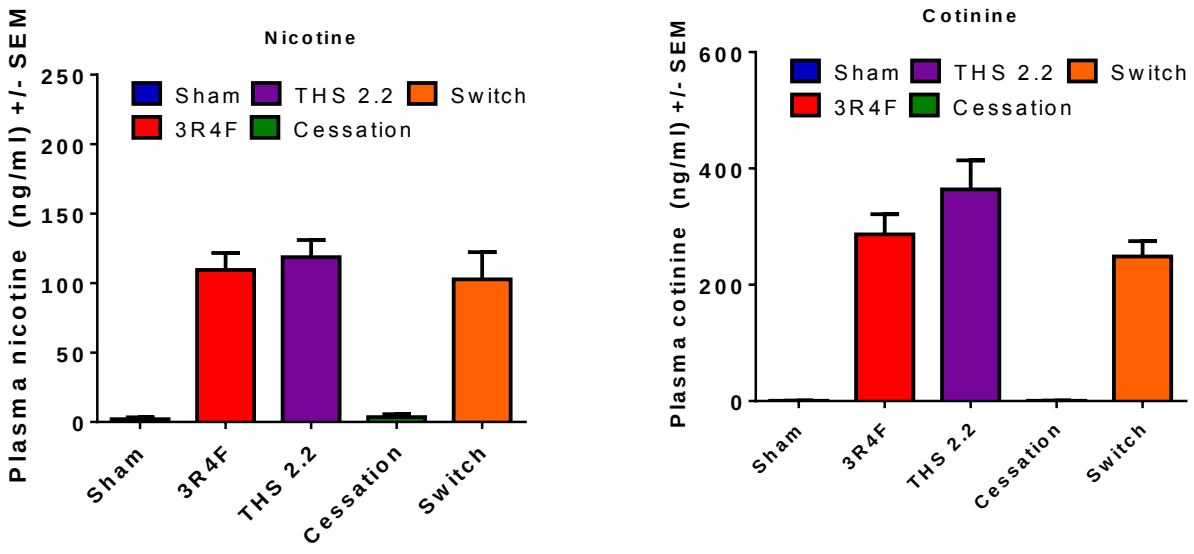


Figure 9. Plasma nicotine and cotinine concentrations
Mean ± SEM, n = 8.

12.2.3 Representative nicotine metabolites in mouse urine

To demonstrate aerosol uptake, the animals were placed into metabolic cages for an 18-h collection period immediately following exposure, and until exposure on the following day. The following metabolites were quantified in the urine samples:

i) 3'-Hydroxycotinine
ii) Norcotinine
iii) Cotinine
iv) Nicotine-N'-oxide
v) Nornicotine

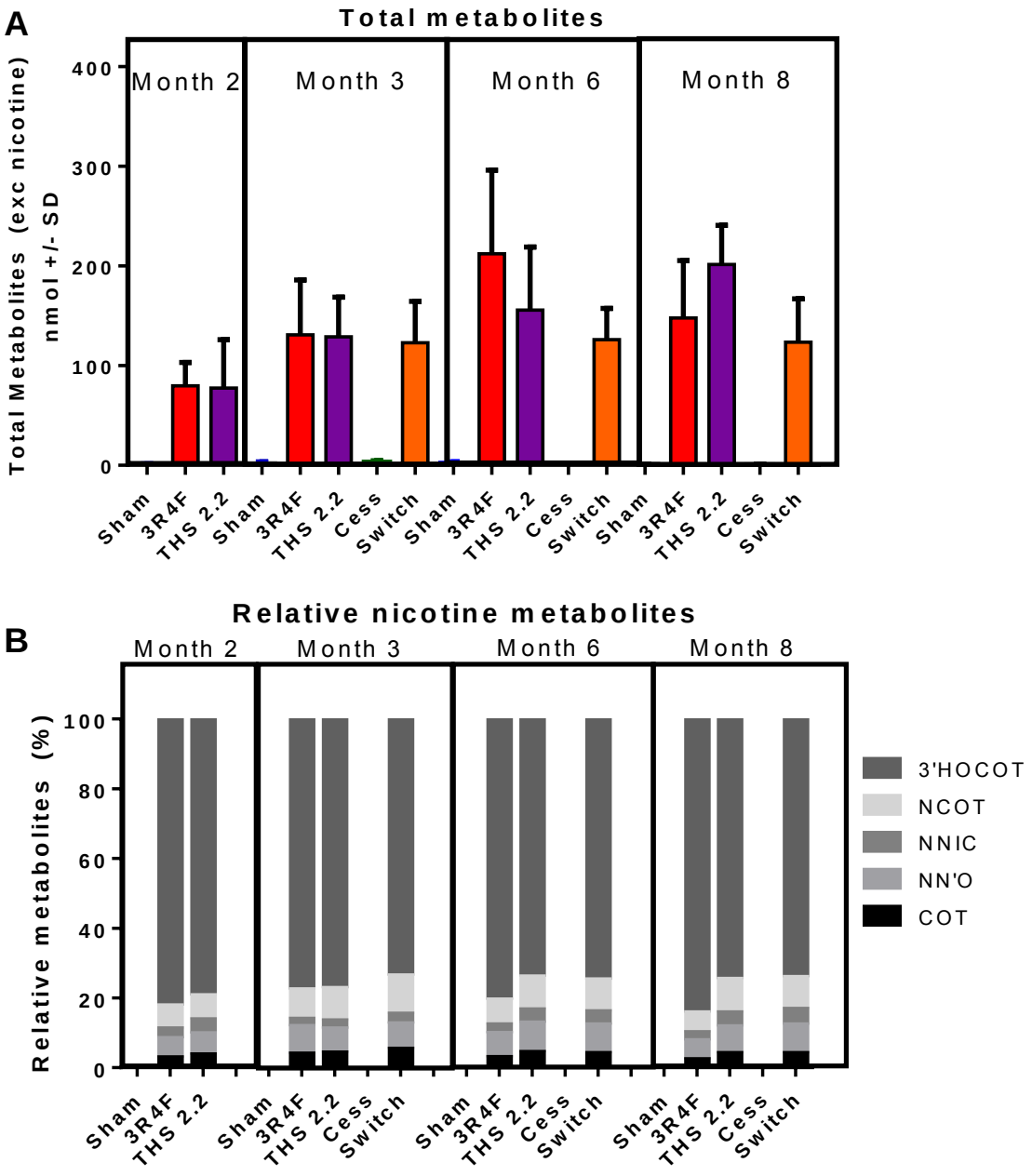


Figure 10. Nicotine metabolites in urine.

A) Total metabolites per mouse (mean ± SD). B) Relative metabolites from groups exposed to nicotine-containing smoke or aerosol (mean, no error; n = 8). 3'HOCOT, 3-hydroxycotinine; NCOT, norcotinine; NNIC, nornicotine; NN'O, nicotine-1'-N-oxide; and COT, cotinine.

Predictably, nicotine metabolites were only recovered in the animal groups exposed to either 3R4F or THS2.2 test atmospheres, including the switch to THS2.2 group (Figure 10, Figure 11). Individual metabolites (Figure 11) demonstrated the same general trends as depicted in the total metabolite values.

Although animals in the 3R4F and THS2.2 groups inhaled aerosols with similar nicotine levels (Table 3), there remained some variability in total nicotine measurements. The following factors may have limited the quantitative aspect of this assay:

i) Month 2 urine collections were performed using metabolic cages with four mice. Subsequent collections were performed in metabolic cages housing individual mice (deviation 5). These individual cages enabled improved urine recovery compared with the group metabolic cages, so metabolite recovery is higher from month 3 onwards.

ii) Urine was only collected post-exposure. It is likely that metabolites were secreted in the urine during the exposure period, so would not be accounted for in this analysis. This likely resulted in variability from sample to sample.

iii) Only five metabolites were evaluated, and nicotine itself was excluded because of the possibility that environmental nicotine would contaminate the sample.

Despite the above limitations, substantial nicotine recovery was still observed in all groups subjected to nicotine exposure. Animals in the sham and cessation groups generally had nicotine values below the LLOQ. In addition determinations of nicotine and cotinine in blood, corroborated the correct exposure of the mice to the aerosols.

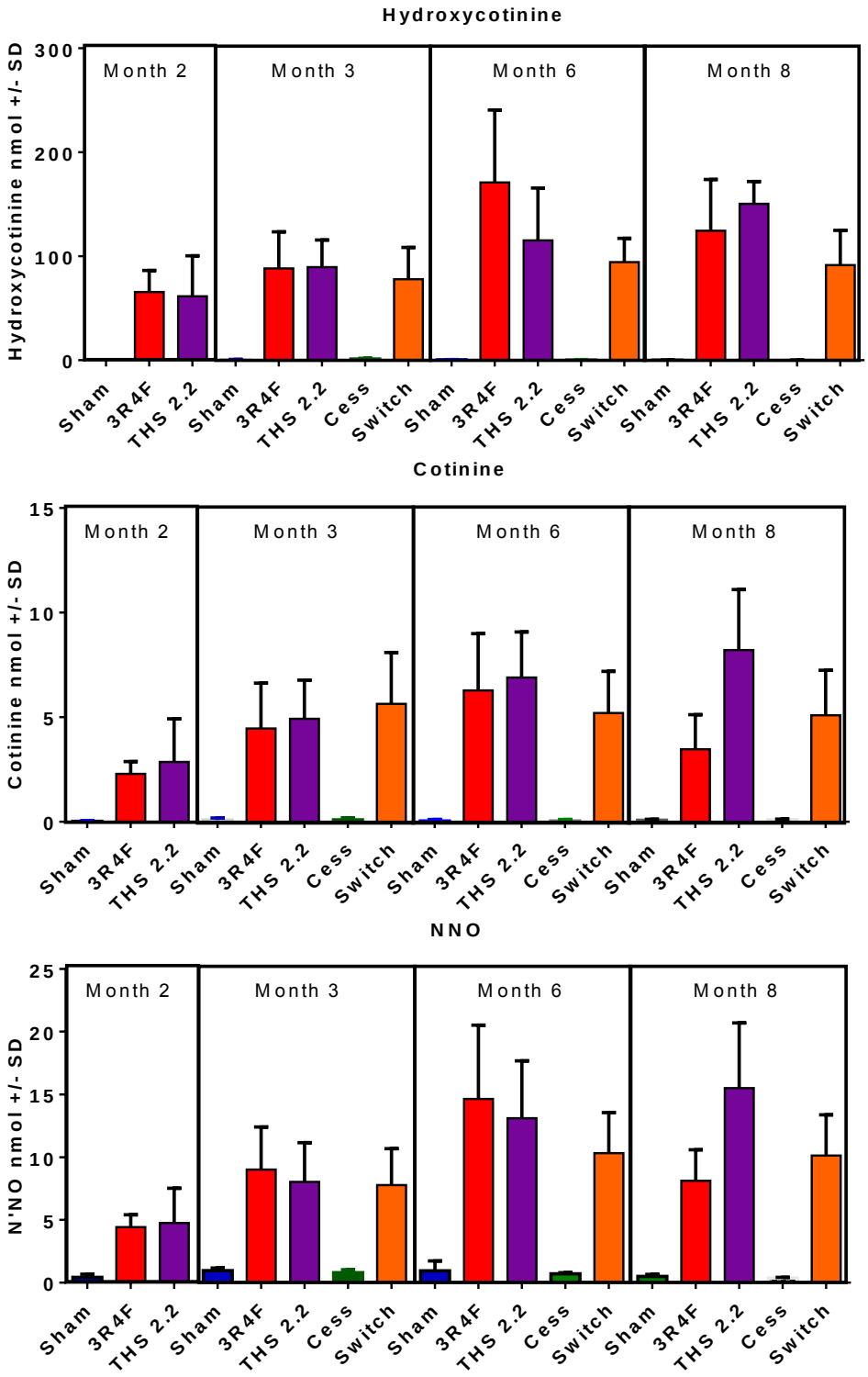


Figure 11 (continued on next page)

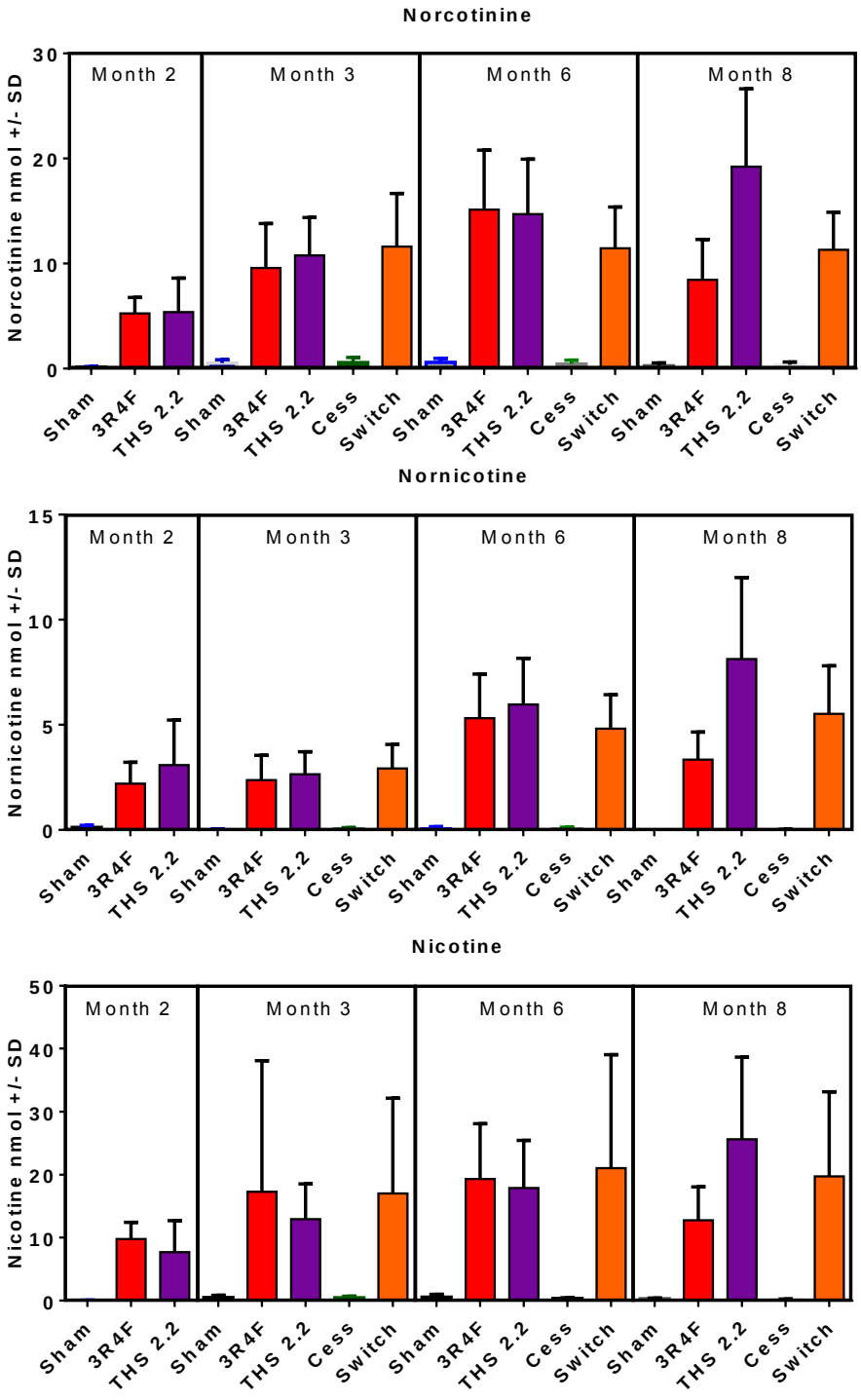


Figure 11. Nicotine and its metabolites in the urine (individual metabolites).

Mean ± SD, n = 8.

12.2.4 Additional smoke constituent metabolites in mouse urine

In addition to nicotine and its metabolites, urine samples were also evaluated for additional metabolites of smoke constituents, oxidative stress biomarkers, and eicosanoid levels (Table 19 to Table 34).

Four non-nicotine cigarette smoke metabolites were selected (HPMA, NNAL, S-PMA, and CEMA) because they are commonly used in clinical studies as biomarkers of CS exposure, and represent metabolites of known potentially harmful smoke constituents. HPMA is a metabolite of acrolein, NNAL a metabolite of 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), S-PMA a metabolite of benzene, and CEMA a metabolite of acrylonitrile (Figure 12). Sham-exposed animals had an average urine basal HPMA level of 2000 ng (range, 1829–2500 ng depending on the month), and showed no significant difference from THS2.2-exposed animals. The basal level in the sham animals reflects the normal physiological presence of acrolein as a product of lipid peroxidation (Carmella et al, 2009).

CS increased HPMA levels relative to sham across all study time points. Affected urine levels of the three other non-nicotine smoke metabolites were more noticeable because of the absence of endogenous metabolites. The level of NNAL was much higher in CS-exposed animals at each time point, with generally very low levels detected in THS2.2-exposed animals (chronic THS2.2 or switching groups) reflecting the trace amounts of NNK in the aerosol. Similar detection patterns were seen for S-PMA and CEMA, again reflecting the significantly reduced levels in THS2.2 aerosols. Again, the values from month 2 were generally lower than those from months 3, 6, and 8 because of the change from pooled urine samples from four mice in month 2 to the more efficient single urine samples from month 3 onwards.

As the analysis of these metabolites of smoke constituents used the same urine as was used for the nicotine metabolites, the same limitations apply. Mainly that only a post-exposure urine sample was collected, resulting in variability as metabolites in urine secreted during exposure were not accounted for.

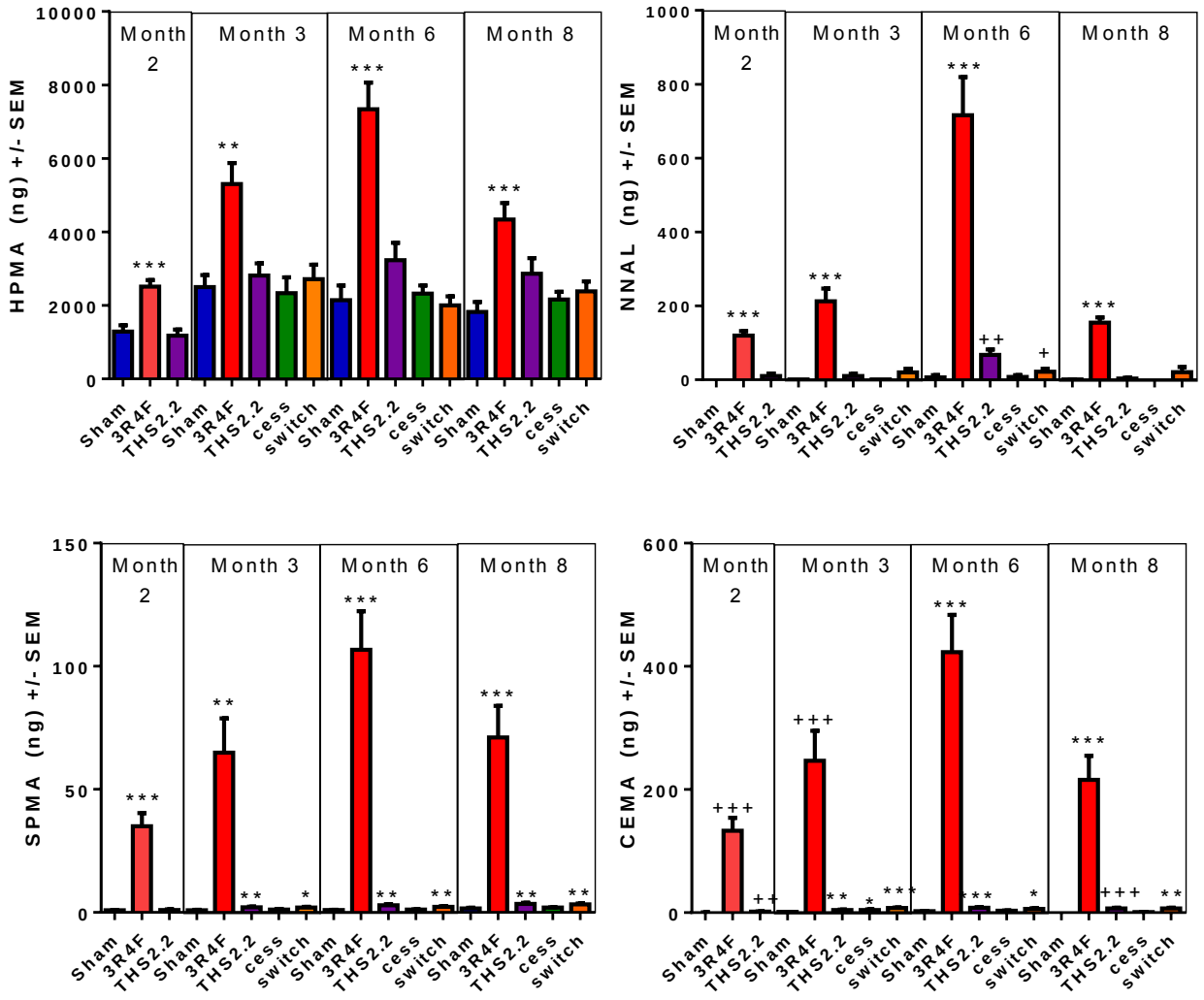


Figure 12. Urinary biomarkers of exposure (non-nicotine exposure metabolites).

Mean ± SEM, n = 8. *P*-values against Sham obtained by t-test accounting for variance heterogeneity (*) or by Wilcoxon exact MC (+). ***/+++$p < 0.001$; **/++$p < 0.01$; */+$p < 0.05$.

Additional biomarkers of exposure were grouped as markers of oxidative stress (Figure 13) and eicosanoid levels (Figure 14). The DNA adduct 8-OH-dG was previously shown to be associated with increased oxidative stress following CS exposure in clinical subjects (Doruk et al, 2011). Analysis of post-exposure urine in this study showed that 8-OH-dG levels trended slightly higher in CS-exposed animals compared with sham animals; however, these observations were not significant ($p>0.05$) and there were no obvious effects of either THS2.2 exposure or from switching/cessation.

Both MDA and 4-HNE, representing markers of oxidative stress, were detected in significantly higher levels in CS-exposed mice (relative to sham-exposed animals) in months 6 and 8 (p<0.05).

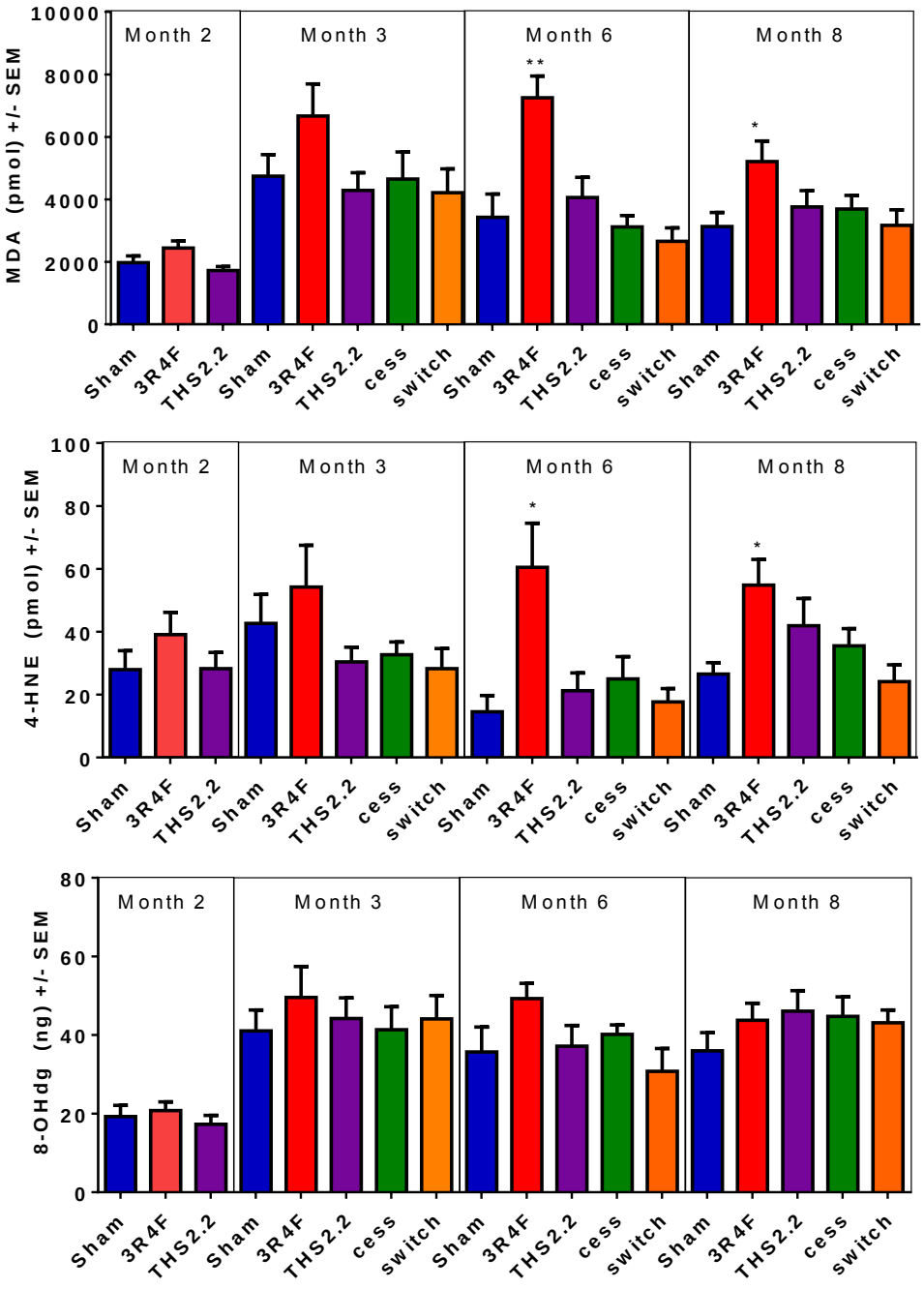


Figure 13. Urinary biomarkers of exposure (markers of oxidative stress).

Mean ± SEM, n = 8. *P*-values against Sham obtained by t-test accounting for variance heterogeneity (*) or by Wilcoxon exact MC (+). ***/+++p < 0.001; **/++p < 0.01; */+p < 0.05.

A series of nine eicosanoid species was evaluated (Figure 14), and some significant differences were observed between 3R4F and sham groups ($p < 0.05$) in month 6 (increased T-pGEM, 2,3-di-PGF2a, 11-dh-TXB2, and PGF2a), there were no overall persistent trends at the different time points evaluated, nor were there trends related to aging effects.

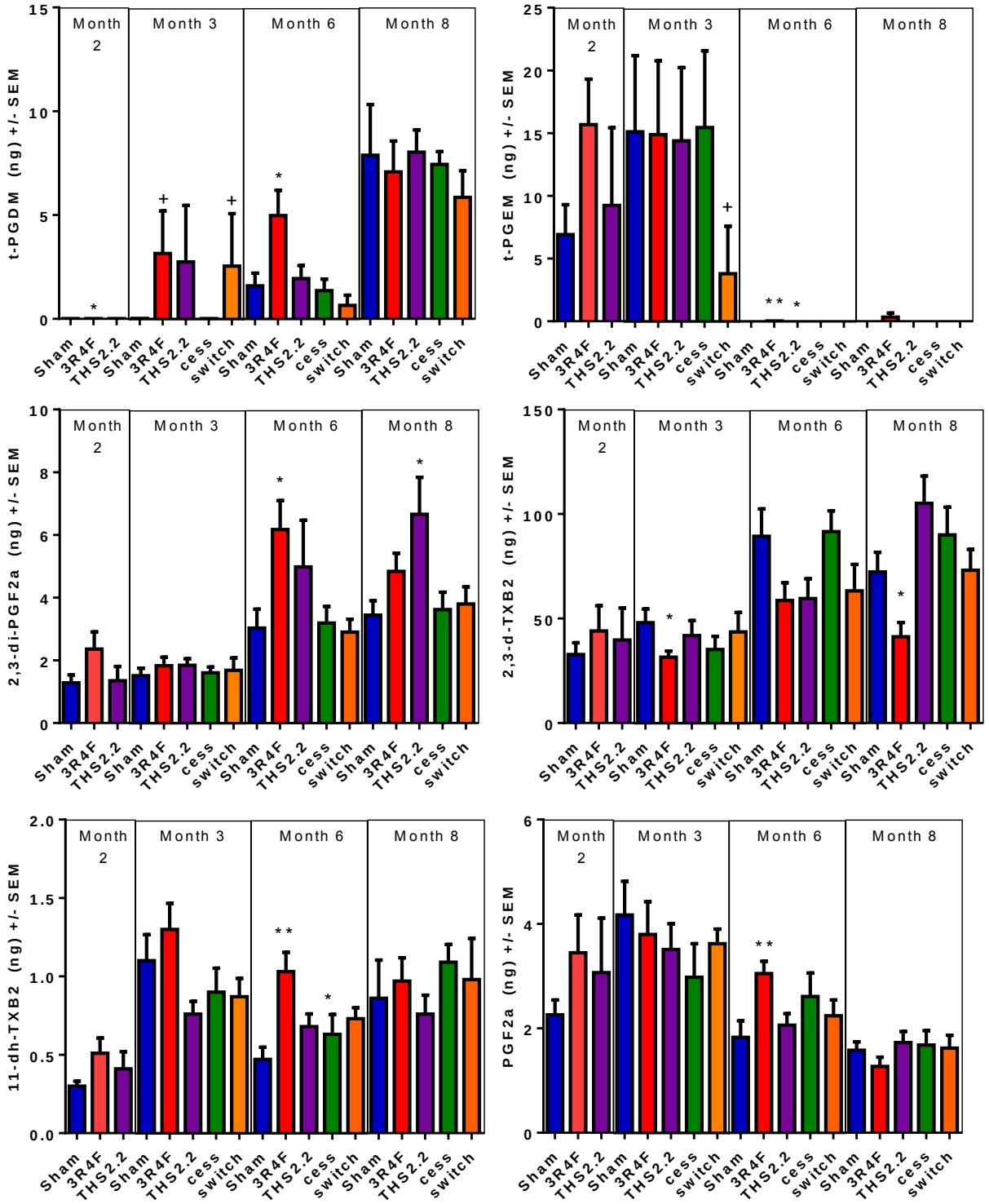


Figure continued on next page.

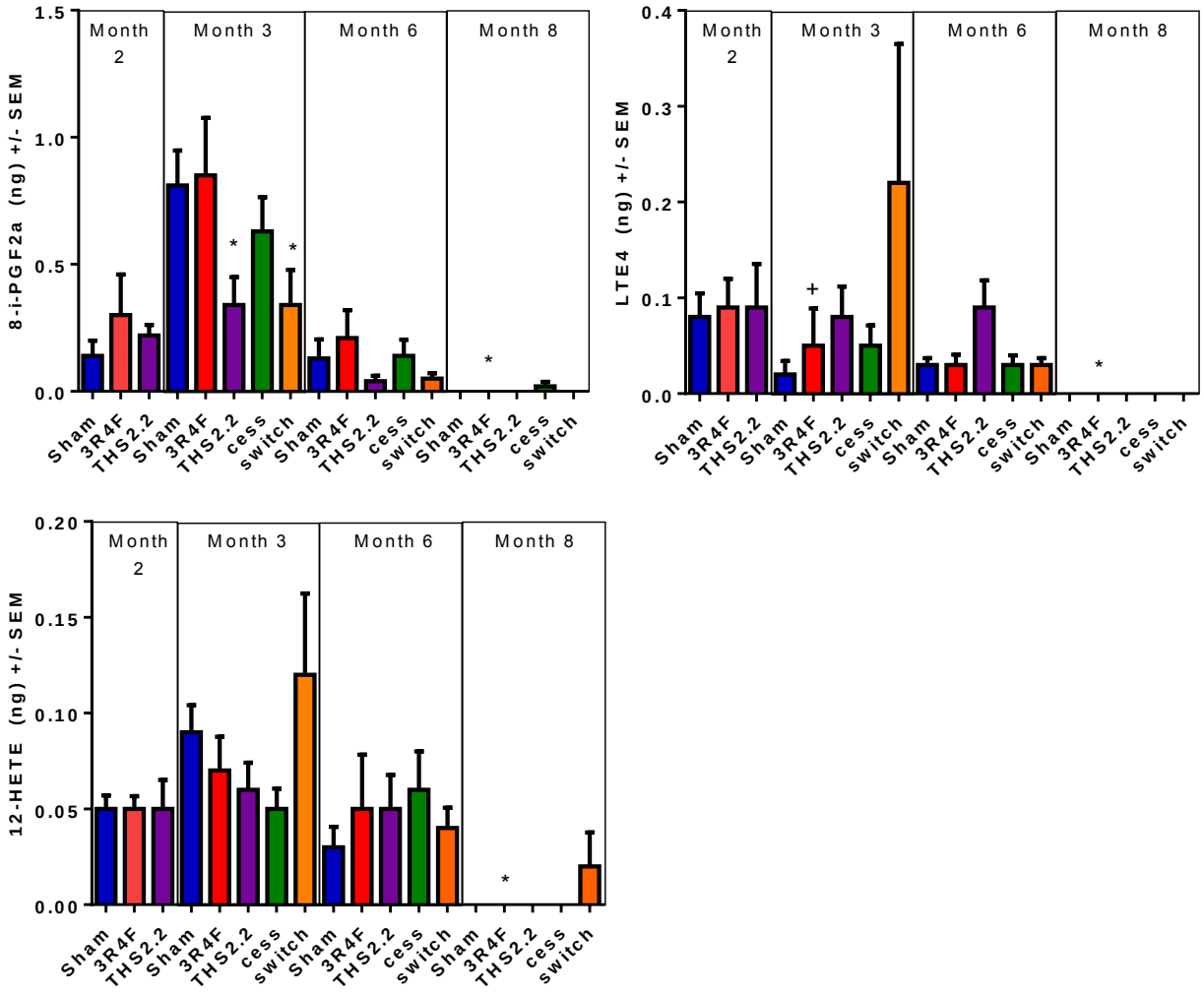


Figure 14. Urinary biomarkers of inflammation and eicosanoids.

Mean ± SEM, n = 8. *P*-values obtained by t-test accounting for variance heterogeneity or by Wilcoxon exact MC. ***/+++$p < 0.001$; **/++$p < 0.01$; */+$p < 0.05$.

12.3 Lung Function Measurements

To measure lung function, the animals were anaesthetized, tracheotomized, and cannulated, with the cannula connected to the flexiVent rodent ventilator set (Scireq, Montreal Canada). A series of perturbations was performed (Table 35 to Table 52), and the results are reported in the following sections.

12.3.1 Snapshot perturbations (single compartment model)

The linear first-order single compartment model of respiratory function is one of the standard models of respiratory mechanics, and produces the commonly used dynamic resistance and compliance parameters. In this forced oscillation technique, a sinusoidal pressure waveform is produced on the subject's airway to produce resistance and compliance measurements at a single defined frequency.

The dynamic lung resistance (R) reflects the sum of both airway and tissue resistance, and is used to indicate the level of pulmonary constriction. Dynamic elastance (E) is the elastic rigidity of the lungs, and compliance (C) is the ease with which the lungs may be extended (and is the reciprocal of 'E'). Experimental emphysema has been shown to result in lower dynamic resistance and higher compliance compared with healthy subjects in murine models (Vanoirbeek et al, 2010).

The snapshot perturbation manoeuvres in 3R4F group showed a significantly lower R (p<0.05 in month 2) and E (p<0.05 at the month 2 and 3 time points) and higher C (p<0.05 at the month 2 and 3 time points) compared with the sham-exposed group (Figure 15). All three parameters showed a time/aging effect, which may have contributed to the convergence of the measurements, resulting in no significant differences between CS-exposed and fresh air-exposed animals at the final two study endpoints (months 6 and 8). This suggests that the effect of aging dominates and possibly masks the effect of the exposure in later stages at later time points. Exposure of the animals to the THS2.2 aerosol had no obvious impact in any of the snapshot perturbations relative to the sham-exposed group (p>0.05 for all parameters). Neither switching nor cessation resulted in any obvious effects in the snapshot perturbation parameters, largely because of the absence of any exposure group effects in the latter part of the study, when the switching/cessation effects would normally be most evident. Note that the differences in the 3R4F group relative to the sham was higher in month 2 relative to month 1 or 3. This may reflect month to month experimental variability, though there were no identified causes of this variability. To minimize this variability, we had randomized the animals (and groups) during the necropsy, and efforts were made to minimize a handler bias, and prior to every animal, the equipment had a validation cycle ensuring that the system was air tight, and recording pressure and volume readings at acceptable levels.

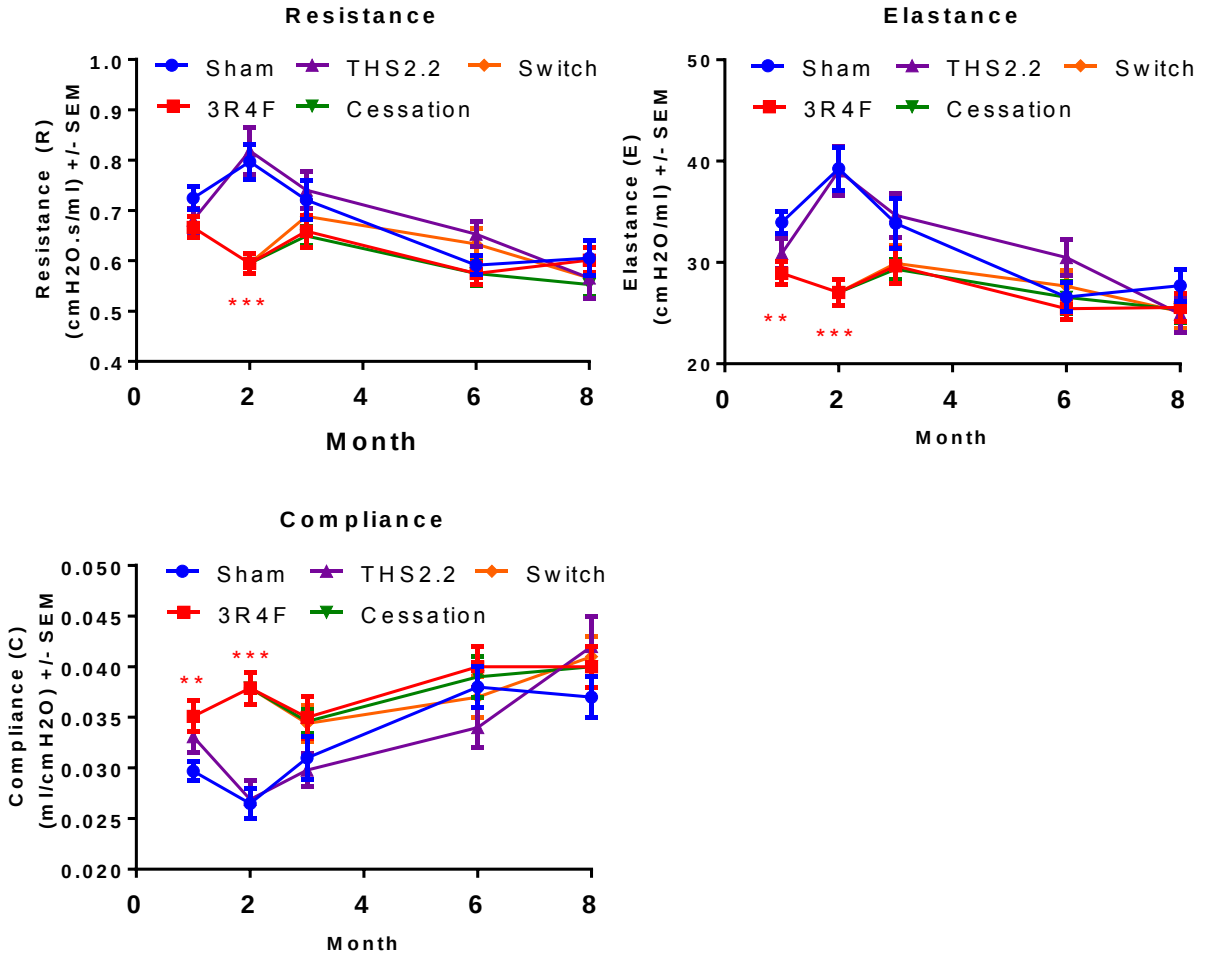


Figure 15. Lung function (snapshot perturbations).

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.3.2 Primewave-3 (constant phase model)

The constant phase model is a more comprehensive model of respiratory physiology that enables a distinction to be made between central and peripheral respiratory mechanics. Newtonian resistance (Rn) represents the resistance in the central airways, whereas tissue damping (G) is closely related to tissue resistance, and reflects the energy dissipation in the lung tissue. Experimentally induced lung emphysema in mice results in lower airway resistance and tissue damping (lung tissue resistance) compared with healthy subjects. Tissue elastance (Htis) is closely related to dynamic elastance, and reflects the energy conservation in the lung tissue and ultimately describes the ‘stiffness’ of the lung. A decrease in Htis is indicative of emphysema and reflects the reduced elastic

rigidity or stiffness of the lung tissue. Tissue hyperesivity (eta=G/H) characterizes the ratio of energy dissipation to energy conservation in the lung tissue, and is unaltered in an emphysematous lung.

The primewave-3 model showed a reduction of resistance of the central airways (Rn, $p<0.05$ at months 2, 3, and 6 timepoints), and in the lung tissue (G, $p<0.05$ in months 1 and 2), and a decrease in tissue elastance ($p<0.05$ in months 1 and 2) in 3R4F group (Figure 16). Following a similar trend as that seen in the snapshot perturbations, the differences were observed in early study time points, but were no longer detected by the end of the study. Therefore the effect of cessation or switching was observed only in Newtonian resistance, where the CS effect persisted to month 6, and a significant difference from CS emerged for both cessation and switching groups ($p<0.05$). Tissue hyperesivity results were similar across all groups and time points, with only occasional values achieving significance; this is likely to reflect incidental variance in the measurement. Similar to the month to month variability observed in the snapshot perturbations, the primewave-3 model also had greater sham to CS differences in month 2 relative to month 1 and 3, with again no obvious technical reasons.

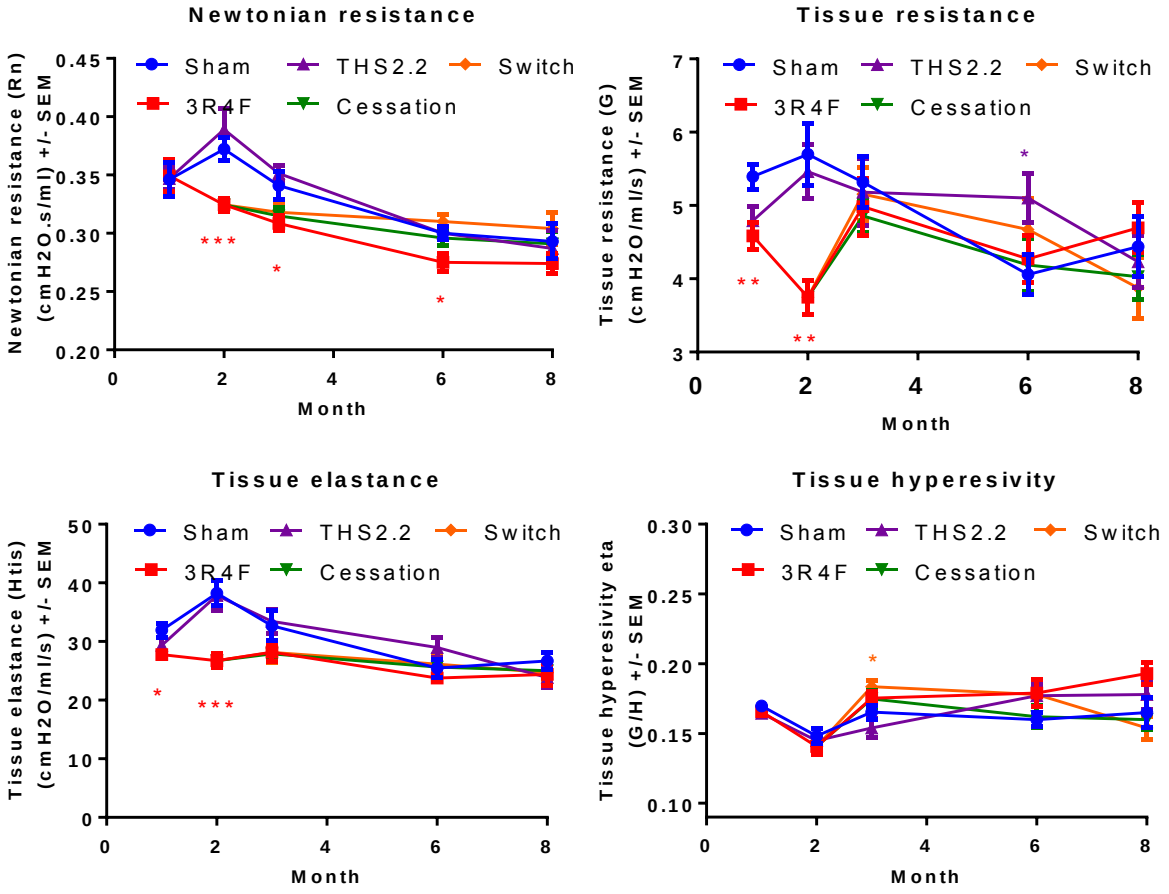


Figure 16. Primewave-3 perturbations

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.3.3 Quasi-static pressure volume loop (Salazar–Knowles analysis)

Pressure volume loops (P–V loops) capture the quasi-static mechanical properties of the respiratory system. The Salazar–Knowles equation can be fitted to the P–V loops, enabling the calculation of: quasi-static elastance (Est), reflecting the static elastic recoil pressure of the lungs at a given pressure; quasi-static compliance (Cst, the reciprocal of Est); the Salazar–Knowles parameter, A, which is an upper bound estimate of the difference between total lung volume and zero volume; and K, reflecting the curvature of the upper portion of the deflation P–V curve. Additionally, hysteresis (the area of the P–V loop) can be calculated and, together with A and K, indicates enlarged alveolar spaces, loss of elastic recoil, and hyperinflation of the lungs. Upon the induction of

experimental emphysema in mice, parameters A and K and hysteresis are typically increased, and Est decreased (and consequently Cst increased) relative to healthy subjects.

Exposure of the mice to 3R4F CS resulted in a leftward shift of the P–V loops for both the inflation and deflation phase of the manoeuvre (Figure 17) from month 2 onwards. The P–V loop also showed an increased lung volume at the maximum pressure (30 cm H_2O) in 3R4F-exposed animals compared with the sham-exposed group (Figure 17). These changes persisted from month 2 until the end of the study period, and were further reflected by changes in parameter A (Figure 18) where differences were seen for CS exposed animals from month 1; THS2.2 and sham-exposed animals showed similar levels of this parameter ($p>0.05$). The switching and cessation groups both recovered similarly for parameter A, and separated from CS-exposed animals after 3 months' of cessation/switching. A similar pattern was observed for hysteresis, whereby CS-exposed animals demonstrated a higher hysteresis value from month 1 relative to sham ($p<0.05$). Moreover, a recovery towards the sham hysteresis values in months 6 and 8 for both cessation and switching groups was observed ($p<0.05$ compared with 3R4F). The curvature of the P–V loop, represented by K, showed no major or persistent differences between the exposure groups. CS-exposed animals generally displayed a lower K value towards the later months of the study, but this was only significant at the month 6 time point ($p<0.05$).

The quasi-static elastance, Est, in the 3R4F groups was significantly lower ($p<0.05$) than the sham groups from 1 month post-cigarette exposure, while THS2.2 values were similar to those of the sham group. The switching/cessation groups demonstrated a recovery, in which Est values settled between those of the 3R4F and sham groups ($p<0.05$ versus 3R4F for both groups at the month 6 time point only). The quasi-static compliance (Cst) showed a similar trend over time.

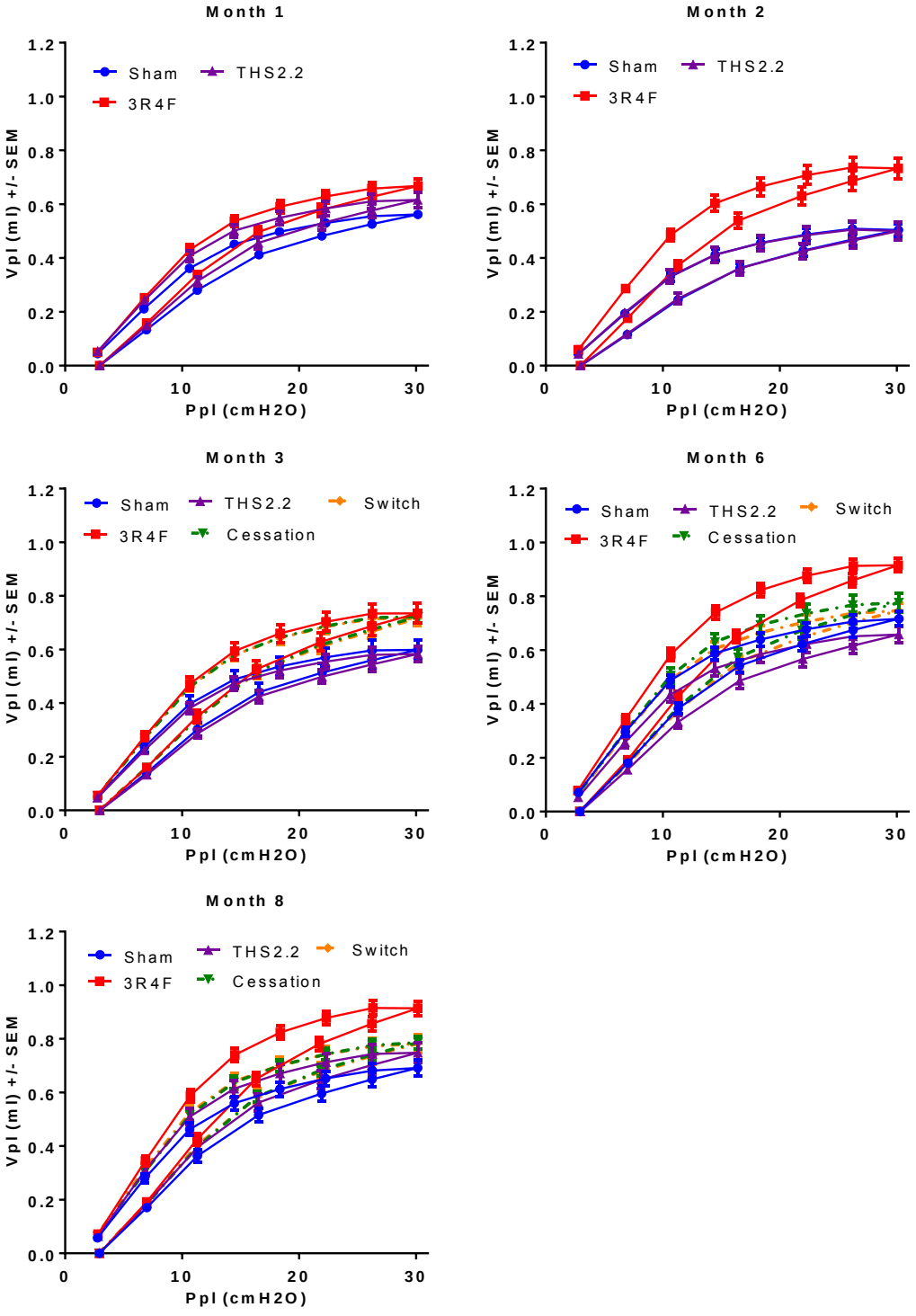


Figure 17. Pressure volume loops.

The relationship between pressure (P_{pl}, pressure plethysmography) and the resultant volume (V_{pl}, volume plethysmography) over an inflation/deflation cycle is shown. Mean ± SEM, n = 9–10.

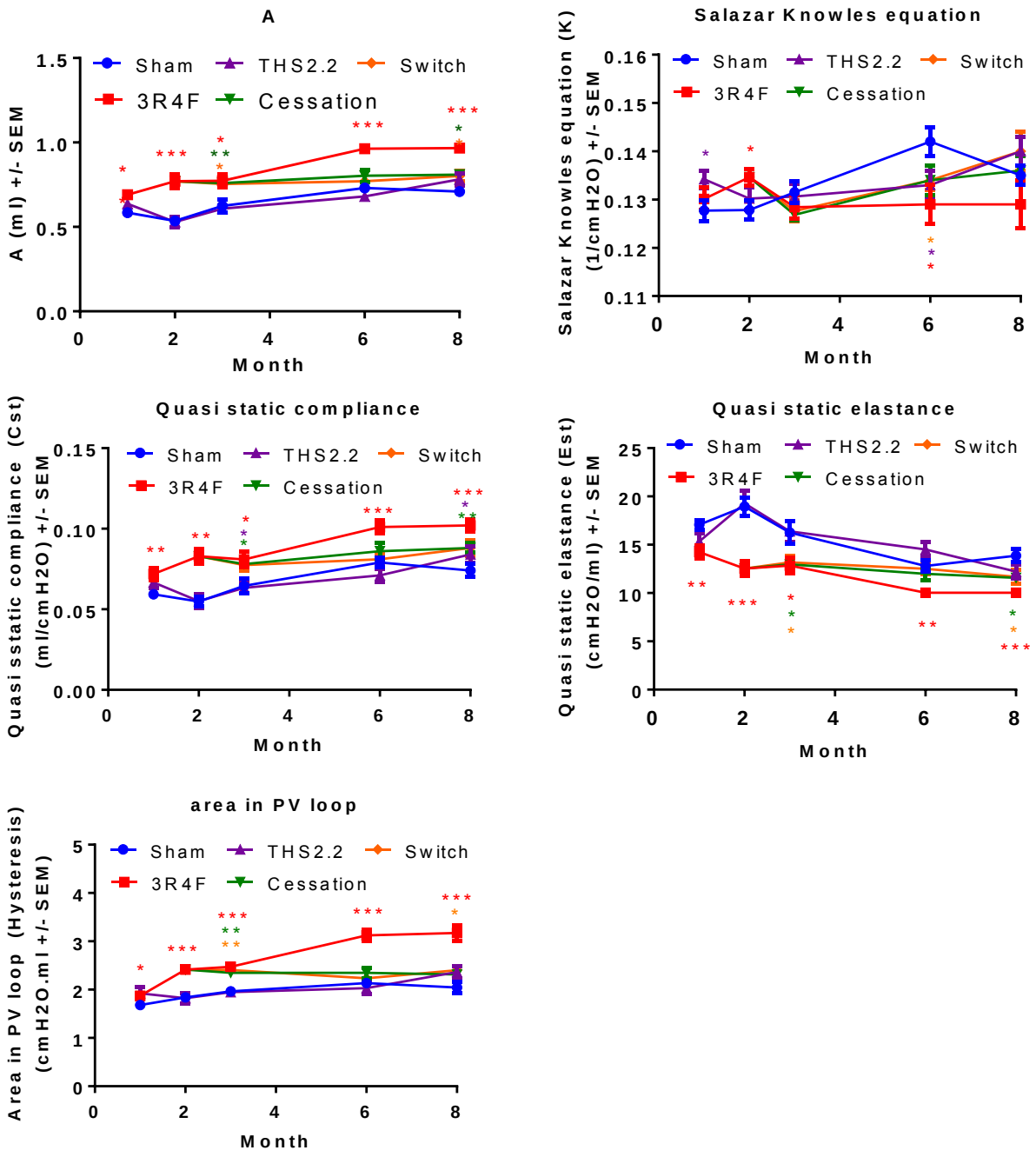


Figure 18. Salazar–Knowles analysis

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.3.4 Negative pressure forced expiration

These perturbations are produced using a slow inflation of the lung to 30 cm H_2O, followed by the rapid switching to a negative pressure. In humans, the ratio FEV_1 represents the proportion of a subject's vital capacity that they are able to expire in the first second of a forced expiration to FVC. Obstructive pulmonary diseases are diagnosed when the ratio of FEV_1 to FVC is reduced, primarily based on the increased resistance of the airways and thus the decreased capacity of the patient to expire a significant proportion of the lung volume in a short time. In the mouse model, $FEV_{0.1}$ or $FEV_{0.2}$ (time period of 0.1 or 0.2 s, adapted to the respiratory frequency and lung volume of the mouse) and FVC can be measured using the above-mentioned technique. The observed reduction in airway resistance upon smoke exposure and the increased pulmonary volume reported above should result in an unaltered $FEV_{0.1}$ or $FEV_{0.2}$/FVC ratio.

The parameters $FEV_{0.1}$, $FEV_{0.2}$, and FVC (Figure 19) were all significantly higher ($p<0.05$) in CS-exposed animals than in sham animals at all time points. However, this is likely to represent differences in lung volumes, because no consistent differences were observed between the different exposure groups when standardized to the FVC. Furthermore, it has previously been noted that these key human variables are less useful when evaluating mouse models of emphysema because of technical limitations of the experimental system (Vanoirbeek et al, 2010).

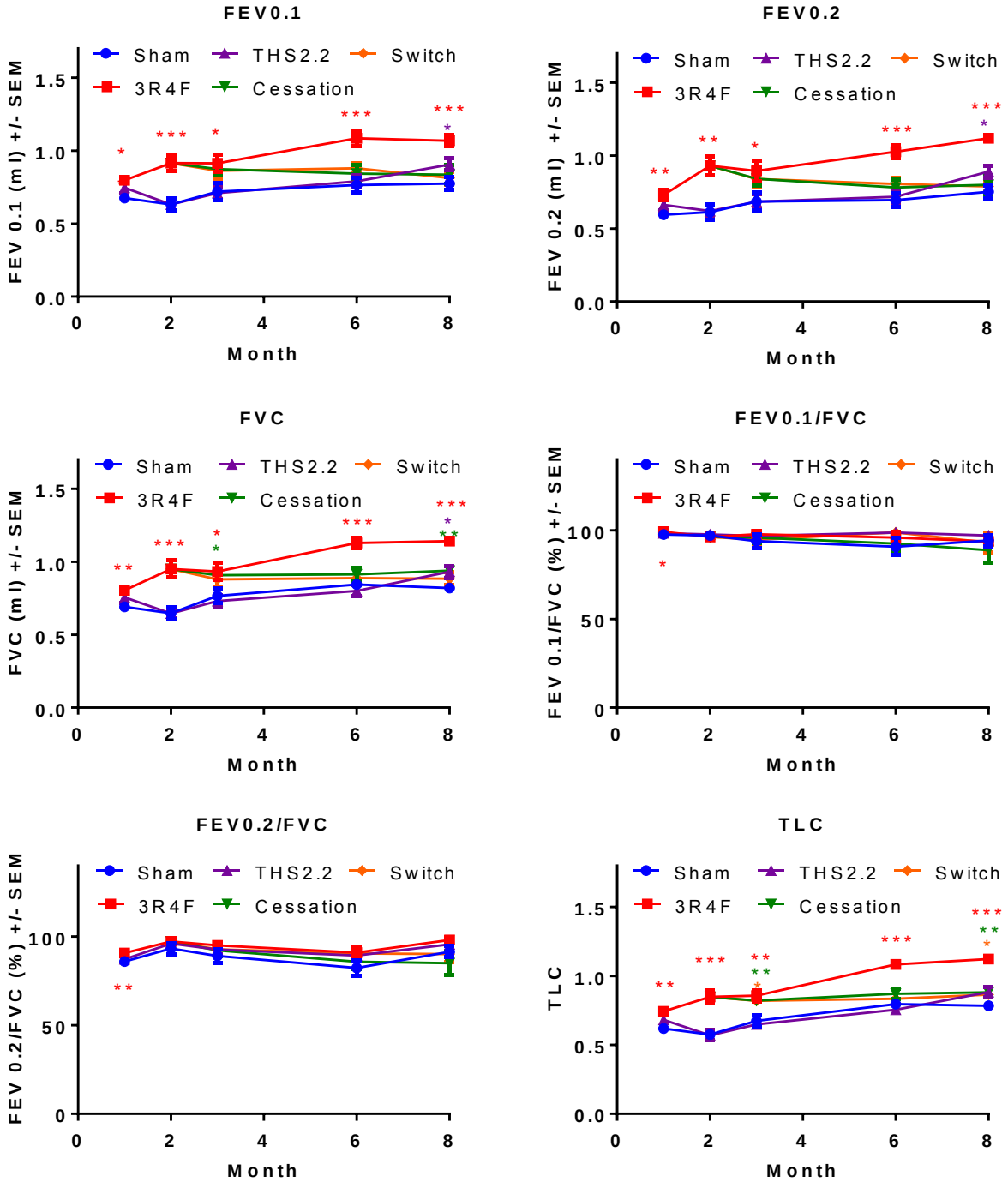


Figure 19. Negative pressure forced exhalation perturbations

Mean ± SEM, n = 11–12. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.4 Haematology

During necropsy of the animals, a terminal bleed was performed and fresh blood samples were sent for complete blood count analysis.

12.4.1 Red blood cells

Analysis of the red blood cells demonstrated an increase in red blood cell count and haematocrit typical for animals exposed to CS (Figure 20, Table 53 to Table 58). This has been seen in previous cigarette smoke studies, and is thought to be a consequence of the CO present during exposure periods. We also observed a corresponding increase in net haemoglobin concentration and mean haemoglobin volume in 3R4F group, though this appeared to be representative of the increased cell number, because the mean haemoglobin content (haemoglobin content as a function of packed cell volume) was similar across groups (p>0.05). Exposure of the animals to the THS2.2 aerosol resulted in values of red blood cell parameters that were very similar to those of sham-exposed animals. This is likely to reflect the lower CO levels emitted by THS2.2 sticks compared with 3R4F cigarettes. Both cessation and switching resulted in a rapid reversion back to sham levels within 1 month of cessation/switching, although a lag was seen for the mean erythrocyte volume parameter, which returned to sham levels only 3 months after switching/cessation.

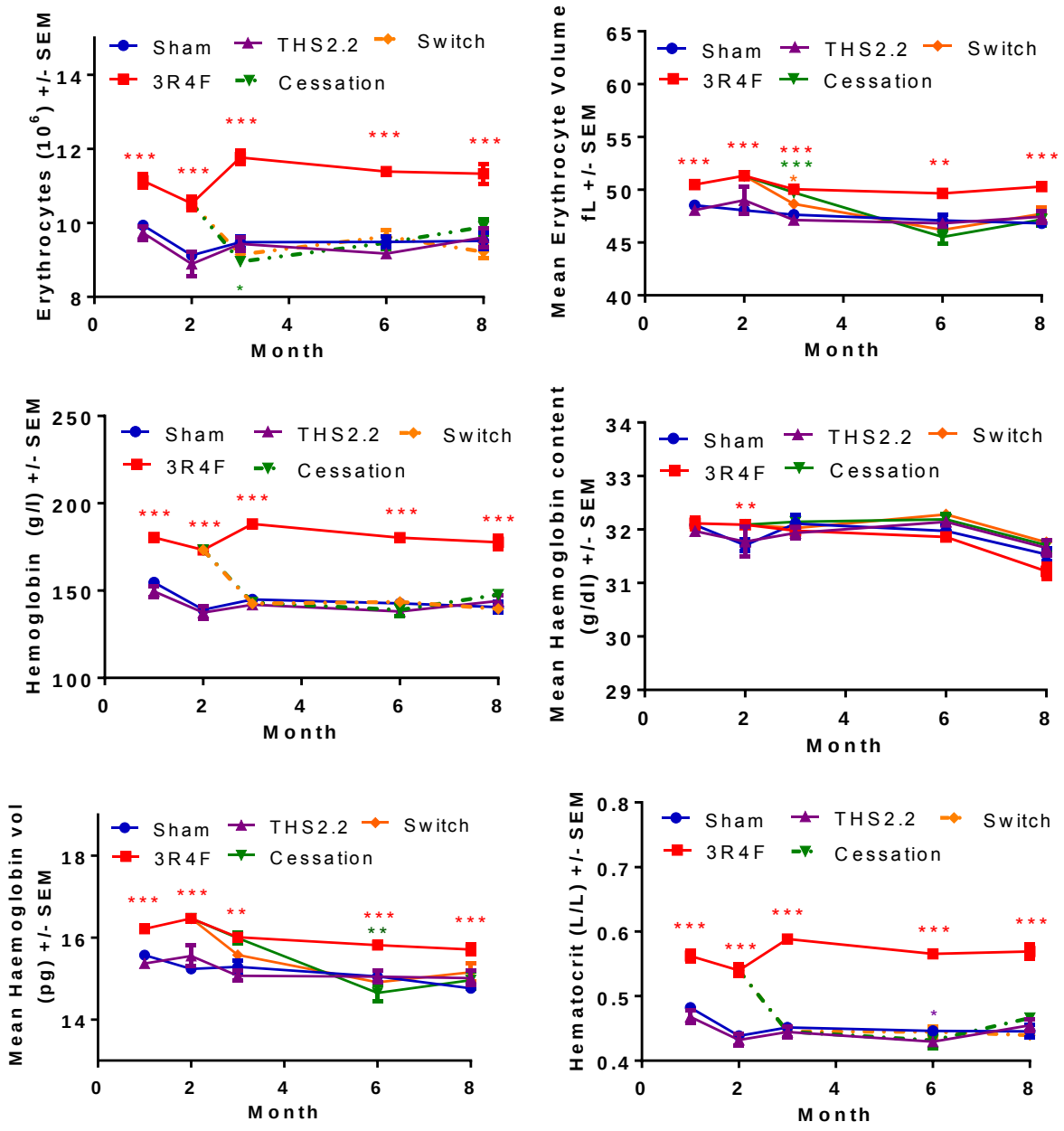


Figure 20. Haematology, red blood cell parameters

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.4.2 White blood cells

There were no obvious trends or differences in white blood cell counts between the different exposure groups throughout the exposure period (Figure 21, Table 59 to Table 72).

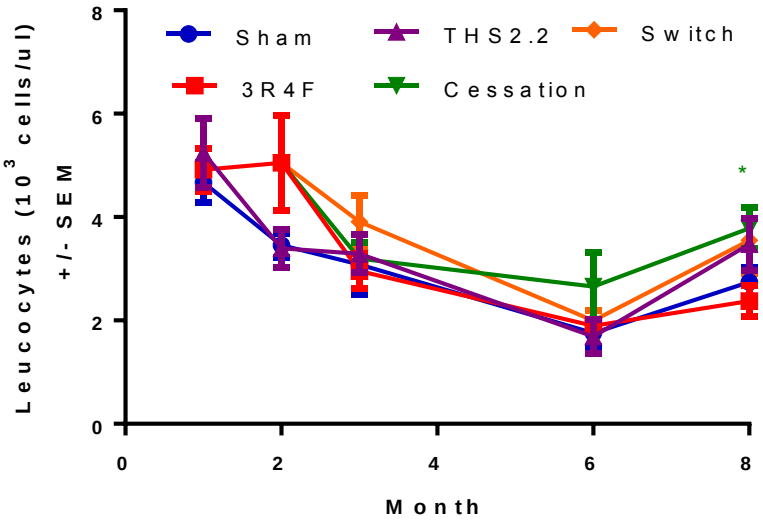


Figure 21. Total number of white blood cells

Mean ± SEM, n = 9–10. *p<0.05 compared with sham-exposed animals.

Differential white blood cell counts also revealed a lack of any major smoking-related influence. Both absolute and relative counts of neutrophils, lymphocytes, and eosinophils were similar between exposure groups throughout the exposure period (Figure 22). Over time, we observed a trend of increasing platelet count and decreasing lymphocyte count up to month 6, which may reflect an aging effect (Figure 23). Additionally, a decreased number of reticulocytes (both absolute and relative) was detected in CS-exposed animals during the first two exposure months (Figure 24), after which there were no obvious or distinguished differences between any of the groups. Based on the increased red blood cell counts as an adaptive response to the increased CO levels (Figure 20), the expectation was that CS would result in increased reticulocyte numbers. The animals were randomized during necropsy, and the handler was the same throughout the dissection months, ruling out any obvious technical problems related to the low reticulocyte numbers seen in the first 2 exposure months.

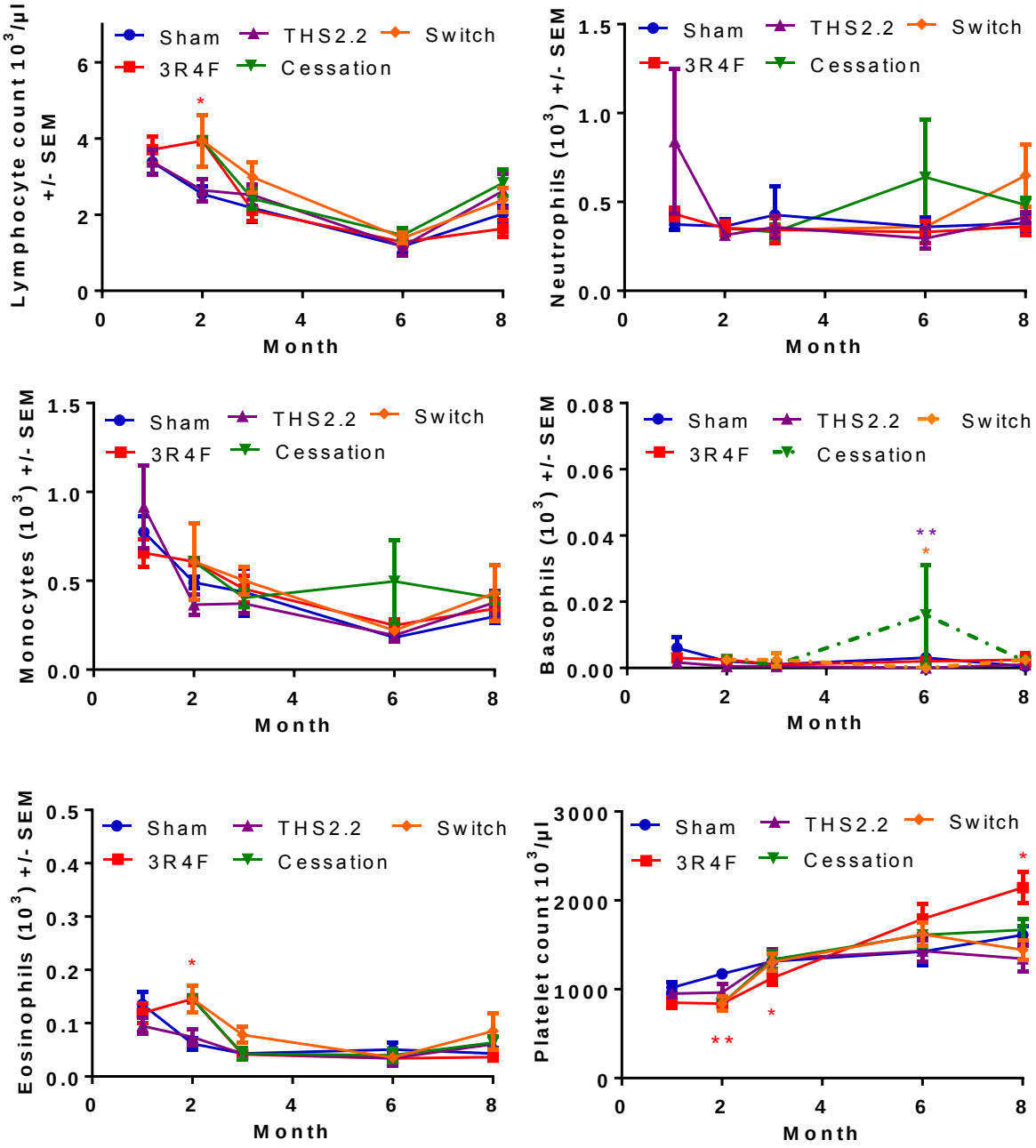


Figure 22. Differential cell counts (absolute) of white blood cells and platelets.

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

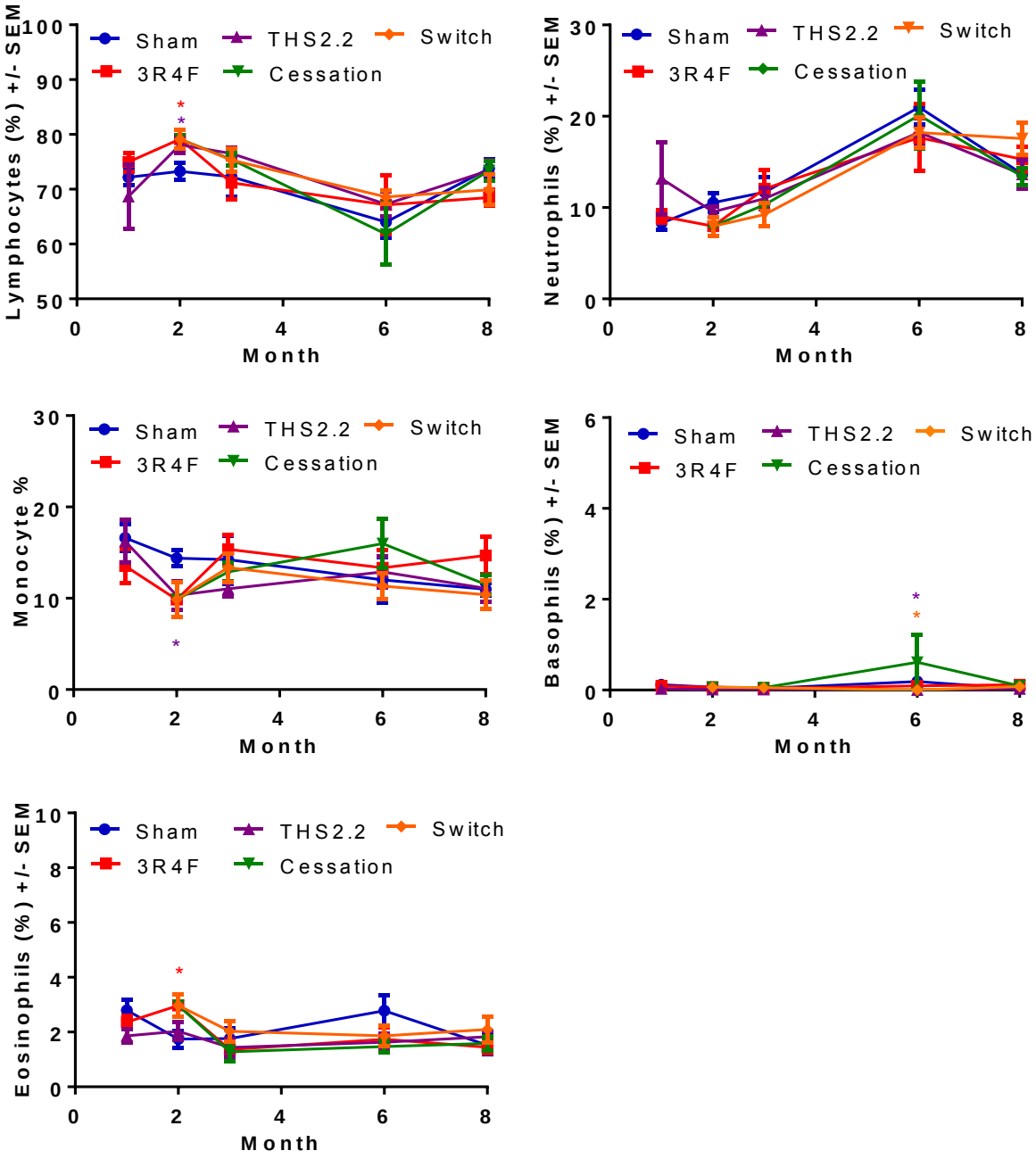


Figure 23. Differential cell counts (relative) of white blood cells.

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

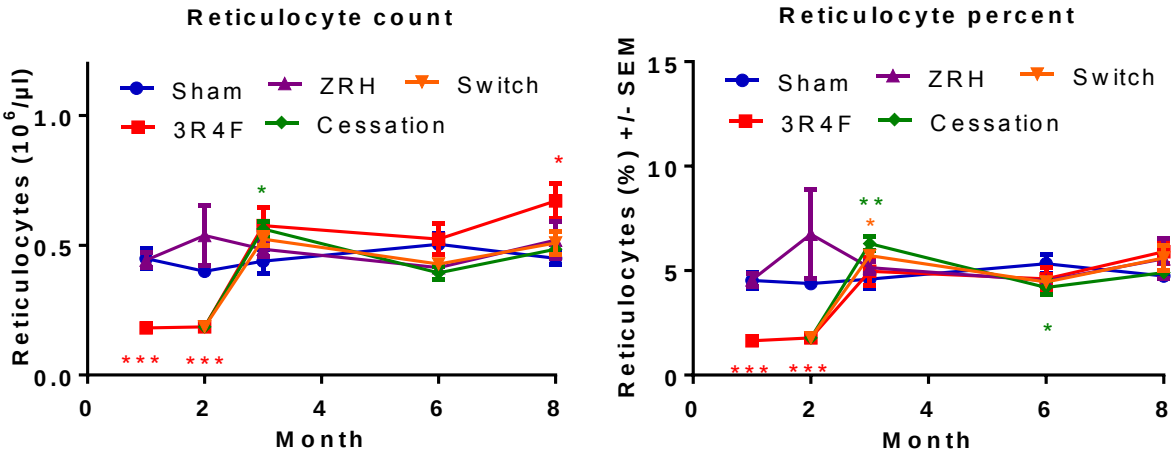


Figure 24. Reticulocyte counts (absolute and relative amounts).

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.5 Lung Inflammation

12.5.1 Absolute and relative numbers of FLCs

The degree of lung inflammation can be accurately represented by the analysis of FLCs following bronchoalveolar lavage. The quantity and composition of FLCs was analysed using flow cytometric separation of cell populations based on morphology and cell-specific surface marker expression (Table 73 to Table 77). The absolute number of FLCs (total) was dramatically increased 1 month post-CS exposure (Figure 25), with continued elevated cell numbers observed throughout the study. In contrast to the CS smoke effect on FLCs, even prolonged exposure to the THS2.2 aerosol had no effect relative to sham-exposed animals.

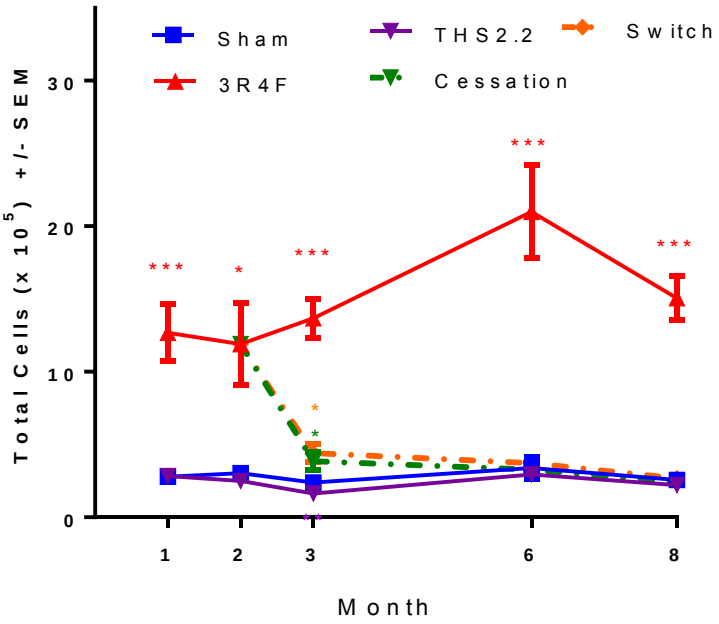


Figure 25. Total FLCs in BALF

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01,***p<0.001.

Cessation resulted in a rapid decline in total FLC counts, which approached levels of sham- or THS2.2-exposed animal levels by month 6, with a considerable reduction in numbers even after the first month post-CS exposure. The switching group showed a very similar response to the cessation group, also approaching sham FLC counts after 1 month. By month 6, both the cessation and switching groups reached the level of the sham- and THS2.2-exposed animals (p>0.05), but the trends indicate that this might already have happened earlier on.

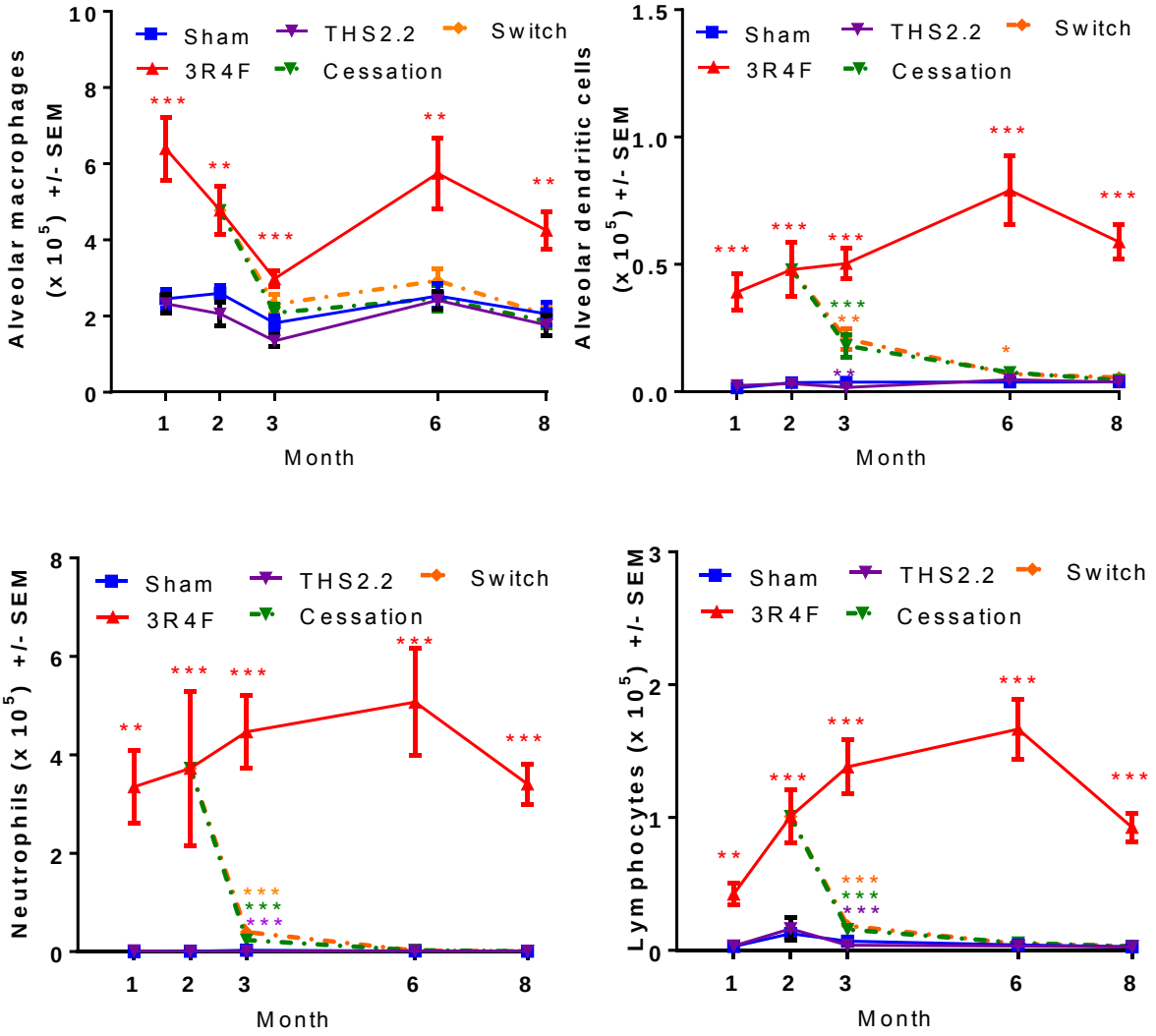


Figure 26. BALF FLC composition: absolute counts of principal inflammatory cells

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

Evaluation of the differential cell count (Figure 26) showed that the increase in FLCs was the result of increased numbers of macrophages, neutrophils, and lymphocytes, as seen in a previous inhalation study (Phillips, 2013). The increased neutrophil and lymphocyte counts resulted in a net relative decrease in macrophages (Figure 27), while the absolute cell counts showed that both cessation and switching resulted in an equally rapid return to cell count numbers in sham- or THS2.2-exposed groups by month 3. The relative cell counts (Figure 27) showed that the composition of cells still differed from that of sham-exposed animals in month 3, despite the overall decrease

in absolute cell number. This might be related to the fact that distinct cell types survive longer than others. However, by month 7, the relative cell counts were indistinguishable between the sham, THS2.2, cessation, and switching groups. Many cells (~35%) were not easily categorized as neutrophils, lymphocytes, or macrophages, particularly in the 3R4F group, so that the 'total cell' number did not sum to 100%. All cell types responded rapidly to either cessation or switching, with neutrophil levels rapidly decreasing to sham levels at month 3, and macrophages and lymphocytes reaching sham levels at month 5.

Dendritic cells were quantified based on their positivity for the surface markers CD11c and MHCII. The putative dendritic cell population was small relative to the total number of BALF cells, but the large influx of cells following CS exposure resulted in a large net increase in dendritic cell numbers in the 3R4F group in particular. Following cessation or switching, the absolute numbers decreased dramatically (by month 3), as observed for the differential white blood cell counts (Figure 27). The relative levels, however, showed that the dendritic cells persisted as a component of FLCs for up to 4 months post-cessation or switching.

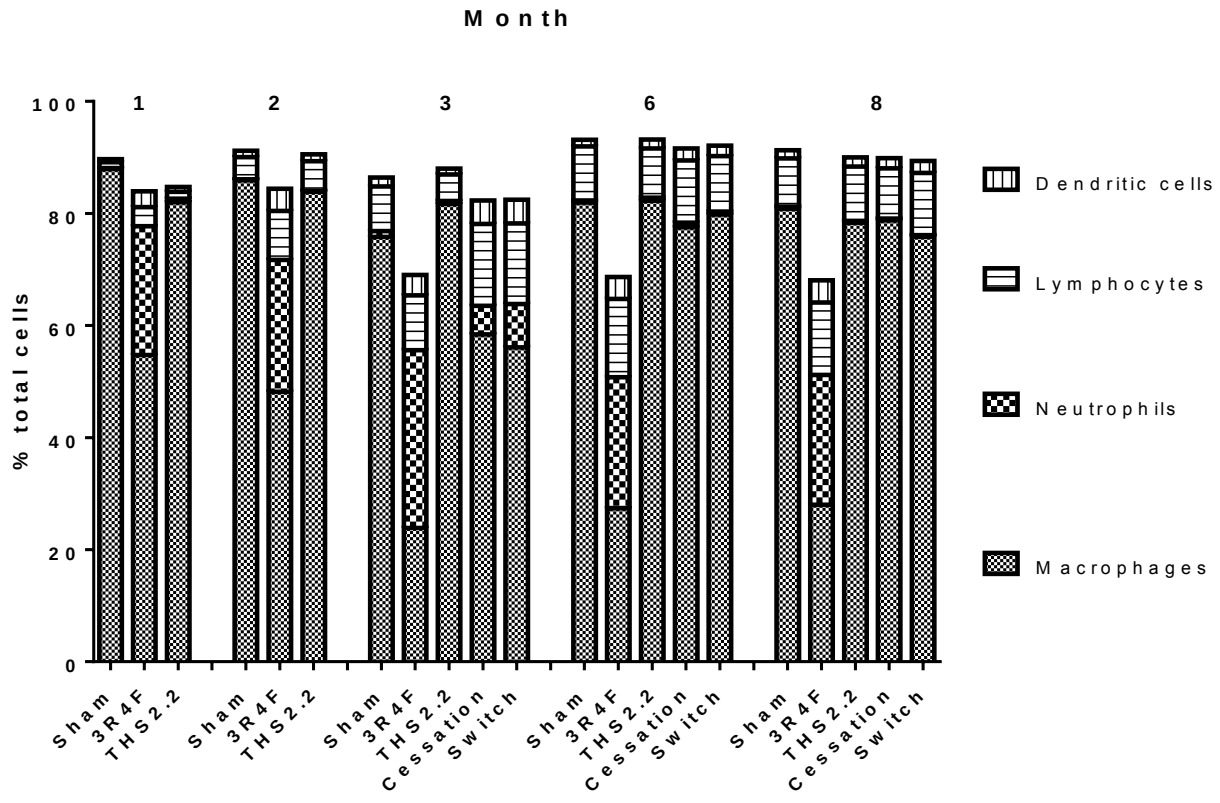


Figure 27. BALF FLC composition: relative levels of inflammatory cells

Mean (no error), n = 9–10.

12.5.2 Differentiation of lymphocytes

Following THS2.2- or sham-exposure, very few lymphocytes were present in the BALF. The elevated lymphocyte numbers following CS exposure were represented by an increased absolute number of $CD4^+$, $CD8^+$T-lymphocytes and B-cells (Figure 28, Table 78 to Table 84). At most time points, each of the lymphocytic subsets was increased relative to sham following CS exposure, although relative levels of $CD4^+$ lymphocytes were the most prominent, and found to increase over time. Switching and cessation resulted in a decreased relative level of $CD4^+$ and $CD8^+$ lymphocytes, both returning to sham levels by month 8. Interestingly, the relative B cell level increased following 1 month of either switching or cessation; this trend was also observed in the slow return to sham levels seen for absolute numbers of B cells (Figure 28).

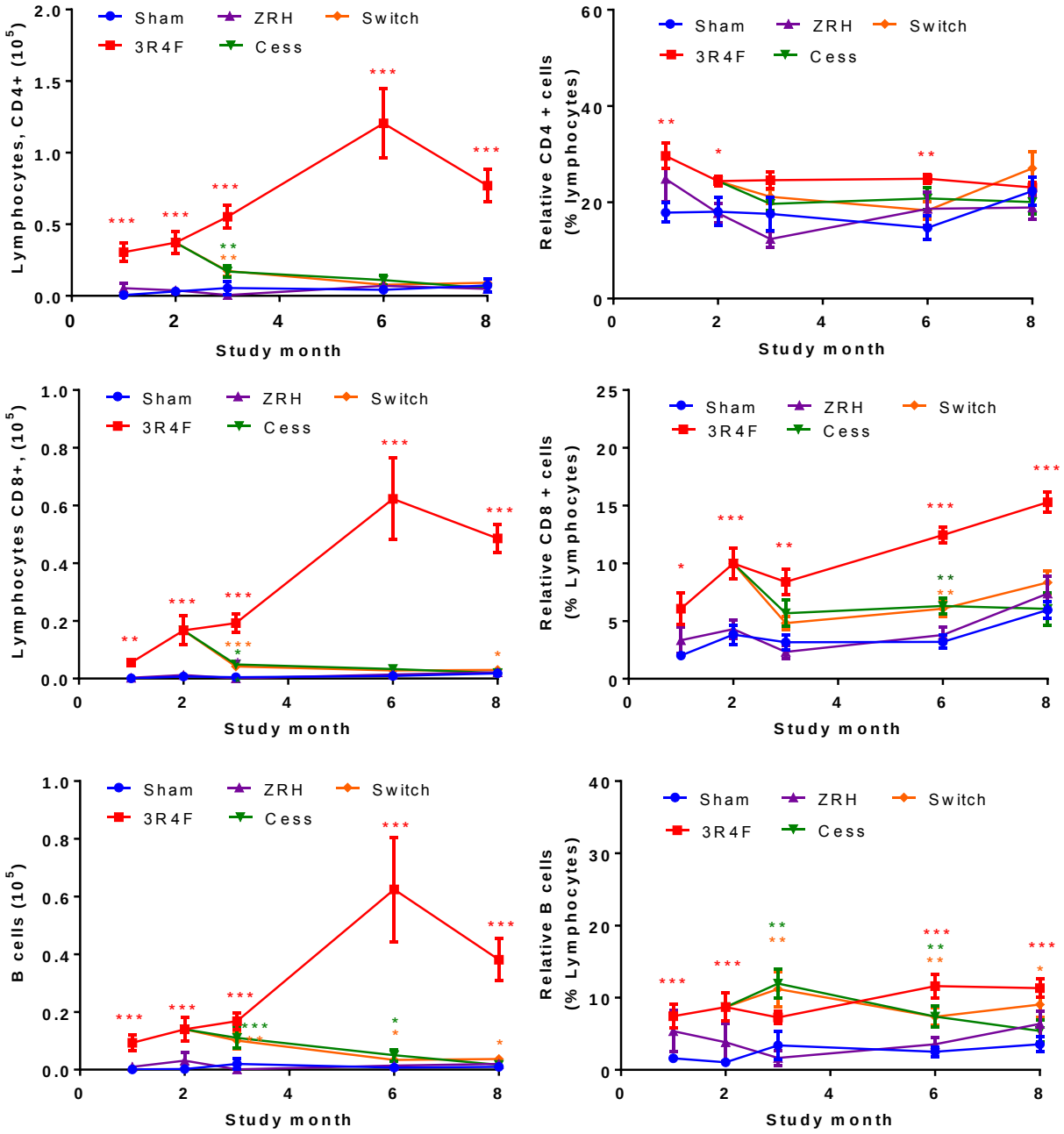


Figure 28. Lymphocyte differentiation

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.5.3 Macrophage activity

Flow cytometric analysis of surface activation markers on isolated macrophages showed a trend following CS exposure similar to that seen in previous studies (COPD II) (Phillips, 2013). Following just 1 month of exposure to CS, increased expression of CD54, CD86, and CD11b (based on median fluorescent intensity shifts) was observed (Figure 29, Table 85 to Table 87). Cessation and switching resulted in a recovery to sham-exposed group levels by month 6. In contrast, CD86 showed a continued increase for the first month following switching or cessation, before recovering to sham levels, also at month 6. The absolute numbers of alveolar macrophages decreased substantially in the first month following switching or cessation (Figure 26), combined with the results of the macrophage activation markers is suggestive that the CD86 positive cells are more likely to persist following smoking cessation or switching. However, by month 6 the expression of all markers was close to the sham-exposed levels.

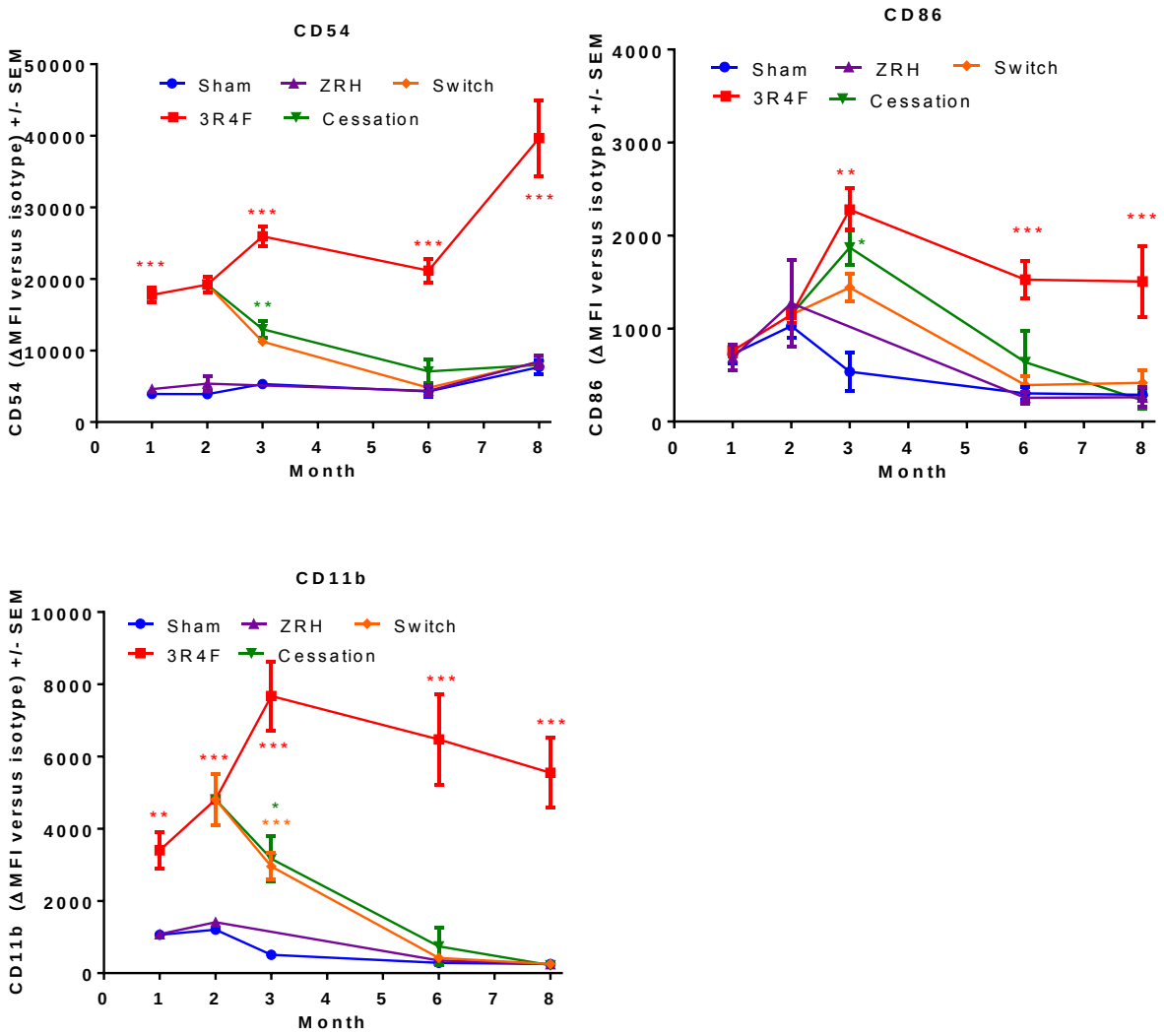


Figure 29. Markers of macrophage activation

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.5.4 Gelatinolytic activity in BALF supernatant

Exposure of animals to CS resulted in an overall persistent increase in gelatinolytic activity (Figure 30, Table 88) relative to sham or THS2.2-exposed animals. The gelatinolytic activity reached a plateau by month 3, and remained stable at just over 2 mU/ml throughout the remainder of the study. Cessation and switching resulted in the return of both groups to near sham and THS2.2 levels after just 1 month post-3R4F exposure.

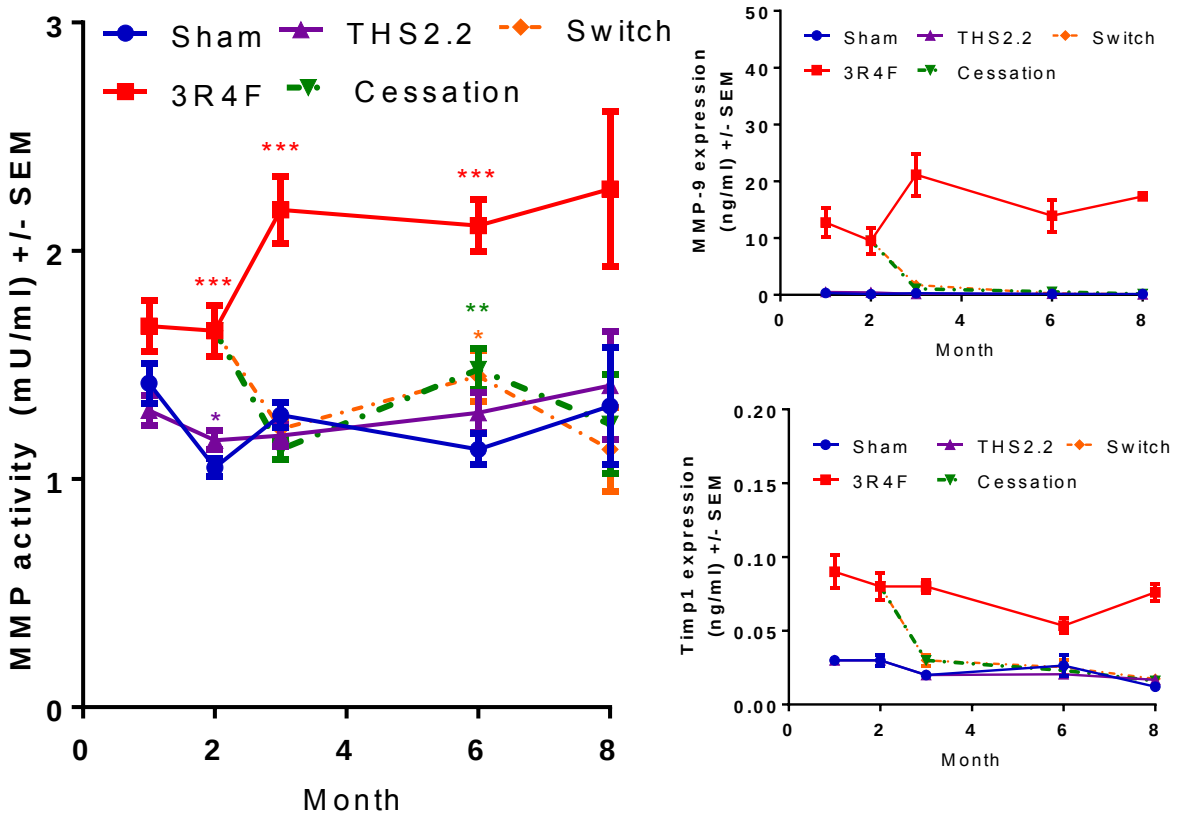


Figure 30. MMP-9 and Timp-1 protein levels and gelatinolytic activity in lavage fluid.

A) Gelatinolytic activity in the BALF fluid of mice in each exposure group. Levels of MMP-9 protein (B) and its inhibitor Timp-1 (C) in the BALF fluid of mice in each exposure group. Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

Analysis of proteolytic activity produced results that mirrored the protein expression levels of key proteolytic enzymes from corresponding BALF samples (compare with

Figure 30, right hand panel, see also Figure 31). This was highlighted by the up-regulation of the proteolytic enzyme, matrix metalloproteinase-9 (MMP-9) (Table 89), a key enzyme associated with tissue destruction during

emphysema (Elkington et al., 2006; Overbeek et al, 2013). The protein levels of tissue inhibitor of metalloproteinases-1 (Timp-1) showed a similar pattern to that of MMP-9 (Table 90). Exposure to CS resulted in increased Timp-1 levels in the BALF compared with sham or THS2.2 exposure. Following cessation or switching, Timp-1 levels in the BALF returned to similar levels as seen in sham or switching groups by month 5 (3 months post-exposure).

12.5.5 Inflammatory mediators in BALF supernatant

Samples of cell-free BALF were analysed for the protein levels of 63 analytes. While many of the analytes were below the limits of quantification of the assay, the results for the 51 detectable analytes are depicted in Figure 31 in the form of a heat map. The most common trend was the rapid increase in expression of the majority of analytes following 1 month of CS exposure to levels that generally persisted until 8 months. The overall expression of the analytes from THS2.2-exposed animals was very similar to those from the sham group. Both switching and cessation resulted in the return of the protein levels towards sham/THS2.2-exposed group levels, reaching sham levels in most but not all cases. The analytes which had exposure-related changes were mainly associated with inflammation, including many proteins observed previously in response to CS, such as MCP-1, -3, and -5, interleukins, as well as several tissue remodelling enzymes and growth factors (VEGF, MMP-9 and Timp-1). This is therefore well aligned with the observed influx of inflammatory cells also detected in the BALF (Figure 25). Metabolic hormones including insulin and leptin were unaffected by any of the exposure regimes. Overall, these results are similar to those observed in previous studies with similar study design (Phillips, 2013).

12.5.6 Inflammatory mediators in serum

Serum samples were analysed for the protein levels of 63 analytes (the same as described in section 12.5.5). As seen in Figure 32, the effect of CS on the serum inflammatory mediator and analyte profile was considerably less profound as compared to the effect on the BALF (Figure 31), and with little overlap in protein expression profile. Many of the analytes were below the detection limits, and most others showed no significant and consistent treatment effects relative to sham animals.

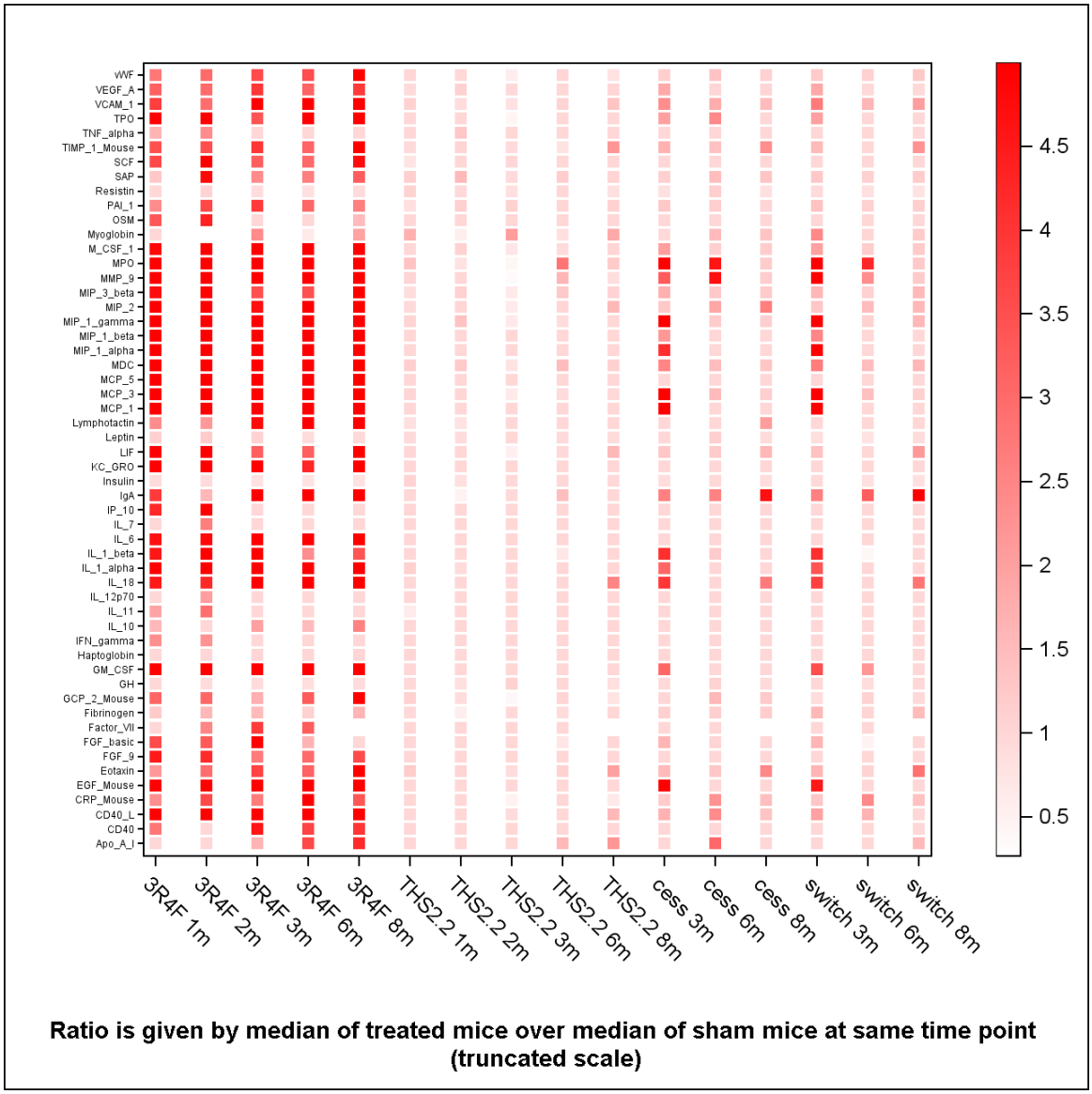


Figure 31. RodentMAP (Myriad/Rules Based Medicine) analyte analysis from cell-free BALF fluid.

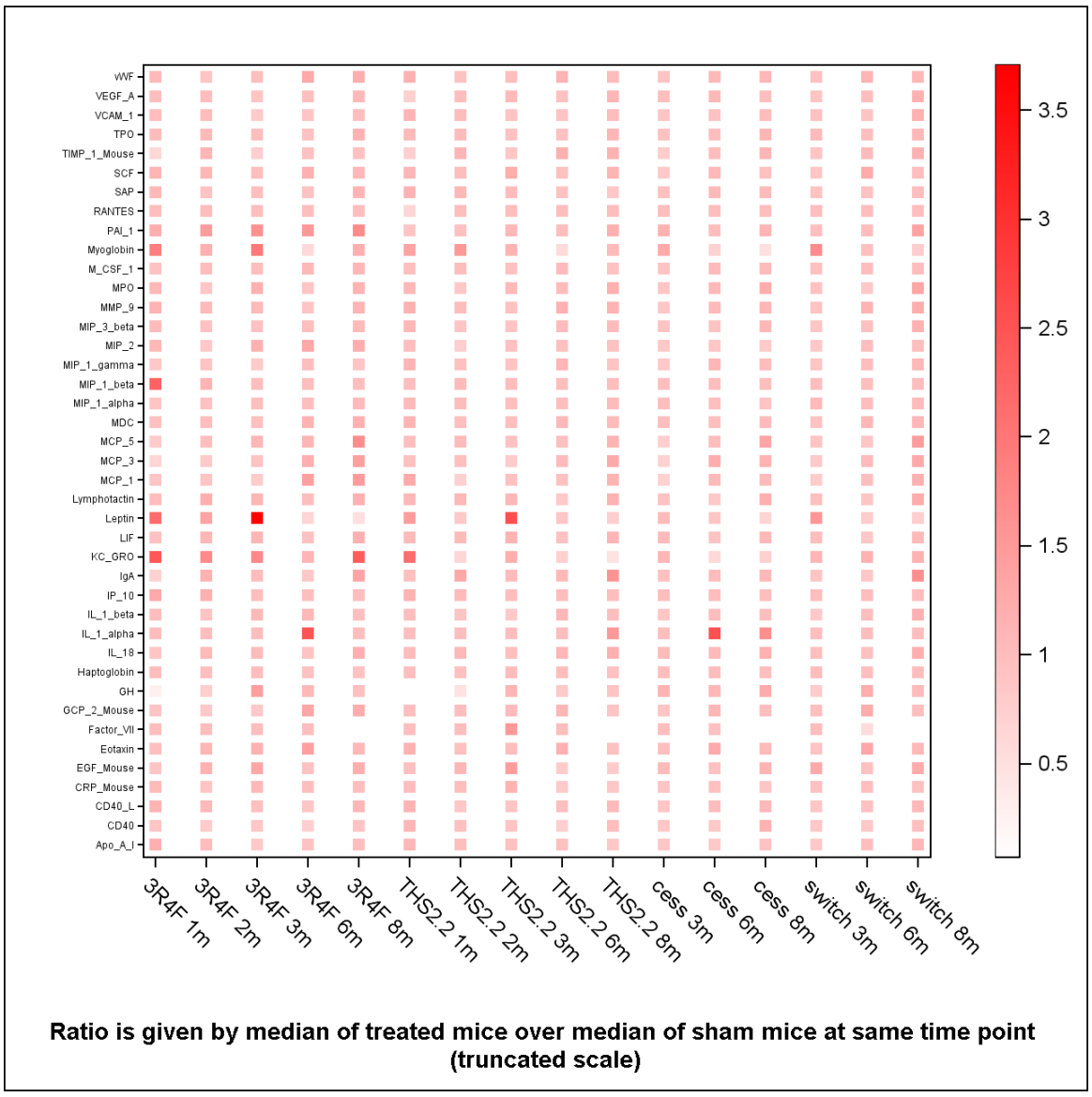


Figure 32. RodentMAP (Myriad/Rules Based Medicine) analyte analysis from serum.

12.5.7 Oxysterols in plasma

Plasma was collected and sent to delegated phase 3 for analysis of oxysterol levels. These results have been reported separately, in the document entitled 'Determination of oxysterols and cholesterol in plasma from a study investigating the effects of Zurich MRTP (DEL/C3/FR1/RDO) on the respiratory and cardiovascular systems of Apo lipoprotein E-deficient mice using LC-HR-MS and LC-CAD' (Report part 6).

A total of 10 oxysterols were evaluated, and normalized to total cholesterol levels. Of these, three oxysterol species were shown to differ in the 3R4F exposure group relative to the sham group (Figure 33):

i) 4β-hydroxycholesterol
   - Lower in 3R4F-exposed group relative to sham.
   - Switching, cessation, and THS2.2 exposure showed levels similar to sham.

ii) 7β-hydroxycholesterol
   - Lower in 3R4F-exposed group relative to sham.
   - Switching and THS2.2 groups trending lower than sham, but not significantly.

iii) 25-hydroxycholesterol:
   - Higher in 3R4F-exposed group relative to sham.
   - Switching, cessation, and THS2.2 exposure showed levels similar to sham.

Also evaluated were 22-, 24- and 27-hydroxycholesterol, 5α6α-, 5β6β-, 7α-hydroxycholesterol, and 7-ketocholesterol, none of which showed significant differences ($p>0.05$) between 3R4F-exposed and sham-exposed animals. Previous studies evaluating oxidative stress in human smokers (Mol et al, 1997; Seet et al, 2011) showed a general increase in total cholesterol oxidation products in plasma from smokers, attributed to increased CS-related oxidative stress. These results did not replicate these human findings, with only a single oxysterol product examined (25-hydroxycholesterol) showed a CS-related increase. Further evaluation of the regulation of oxysterol metabolic enzymes at the transcriptomics and lipidomics level is in progress.

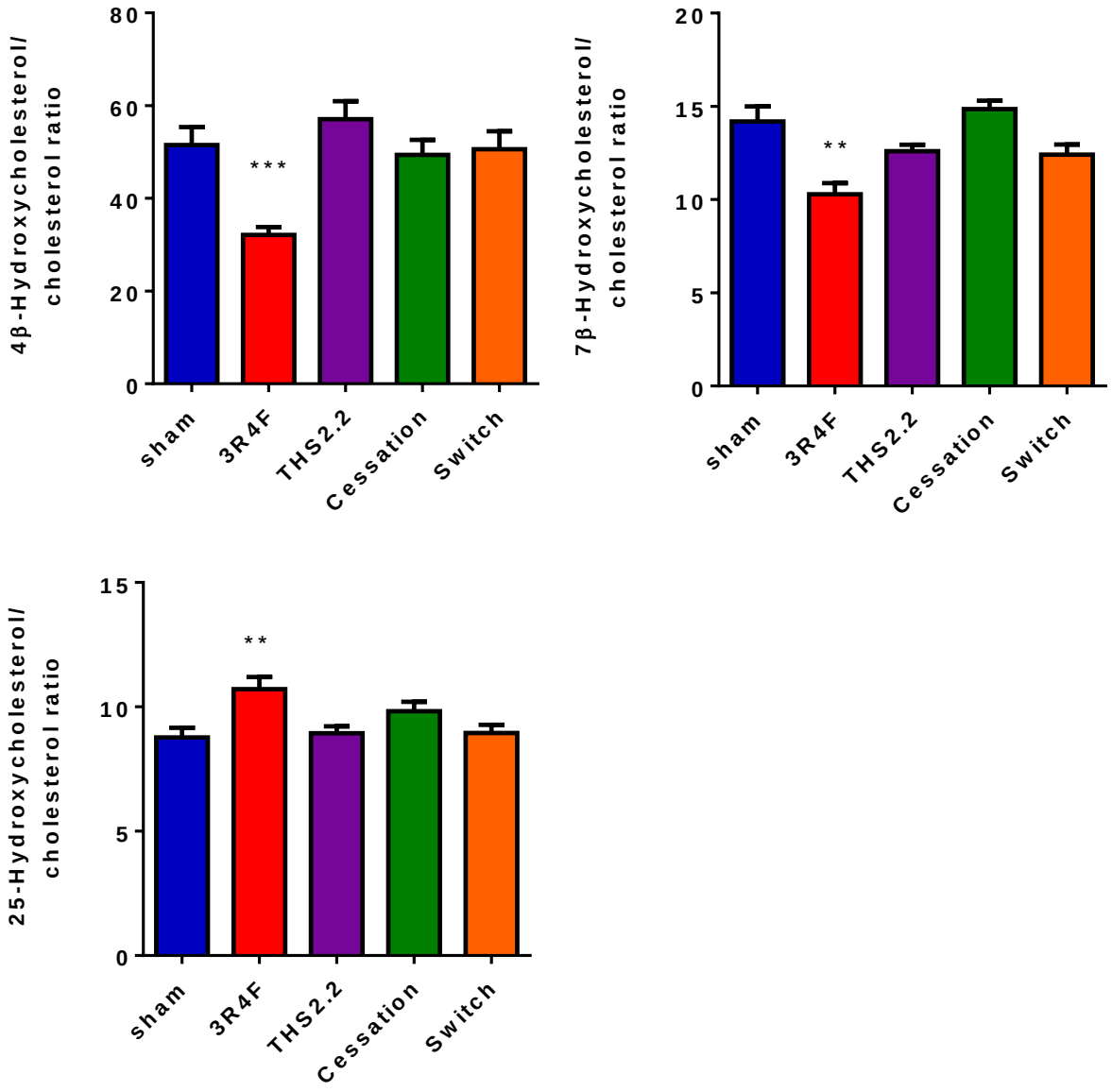


Figure 33. Oxysterol levels, month 8 post-exposure.

Mean ± SEM, n = 10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.6 Atherosclerosis and Clinical Chemistry

12.6.1 Plaque area measurements

The ApoE$^{-/-}$ transgenic mouse model is commonly used for studies of atherosclerosis (Chan et al, 2012; Lietz et al, 2013). Image analysis measurements of the two-dimensional (2D) plaque area revealed an expected and progressive increase in plaque burden in all groups over the 8-month experimental time frame. From month 2 onwards, a significantly increased aortic plaque area was observed in animals exposed to CS compared with either sham or THS2.2-exposed animals (Figure 34,Figure 35). Exposure to THS2.2 aerosols resulted in similar plaque area measurements compared with sham animals (p>0.05 across all time points). Both cessation and switching groups showed relatively similar plaque area measurements to each other, which were between sham and 3R4F levels and were significantly different from the 3R4F exposure group at the month 3 and month 8 time points.

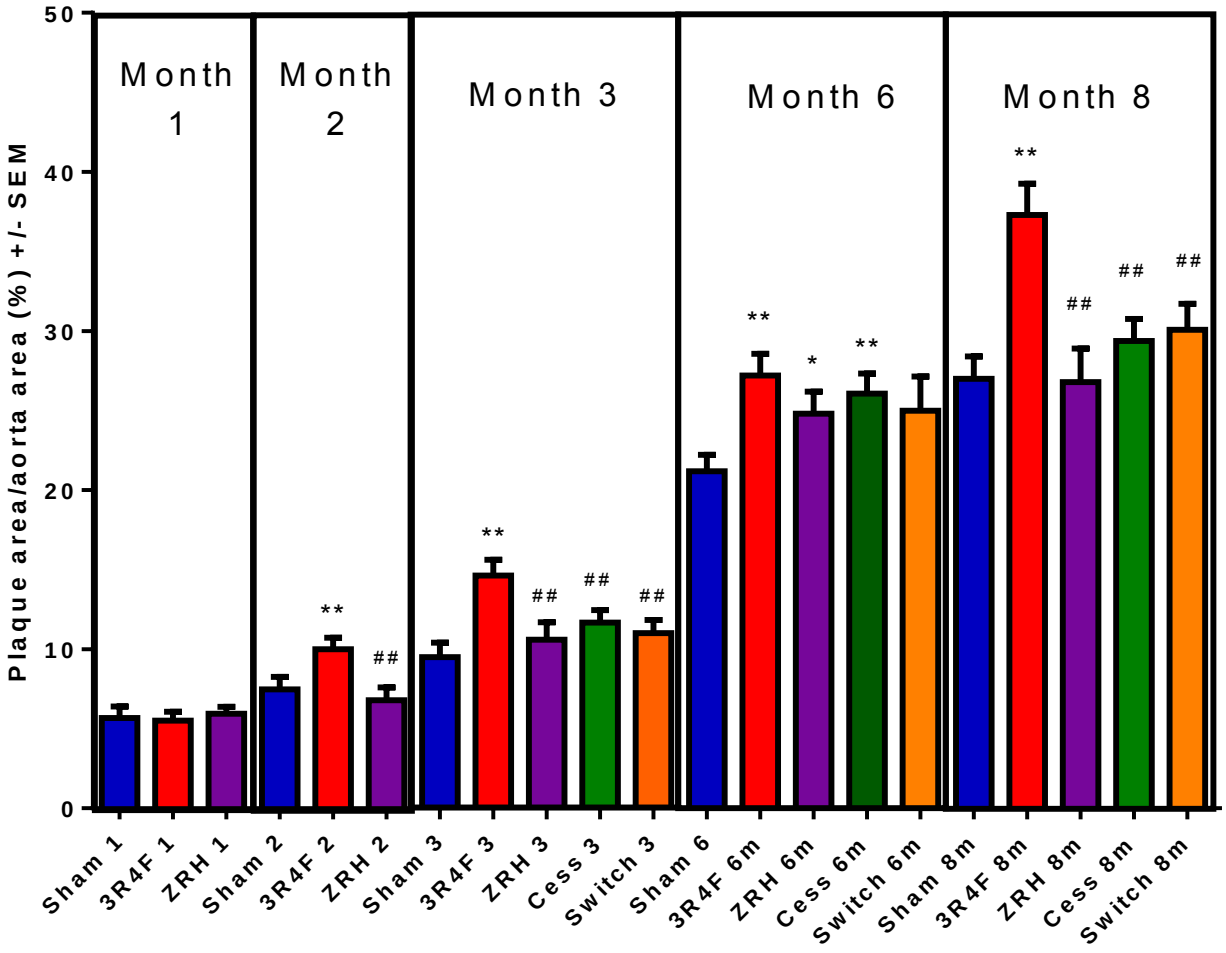


Figure 34. Aortic arch plaque area measurements

Mean ± SEM. Statistics describe comparison with sham-exposed animals *p<0.05, **p<0.01, ***p<0.001, and with 3R4F-exposed animals $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001.

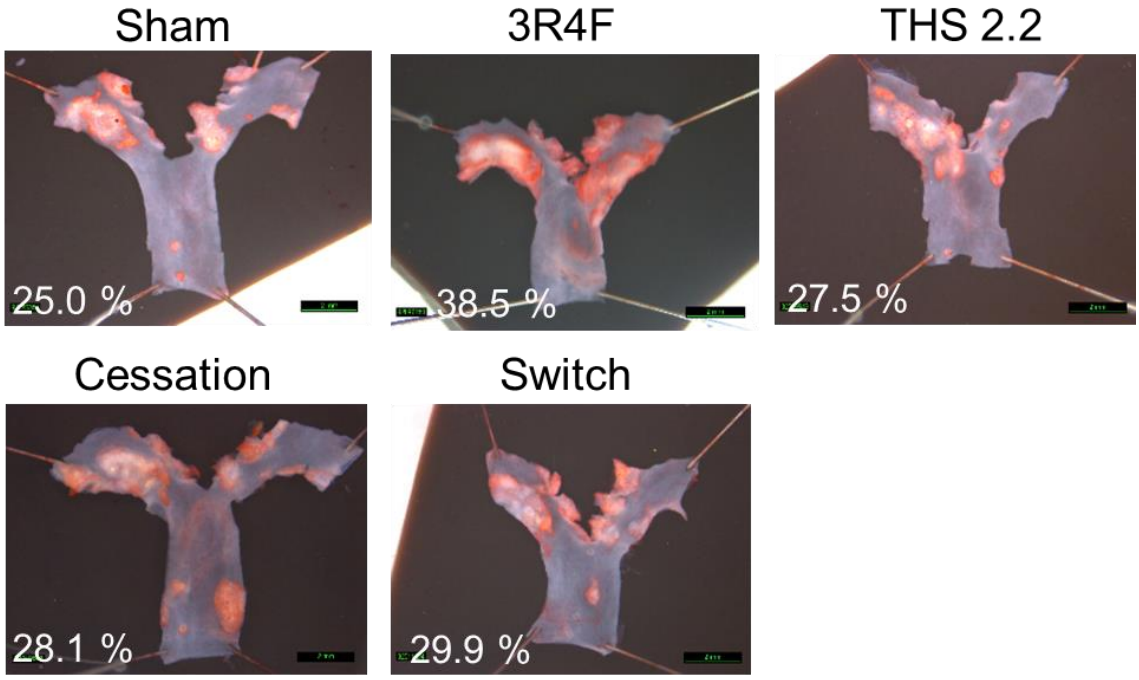


Figure 35. Plaque area measurements in aortic arches.

Representative images are shown from each of the exposure groups. The plaque area (percent, relative to total aortic arch area evaluated) is described for each image.

12.6.2 Plaque volume by micro-CT imaging

In addition to the longitudinal 2D quantification of plaque areas (section 12.6.1), six animals per group (month 8 dissection time point) were sent for micro-CT imaging of plaque development in the aorta and major branching vessels (Figure 35, Figure 36). These results complimented the 2D quantification in that they demonstrated increased plaque volume, surface area, and occlusion in the aorta (Figure 37, A–C), and to a lesser extent the left subclavian artery (Figure 37, J–L). The aorta plaque surface area was the micro-CT parameter most resembling the manual morphometric methods shown in

Figure 34, with both results showing an overall increased plaque area (78% increase versus sham for micro-CT imaging, while manual quantification showed a 38% increase).

The use of comprehensive micro-CT imaging also enabled the evaluation of other vascular regions. However, it was noted that the plaque parameters in brachiocephalic trunk regions (Figure 37, D–F) were fairly similar across exposure groups, and very few plaques were identified in the left common carotid artery (Figure 37, G–I).

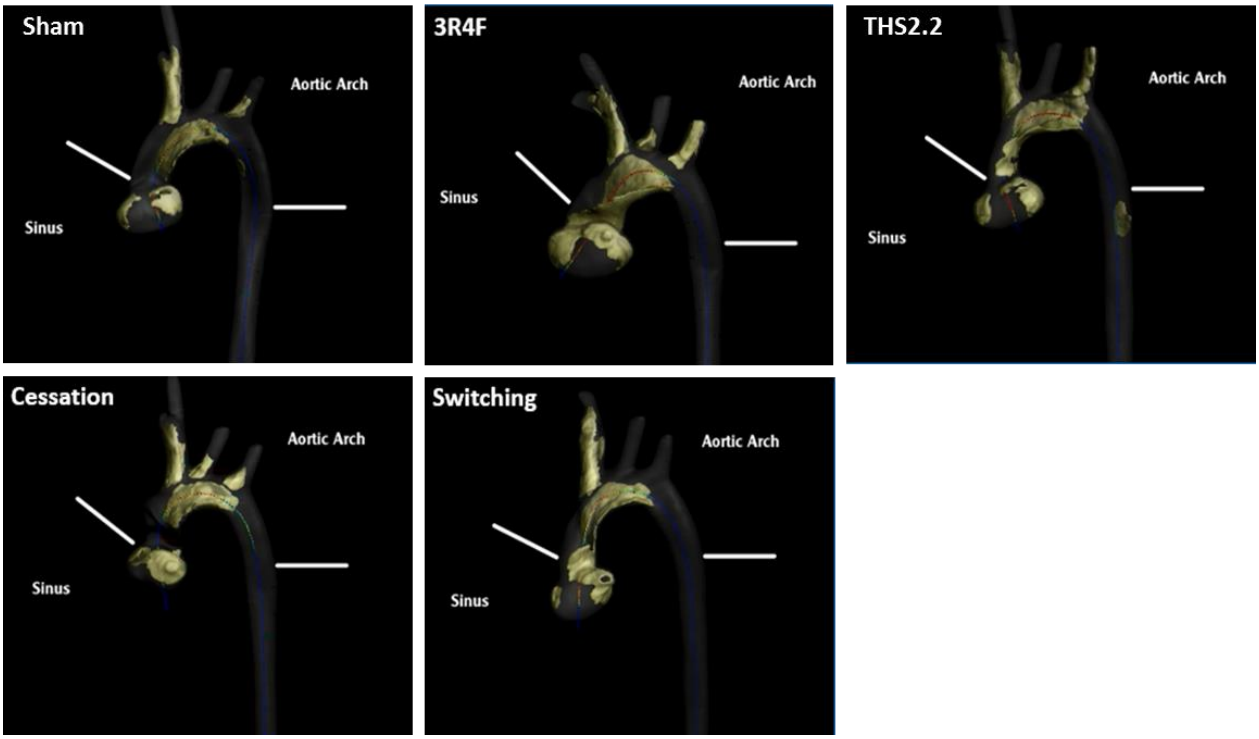


Figure 36. Micro-CT images of representative aorta samples.

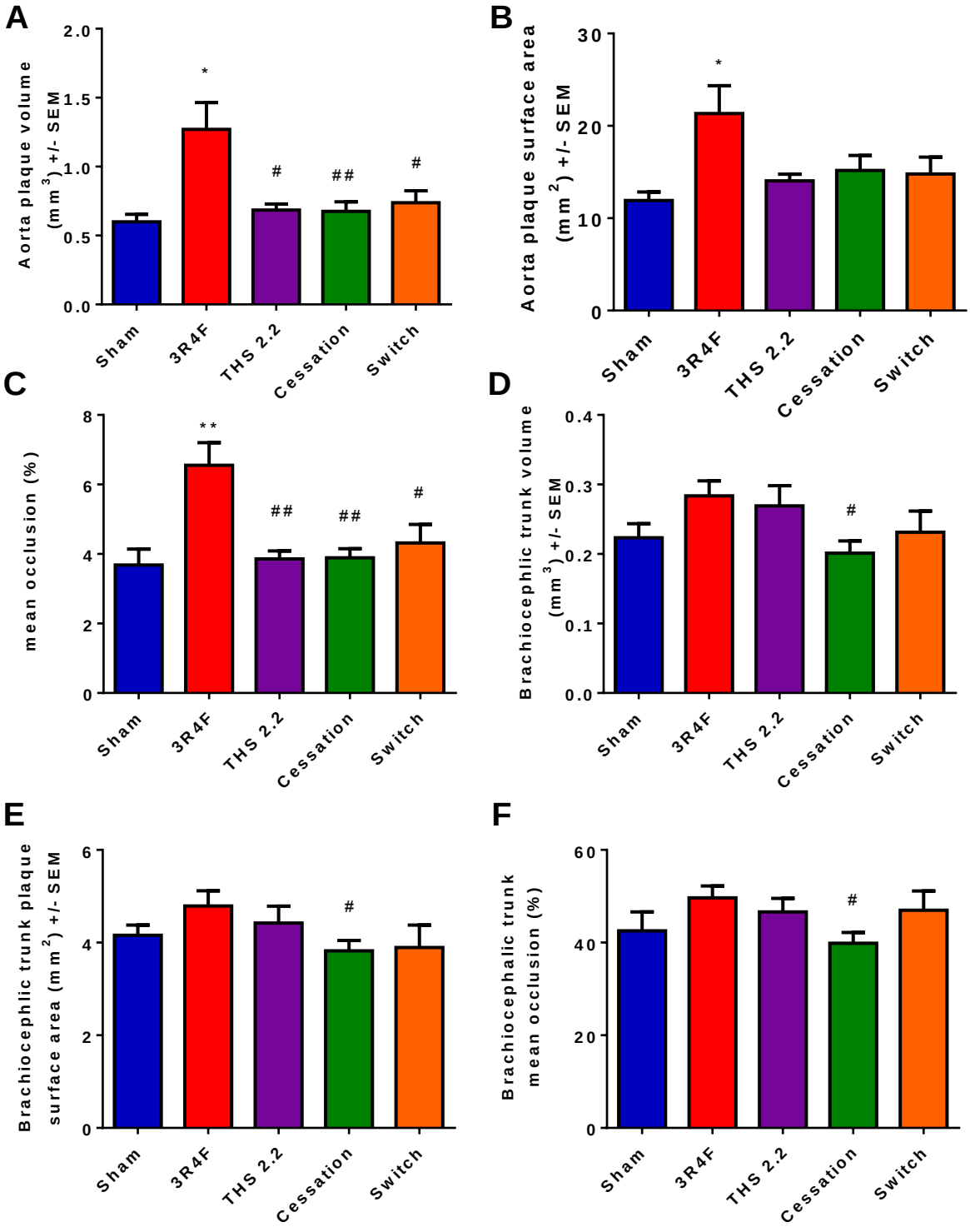


Figure continued on next page.

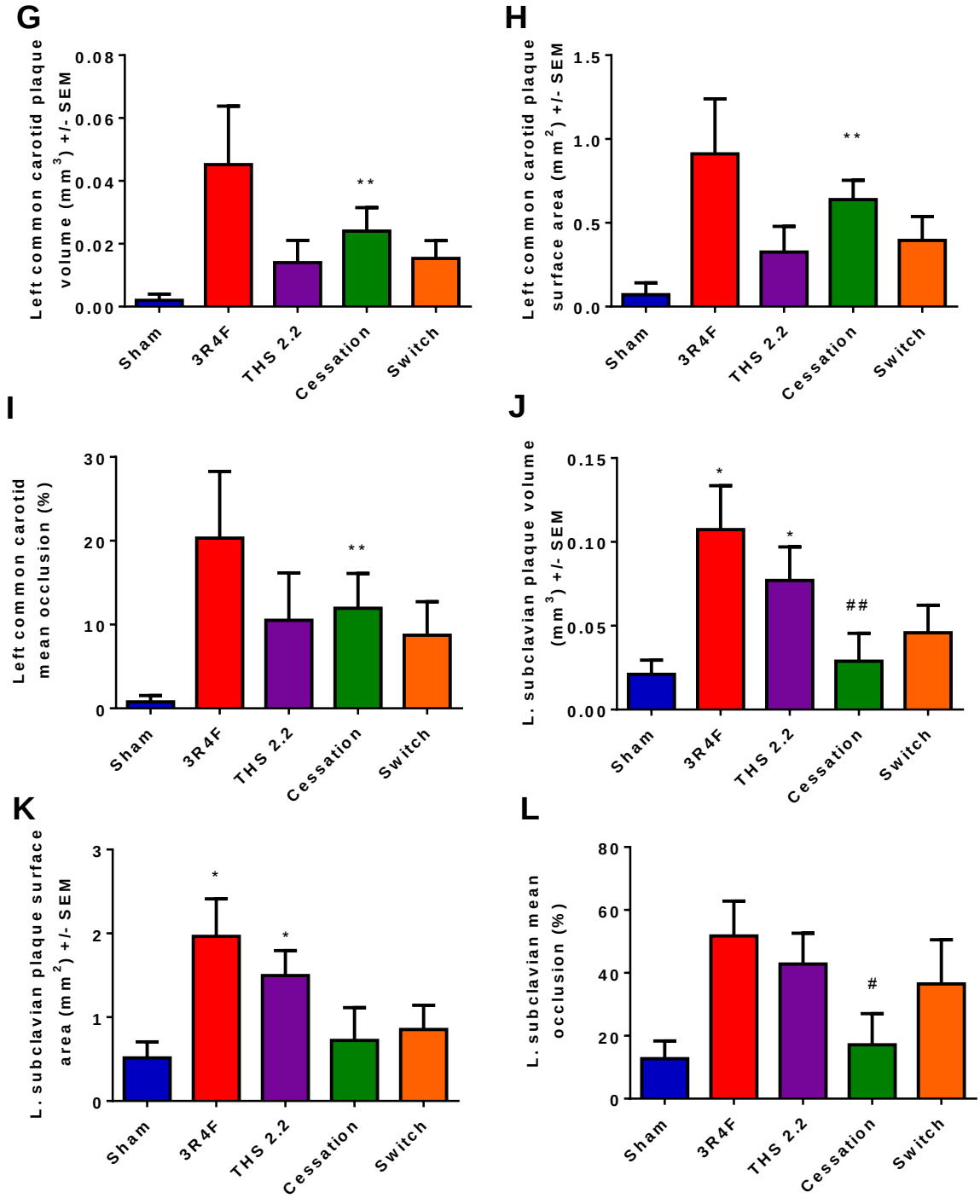


Figure 37. Quantification of aorta (and primary branching vessels) plaque burden by microCT.

Mean ± SEM, n = 6. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.6.3 Clinical chemistry

Non-fasted plasma samples were evaluated using a panel of clinical chemistry metabolic parameters, revealing an expected increase in cholesterol in the CS-exposed group (though statistically significant only up to month 3), with a decrease to sham levels after 1 month of either cessation or switching (Figure 38). Accompanying the increase in total cholesterol, a similar increase in HDL cholesterol was observed (3R4F group increased from months 1–3, though merging to the sham level by month 8). There were no obvious differences in LDL levels among any of the groups at any dissection period. Plasma glucose levels showed a general down trend, likely attributed to an aging effect, because it was observed in all groups including sham-exposed animals (Figure 38). Lower plasma glucose concentrations were also observed in the 3R4F-exposed animals across all time points. Notably, the 3R4F group animals also demonstrated a reduced body weight at all time points, which may have potentially affected these metabolic parameters. Cessation and switching resulted in the return of glucose concentrations to sham levels within 1 month, which again recapitulated the body weight gain observed in the animals. While triglycerides were higher in CS-exposed animals during the first 3 months of the study ($p<0.05$), by months 6 and 8 there were no differences among any of the groups.

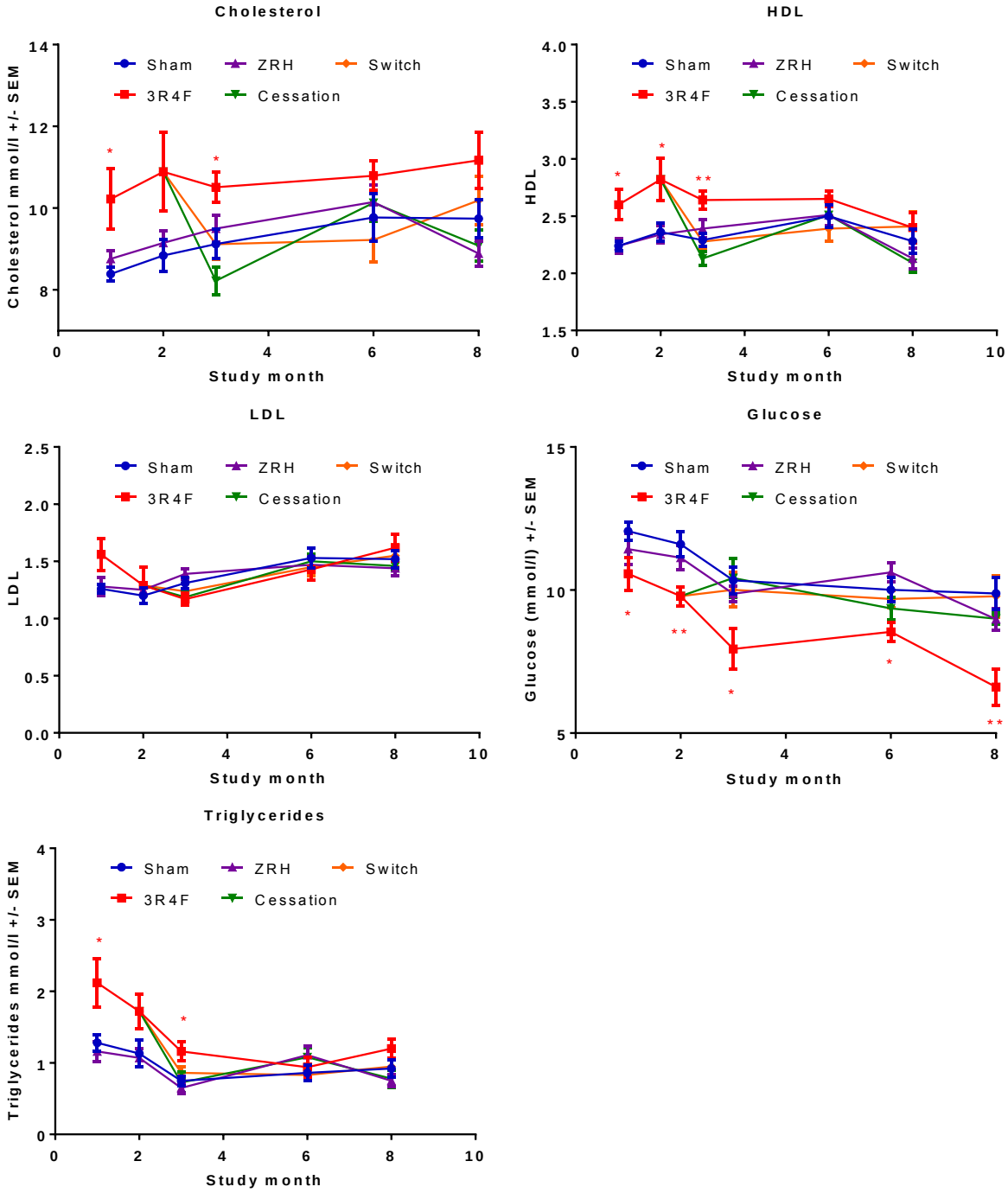


Figure 38. Clinical chemistry parameters

Mean ± SEM, n = 8–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

As the analysis of cholesterol using the clinical anlayzer does not provide information of the VLDL fraction, additional samples (plasma) were sent for analysis using ultracentrifugation – vertical auto profile (VAP) testing (Atherotech, USA, Table 99 to Table 110). In support of the clinical chemistry results, VAP analysis showed an increased plasma cholesterol in the 3R4F-exposed animals relative to the other groups between months 1 and 6 ($p<0.05$ in months 1, 2, and 6). However, by month 8 the total cholesterol was similar between all groups (Figure 39). This was shown to be mainly attributable to the non-HDL sub-fractions (Figure 39).

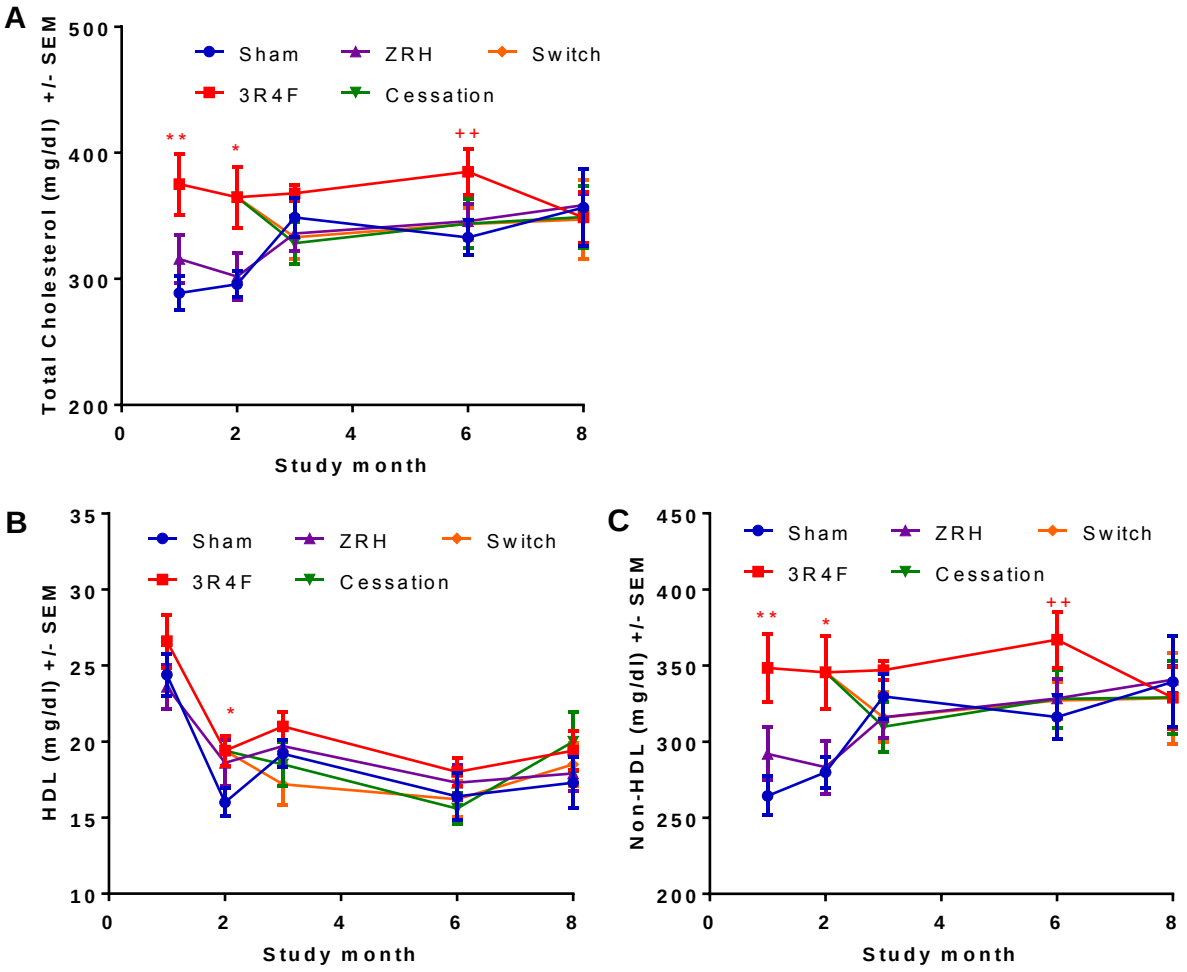


Figure 39. Vertical auto profiling; total cholesterol and HDL/non-HDL fractions

Mean ± SEM, n = 9-10. *P*-values obtained by t-test accounting for variance heterogeneity or by Wilcoxon exact MC. ***/+++$p < 0.001$; **/++$p < 0.01$; */+$p < 0.05$.

Further analysis of the lipoprotein sub-fractions has shown elevated levels of IDL and LDL in the CS-exposed group ($p<0.05$ in month 1 and 6) (Figure 41). As expected and based on previous studies (Lietz et al., 2013), increased plasma levels of VLDL and VLDL3 were observed (Figure 41).

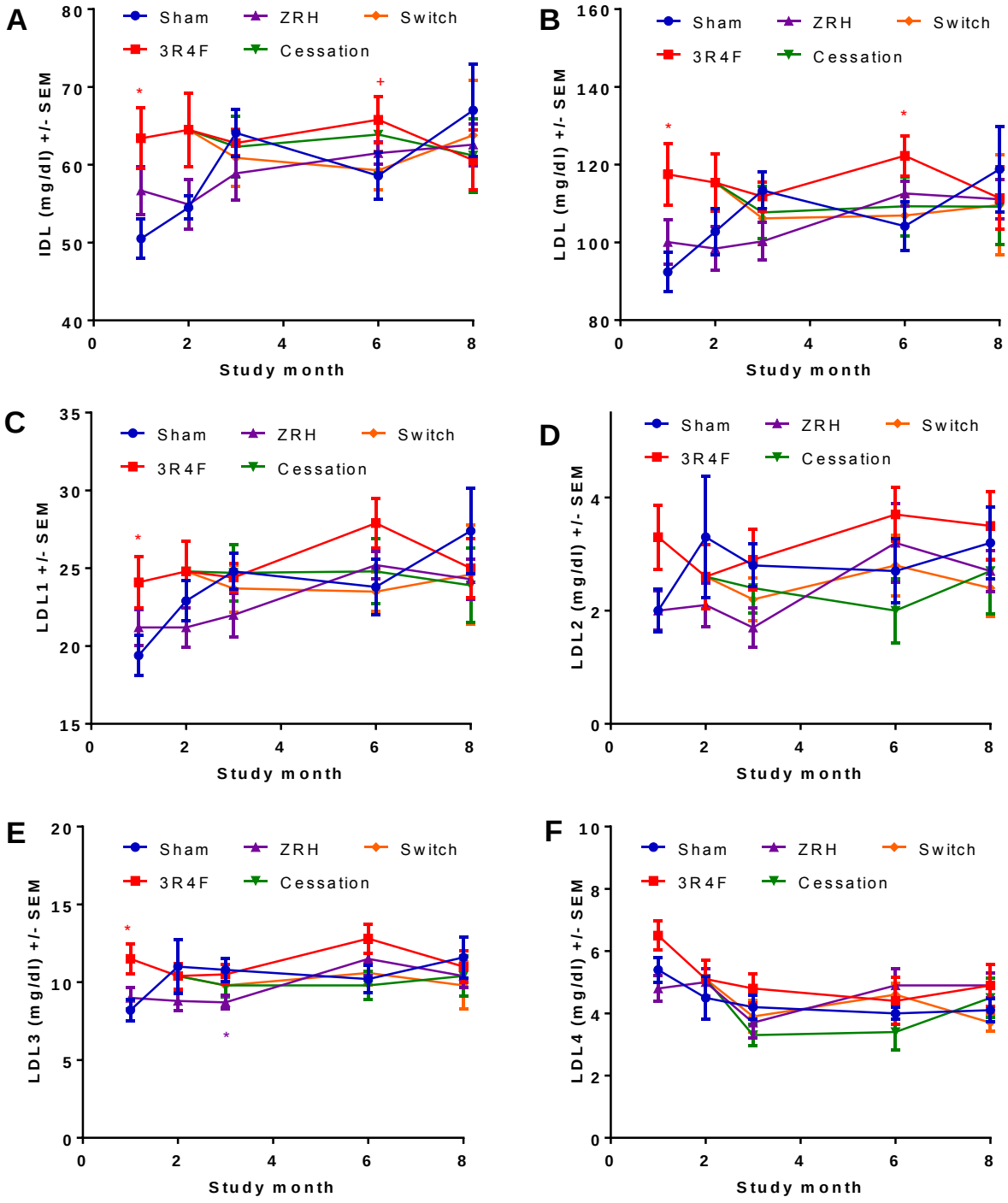


Figure 40. Vertical auto profiling; IDL and LDL cholesterol sub-fractions

Mean ± SEM, n = 9-10. *P*-values obtained by t-test accounting for variance heterogeneity or by Wilcoxon exact MC. ***/+++$p < 0.001$; **/++$p < 0.01$; */+$p < 0.05$.

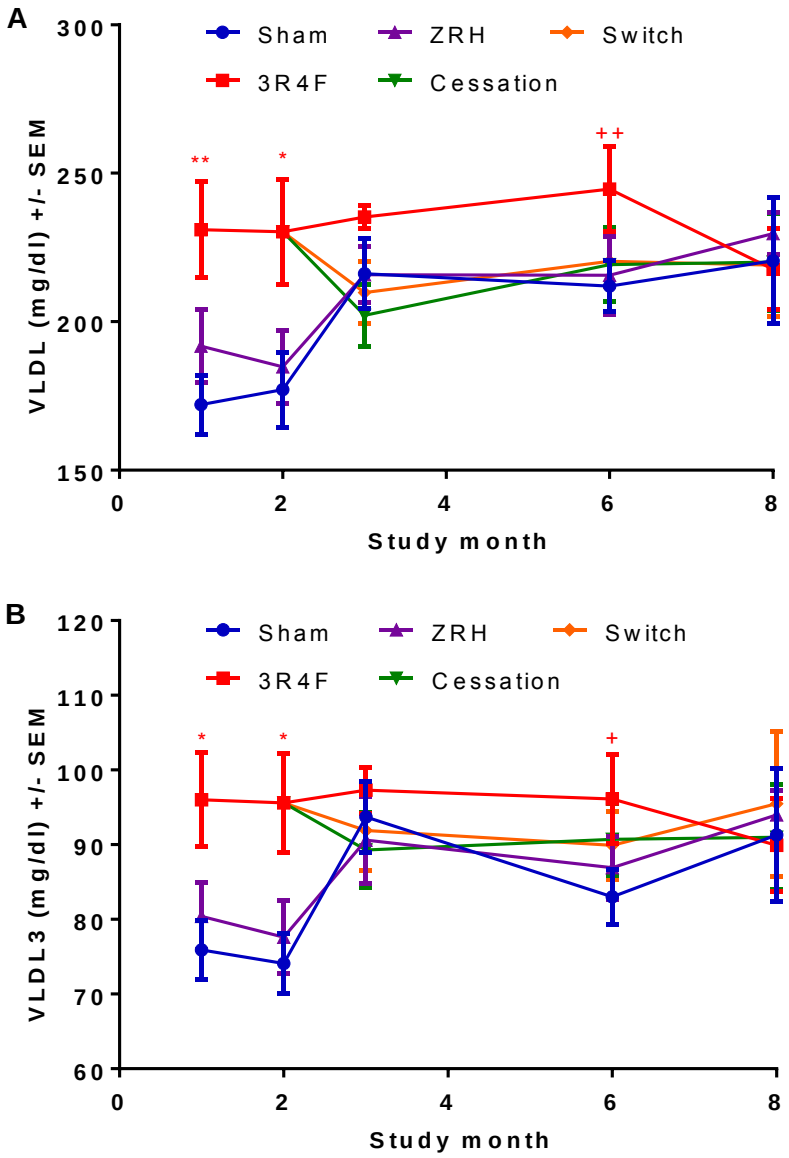


Figure 41. Vertical auto profiling; VLDL cholesterol sub-fractions

Mean ± SEM, n = 9-10. *P*-values obtained by t-test accounting for variance heterogeneity or by Wilcoxon exact MC. ***/+++$p < 0.001$; **/++$p < 0.01$; */+$p < 0.05$.

12.7 Histopathology and Morphometric Analysis

Tissue histopathological assessment was conducted at two separate sites. Test site 4 performed an evaluation of the nose, lung, and liver tissues. These results are summarized here (sections 12.7.2, 12.7.3, and 12.7.4), and are available in more detail in the phase report from delegated phase 4, 'Histopathological and Morphometrical Assessment of Lung, Nose, and Liver of ApoE-Deficient Mice'. The evaluation of the kidneys and pancreas was performed at the test site. The results are summarized in this report (12.7.5), and are available in more detail in the pathology report, 'In vivo evaluation of the effects of THS2.2 on the respiratory and cardiovascular systems of ApoE lipoprotein deficient mice, histopathology of the pancreas and kidneys'.

12.7.1 Lung volume measurement

At the time of necropsy, the lungs were removed and instilled with fixative (to a pressure of 15 cm water column, according to our standard fixation protocols). Within 60 min, they were immersed in fixative to determine the fixative displacement, providing a direct immersion volumetric measurement (Scherle, 1970). Using this technique, the lung volume (as a measurement of displaced fixative weight) of CS-exposed animals was found to be higher than that of sham- or THS2.2-exposed groups (Figure 42, left panel). Both cessation and switching resulted in a rapid return to a lung volume similar to that of sham-exposed animals within 1–3 months. Because CS-exposed animals tended to have a lower body weight than other groups when normalising lung volume to body weight, the group differences were exacerbated. The increased lung volume seen in the CS-exposed animals supports the lung function results, where CS exposure resulted in a leftward shift in the PVsP loop (Figure 17). Similarly, the lung volume, following fixed pressure instillation, is larger in the 3R4F group, due to the exposure-related changes to the lung compliance and elastance.

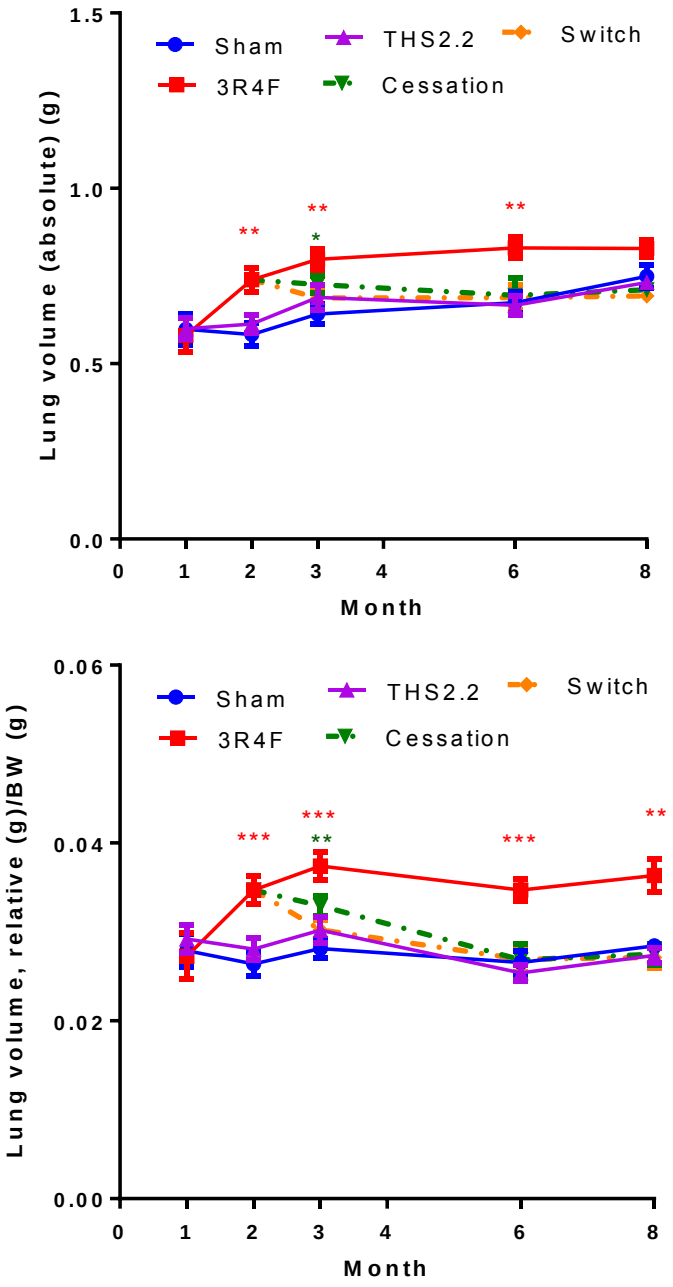


Figure 42. Lung volume measurements

Mean ± SEM, n = 9–10. Statistics describe comparison with sham-exposed animals. *p<0.05, **p<0.01, ***p<0.001.

12.7.2 Histopathology and morphometry of the lung

The overall development and grading of pulmonary emphysema was performed using standard histopathological evaluation of step serial left lung sections in combination with a morphometric analysis (mean cord length, bronchoalveolar attachments, and destructive index (DI)). This evaluation described typical CS-related effects of the lungs of exposed animals, as seen through the infiltration of inflammatory cells into the alveolar and perivascular spaces, as well as through quantitative lung morphometric measurements (Figure 43 and Figure 44) (Phase report 4, 'Histopathological and Morphological Assessment of Lung, Nose, and Liver of ApoE-Deficient Mice'). The overall lung emphysema score (Figure 43, lower right panel) showed an increase relative to that of sham-exposed animals from month 1 onwards, with a progressive increase over time. An aging effect was also observed, as shown by the sham-exposed animal score of 0.16 in month 1 and 0.88 in month 8. No significant differences in the assessment of lung tissue from THS2.2-exposed relative to sham-exposed mice were seen.

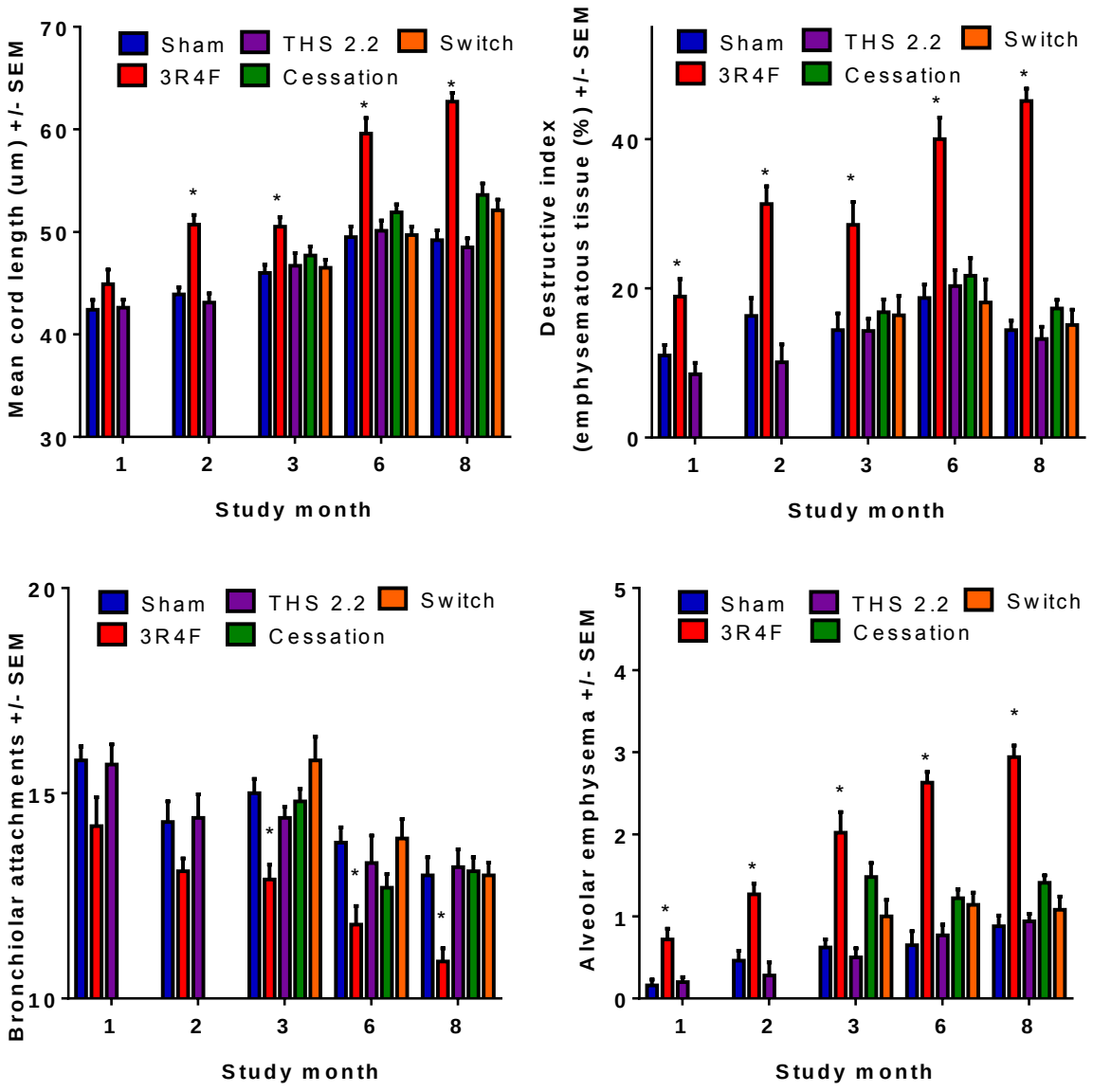


Figure 43. Morphometric assessment of the lung

Mean ± SEM, n = 10. Statistics compared with the sham-exposed group *p<0.05.

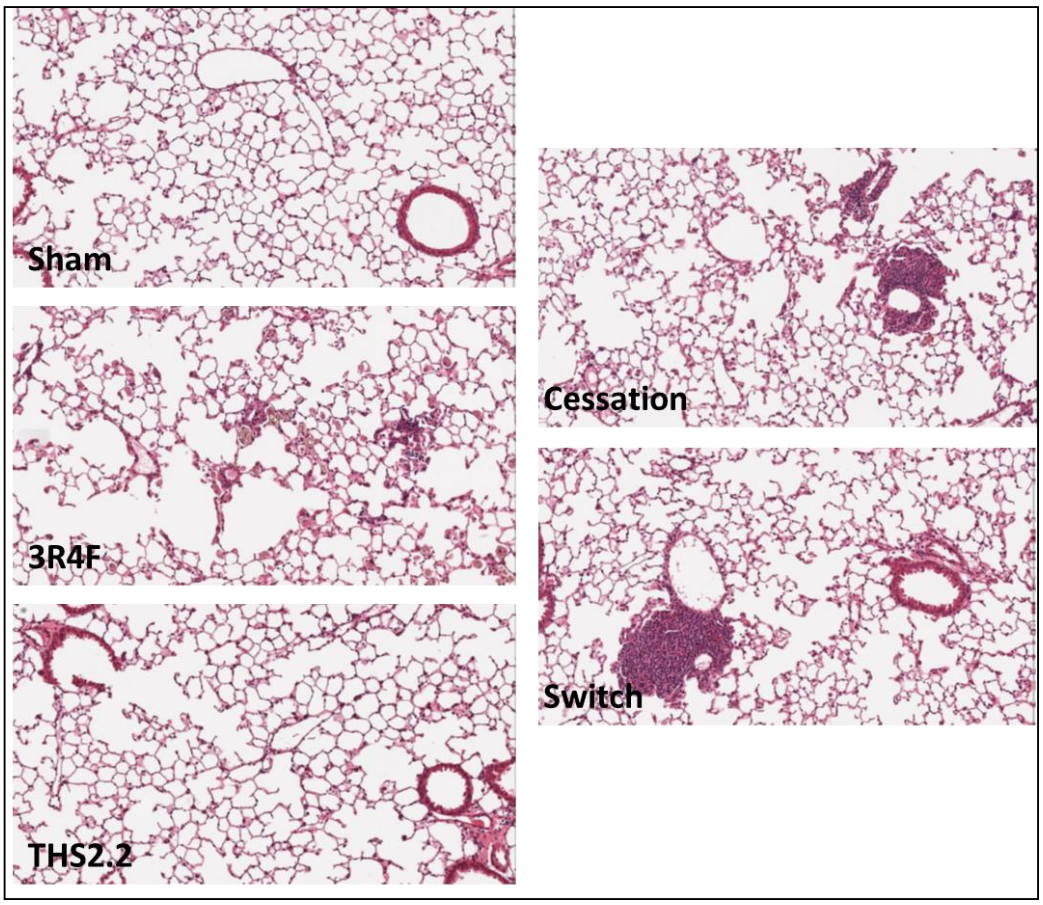


Figure 44. Lung morphology, month 8 timepoint.

Further demonstration of the progression of an emphysematous condition in the lungs was seen in the quantitative morphometric assessment of the lung tissue (Figure 43). Cigarette smoke resulted in increased mean cord length and destructive index, and decreased bronchiolar attachments in the lungs of exposed animals relative to sham-exposed animals. Mean cord length showed a relatively slow recovery to a position close to, but not reaching the cord length in sham-exposed animals. The other morphometric parameters (destructive index, bronchiolar attachments and mean emphysema score) showed a rapid recovery after 1 month post-CS exposure to return to the levels seen in sham-exposed mice. These morphometric parameters, interpreted along with the lung function results, are indicative of the development of emphysema in response to CS exposure. However, it is important to consider that some of morphometric parameters under evaluation, mean cord length in particular, are sensitive to technical artefacts during sample preparation, including differential inflation of the lung during instillation of the tissue (see Figure 42), as well as tissue shrinkage effects during paraffin embedding procedures (Ochs, 2014). Further investigations will therefore evaluate the tissue using volume-independent morphometric parameters.

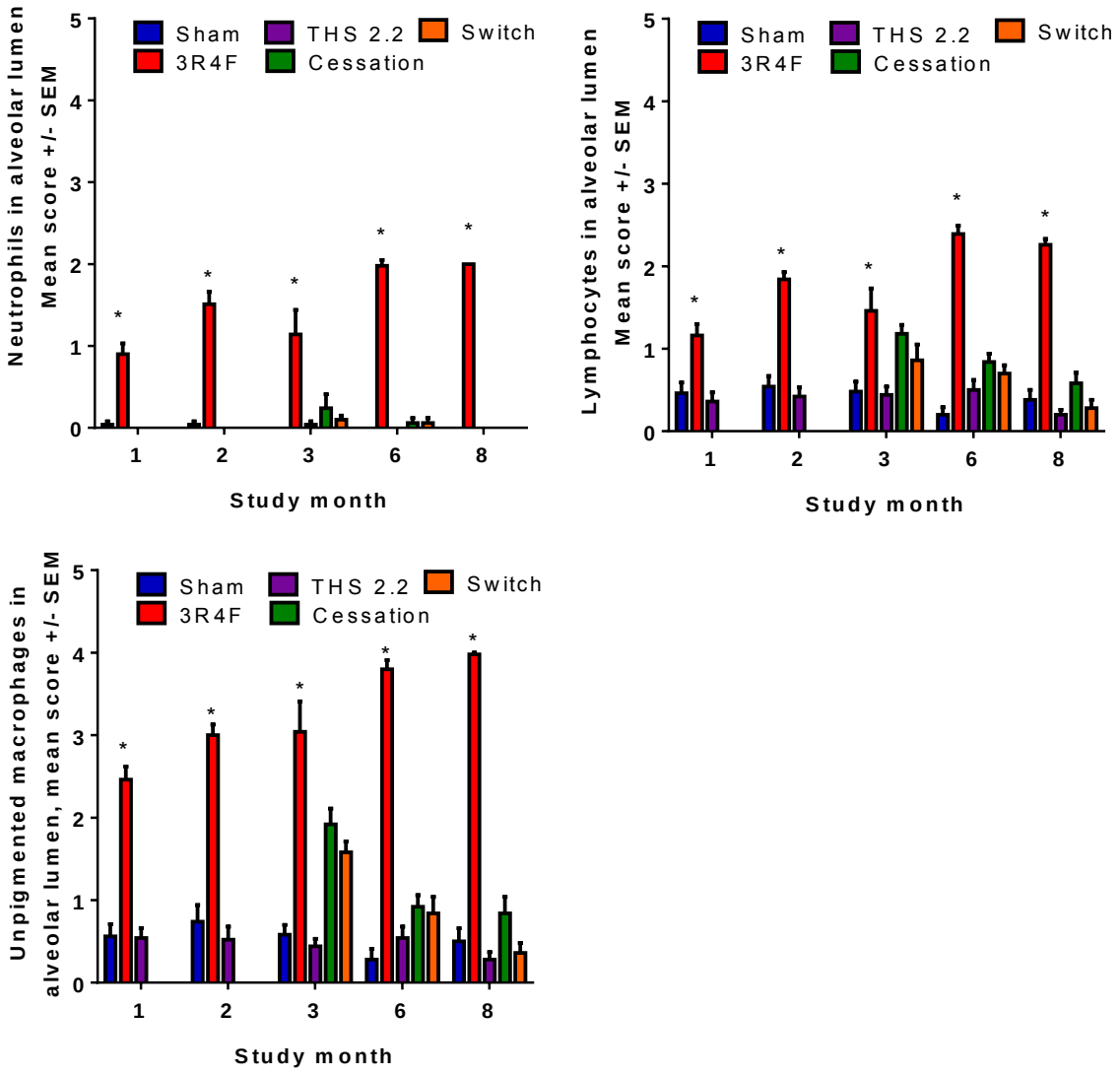


Figure 45. Infiltration into the alveolar lumen

Mean ± SEM, n = 10. Statistics compared with the sham-exposed group *p<0.05.

Histopathological assessment also revealed an infiltration of inflammatory cells into the pulmonary tissue following exposure to CS. Notably, an increased number of neutrophils, lymphocytes, and macrophages were observed in the alveolar space of CS-exposed mice (Figure 45). Recovery to control (sham-exposed) levels occurred within 1–3 months post exposure to CS. These results agree with the analysis of FLC differential counts seen in BALF (section 12.5).

12.7.3 Histopathology of the nose

The evaluation of the nose at three levels revealed typical adaptive effects of the nasal epithelia. This included CS-induced reserve cell hyperplasia and squamous epithelial metaplasia (Figure 46 and Figure 47). These effects were most pronounced in nose level 1 sections, and decreased in severity towards the more distal nasal regions, where at nose level 4 (the most distil nasal region evaluated), there were no differences in either hyperplasia or metaplasia of the epithelium in any of the exposure groups.. The effects were not observed in the THS2.2-exposed group. Similarly, both cessation and switching resulted in a rapid recovery towards sham-exposed group levels within 1 (squamous epithelial metaplasia) or 3 (reserve cell hyperplasia) months post CS-exposure.

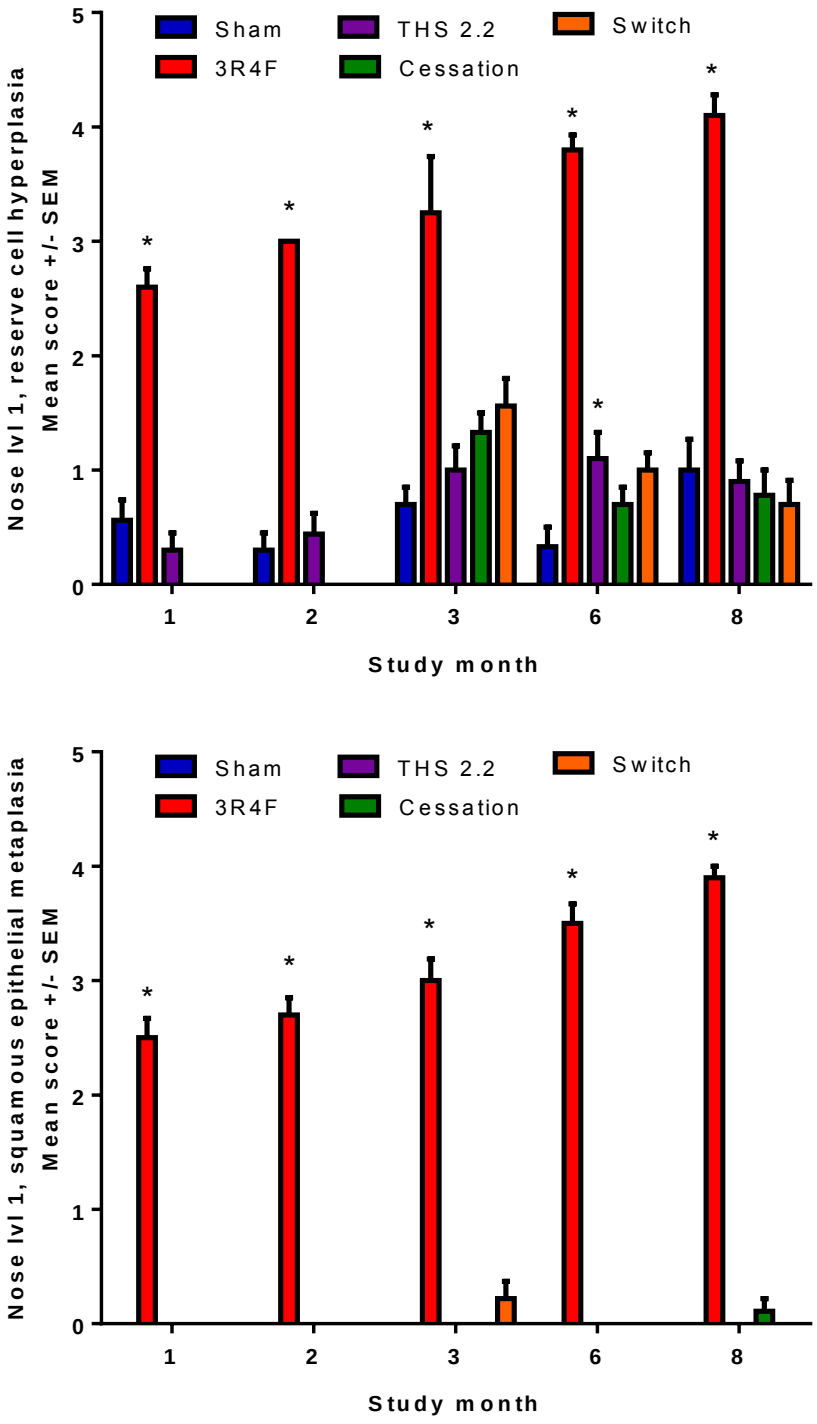


Figure 46. Histopathological assessment of the nose, level 1, respiratory epithelium
Mean ± SEM, n = 10. Statistics compared with the sham-exposed group, *p<0.05.

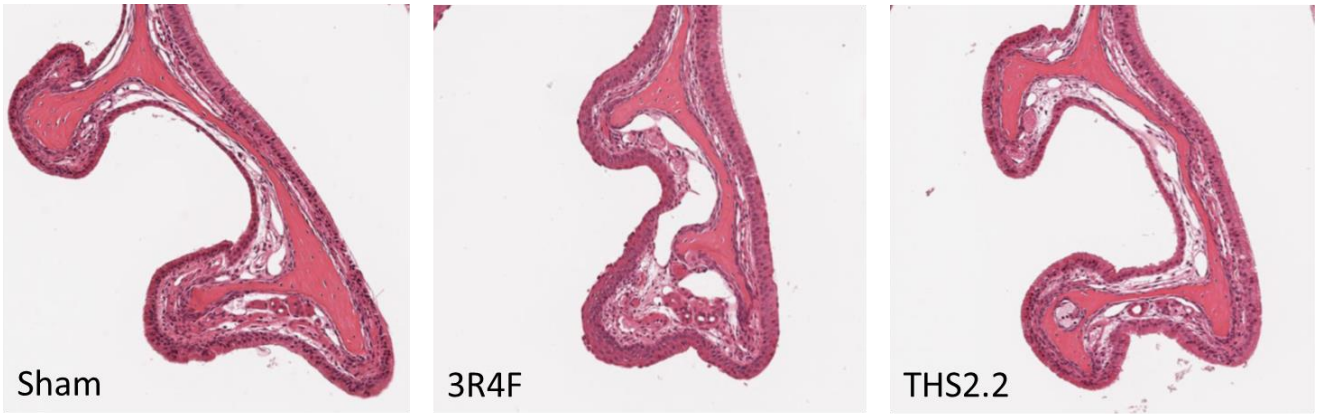


Figure 47. Morphology of the nose, level 1, respiratory epithelium. 8-month time point.

12.7.4 Histopathology of the liver

Histopathological assessment of the liver focussed on hepatocyte fat content (evaluated after oil red O staining), glycogen content (PAS/PASD staining), and additional endpoints including micro-granuloma and micro-haemorrhage. Minimal exposure effects were identified, including a lower mean fat and glycogen content in CS-exposed animals, although this was not statistically significant. Differences between the cessation and switching groups were small, and not statistically significant.

12.7.5 Histopathology of the pancreas and kidney

A histopathological evaluation of the pancreas and kidneys was performed on tissues isolated only in months 6 and 8. These results are summarized in detail in the pathological report, '*In vivo* evaluation of the effects of THS2.2 on the respiratory and cardiovascular systems of ApoE lipoprotein deficient mice, histopathology of the pancreas and kidneys'. A brief summary of these findings follows.

In the kidney, the findings were generally limited to the tissues isolated in month 6, and were characterized by a reduced incidence of thickening of the glomerular basement membrane relative to the sham-exposed groups. There was no difference between THS2.2, switch or cessation in this parameter over any of the time points. Mesangial matrix expansion was also decreased in the CS-exposed animals relative to the other groups. By month 8, the above mentioned CS effects were no longer present and all groups were similar across all parameters evaluated.

The majority of pancreatic parameters evaluated were similar between experimental groups, with exception endocrine cell (Islets of Langerhans) atrophy, where a subtle reduction in incidence was observed in THS2.2, switching, and CS exposure groups. An exposure-related effect cannot be ruled-out entirely for this difference.

12.8 Gene Expression Profiling and Omics

A comprehensive analysis of transcriptomics, proteomics, and lipidomics was performed on multiple tissues:

- Transcriptomics : Nasal epithelia, lungs, liver, thoracic aorta, heart, and kidney
- Proteomics: Lungs

These results are summarized in detail in the following report attachments (see section 1, report structure):

Phase report, delegated phase 1 and delegated phase 2 (PMIRL, Neuchatel), 'Study 150155 report, systems toxicology data from samples harvested in the study 15015: ApoE−/− ZRH switching study to characterize the toxicity of THS2.2 in ApoE−/− mice.'

12.9 Lipidomics Profiling

A comprehensive lipidomics analysis was performed on lung and liver tissues, as well as plasma samples. These results are summarised in detail in the following phase report from delegated phase 3 (PMIRL, Neuchatel, and Zora Biosciences), 'Lipidomic characterisation of ApoE(−/−) mouse tissue and plasma samples after smoke exposure.'

12.10 Insulin Resistance (IR)

In addition to the evaluation of COPD and cardiovascular disease endpoints, this exploratory study arm examined the effects of CS or THS 2.2 aerosol on IR and metabolic parameters (over a period of 3 months) in ApoE$^{-/-}$ mice. These analyses were performed on animals provided a chow diet or a high fat diet (HFD). A detailed summary of the results and conclusions can be found in the following report, '*In vivo* evaluation of the effects of THS2.2 on the respiratory and cardiovascular systems of Apo lipoprotein E-deficient mice. Part 2: Metabolic endpoints – Insulin resistance.'

13 Discussion

As a comprehensive follow-up study to previous disease model studies (COPD I, COPD II) (Lietz et al, 2013), the goals of this study were to evaluate the development and progression of emphysema, COPD, and cardiovascular disease in ApoE$^{-/-}$ mice following chronic exposure to 3R4F CS. This study included a group subjected to chronic pMRTP (THS2.2) exposure, which enabled a comparison of physiological and molecular responses to the novel aerosol to be made across a spectrum of disease endpoints relative to sham- or CS-exposed animals. Following 2 months of 3R4F CS exposure, the effects of cessation or switching to THS2.2 aerosol were assessed for up to 6 months (post-CS exposure).

13.1 Exposure and In-life Observations and Results

Smoke and aerosol generation was performed using automated smoking machines, and delivered according to the Health Canada intense smoking regime with matching nicotine concentrations of 29.9 µg/l for 3 h per day, 5 days per week, for up to 8 months. The average smoke or aerosol delivery to exposure chambers was within 5% from the targeted nicotine concentrations of 29.9 µg/l, which is equivalent to approximately 600 µg/l TPM in CS. Frequent analysis of the test atmospheres demonstrated similar nicotine levels among the groups throughout the exposure period (actual achieved averages ranged from 28.5–29.6 µg/l). The levels of other components of CS, CO, and aldehydes were lower in the THS2.2-exposed group because they are known to be present at lower levels than in CS. Biomonitoring of the animals during exposure demonstrated that the respective aerosols were inhaled, as evidenced by increased COHb levels (mainly in CS-exposed animals because the THS2.2 aerosol has lower CO concentrations) and through the recovery of nicotine metabolites in the urine of CS-exposed and THS2.2-exposed animals. Month 8 plasma samples also showed similar and high nicotine and cotinine concentrations in the 3R4F and THS2.2 exposure groups, to support the urine metabolites data (which analysed only post exposure urine, thereby leading to technical variation within the urine results). Additionally, the presence of other non-nicotine CS components (nicotine, NNK, acrolein, benzene, and acrylonitrile) was mainly observed in the urine of CS-exposed animals, again because of limited concentrations in the THS2.2 aerosol.

The routine and frequent in-life observations performed throughout the study period confirmed that both 3R4F and THS2.2 aerosols were well tolerated by the animals. However, a total of 42 unexpected moribund or unplanned deaths were observed, representing approximately 4% of the total starting animal number. These were found distributed throughout the exposure groups (with the highest incidence in the sham exposure group), and are therefore unlikely to be exposure-related. Many of these animals presented with growths, particularly xanthomas, which were previously observed in this mouse strain (Moghadasian et al, 2001). Exposure of the animals to CS resulted in a predicted reduction in body weight gain over the course of the study. By contrast, chronic exposure to the THS2.2 aerosol had no effect on body weight relative to the sham-exposed animals. Following either cessation or switching to the THS2.2 aerosol, a rapid gain in body weight occurred, whereby the animals recovered to the sham-exposed group body weight after just 3 weeks post CS-exposure, before dropping in weight slightly to just below the sham group body weight for the remainder of the exposure.

Because CS either directly or indirectly induces oxidative stress, 24-h urine samples were also evaluated for markers of oxidative stress and eicosanoid levels in addition to exposure biomarkers. In line with a previous study using the ApoE mouse model (Arunachalam et al, 2010), exposure to CS resulted in increased levels of lipid peroxidation products, 4-HNE and MDA. More importantly, this effect was abolished after switching or cessation.

13.2 Emphysema and Pulmonary Inflammation

The animals were assessed for physiological, histopathological, and molecular evidence of emphysema development. These results collectively identified many effects of CS on the lungs and respiratory system of the animals particularly following CS exposure, but with minimal impact from the THS2.2 aerosol. Additionally, similarities between smoking cessation and switching to THS2.2 were evident.

The evaluation of lung function was performed using unrestrained whole-body plethysmography methods, which indicated that exposure to CS resulted in characteristic patterns of emphysematous changes in the lungs. This was highlighted by decreased lung resistance and elastance, and increased compliance in the snapshot perturbation model. However, these smoke effects were evident only in the first 3 months of exposure, and no significant differences were seen between the exposure groups by month 6, perhaps because they were masked by an aging effect in ApoE$^{-/-}$ animals. Similar observations (both of time and age effect) were made using primewave-8 modelling, which extended the analysis to the peripheral loss of tissue elastance in central airways as well as the lung itself. The changes observed in the early stages (first 3 months) of the study were previously associated with emphysema in enzymatically induced (elastase) emphysema models (Vanoirbeek et al, 2010). In the above analysis, chronic exposure of the animals to the THS2.2 aerosol resulted in no appreciable differences from sham-exposed animals. The aging effect made the observation of a recovery following either switching or cessation impossible. The pressure-volume relationship (PVs-P) also demonstrated a typical emphysema-induced leftward shift following CS exposure. Unlike the other perturbations, the PVs-P loop showed a strong CS effect throughout the study period. There was again no obvious effect of THS2.2 exposure on the PVs-P loop compared with the sham animals at any time points evaluated. These results support the observed CS-induced increase in lung volume measured at various study time points. Switching and cessation resulted in a partial recovery after 4 months followed by a stabilisation, but the PVs-P did not revert back to the sham level. These results differ from the lung physiology measurements obtained in a previous study using C57Bl/6 mice (COPD II study, 15001) in that:

i) The CS effect (compared with sham) for the snapshot and primewave perturbation parameters was lower in ApoE$^{-/-}$ mice compared with C57Bl/6 animals (Study 15001, COPD II).

ii) In the current study, any differences from sham in snapshot perturbations were present and significant only at early study time points.

iii) The PVs-P loops, while showing the same overall smoke and recovery effects, were delayed relative to the C57Bl/6 animals (Study 15001, COPD II).

The differences between these two studies may be attributed to either the different mouse strain used (ApoE$^{-/-}$ mice versus C57Bl/6 mice) or to the lower ApoE$^{-/-}$ mouse exposure dose, which was necessary because this strain is more sensitive to CS and does not tolerate it at the higher concentrations used for the parental strain. To our knowledge, only one study has reported lung function analysis in ApoE-/- mice, using an acute exposure model of 3 days (Arunachalam et al, 2010). In this study, differences in the lung physiology of the two strains of mice were observed. Both strains demonstrated CS effects, but with different baseline values and relative changes from sham animals.

A rapid and sustained progression of pulmonary inflammation was evident in CS-exposed animals in the present study using multiple measured parameters. The observed rapid (within 1 month) influx of inflammatory cells comprising increased numbers of neutrophils, lymphocytes, and macrophages was accompanied by a change in numerous inflammatory mediators identified in the BALF. Additionally, we detected increased levels of key proteolytic enzymes, notably MMP-9 and its inhibitor Timp-1, which have been shown to be linked to the extracellular matrix remodelling that accompanies the alveolar destruction of emphysema (Foronjy et al, 2008). Exposure of the animals to the THS2.2 aerosol caused no changes to lung inflammation parameters relative to sham-exposed animals. Following cessation of switching, a rapid decrease in pulmonary inflammation in terms of cell infiltrate and expression of mediators was seen.

The above results were also confirmed at the histopathological level (see report from delegated phase 4 for a complete discussion and data analysis). Such analysis included the assessment of pulmonary emphysema, including the measurement of morphometric endpoints (mean cord length, DI, and bronchial attachments). The following parameters were observed to increase in a time-dependent manner following CS exposure, and with some reversal following cessation or switching:

i) Intra-alveolar inflammatory cell infiltrate

ii) Emphysema (based on a 5-point scoring system)

iii) Emphysema (based on morphometric changes)

The exposure of the animals to CS also resulted in increased lung volume, following instillation with fixative to a fixed pressure (15 cm H_2O). This may be related to hyperinflation of the lung as a consequence of emphysematous tissue damage and changes to the elastic recoil of the lung following CS exposure. This volume impacts some morphometric endpoints, such as mean cord length (Ochs, 2014), and therefore additional evaluation using volume-independent morphometric analysis is planned.

These results collectively show that CS is a causative agent in the development of atherosclerotic plaque development and of emphysema, as demonstrated by a number of anatomical and molecular disease indicators. The chronic exposure of the animals to a THS2.2 aerosol had minimal biological impact. While the 2-month exposure of mice to CS resulted in early and detectable signs of emphysema and atherosclerosis, both switching and cessation resulted in a partial (lung function, plaque area, and lung morphometry) or even complete (pulmonary inflammation) recovery to sham-exposed levels.

13.3 Cardiovascular Endpoints

An important objective of this study was to evaluate the effects of chronic CS exposure on the development and progression of atherosclerosis, and particularly to examine the impact of smoking cessation or switching to THS2.2. The aortic plaque area in CS-exposed mice was significantly greater than in sham-exposed animals as early as the 2-month time point, and continued through to the end of the 8-month experimental period. Chronic exposure of the animals to THS2.2 resulted in similar plaque areas to sham-exposed animals (with the exception of month 6 where an increased plaque area relative to sham was observed, though not as large as in CS-exposed animals). Following either cessation or switching to THS2.2, a reduction in the plaque area occurred, though they remained slightly, but not significantly, larger than those of sham or chronic THS2.2-exposed animals. These results are in agreement with historical data showing increased atherosclerotic plaque development following CS exposure (Han et al, 2012) and a partial recovery following cessation (Boue et al, 2012; Lietz et al, 2013).

Together with the increased atherosclerotic plaque burden, differences were also observed in the plasma lipoprotein profile of CS-exposed mice compared with sham controls. CS exposure resulted in increased total cholesterol and HDL levels relative to sham-exposed animals, particularly during the early exposure months. Additionally, glucose levels were decreased following CS exposure. The differences were largely reversed within 1 month following either cessation or switching to THS2.2 exposure. These clinical chemistry findings support previous clinical studies, which showed that the cessation of smoking improved the plasma HDL profile (Gepner et al, 2011).

14 Conclusions

This study describes the biological effects of chronic exposure (8 months) of ApoE$^{-/-}$ mice to either CS or to an aerosol from the pMRTP product THS2.2. This model is particularly suitable because of the possibility of evaluating both COPD and cardiovascular disease indicators. Exposure to CS resulted in significant levels of pulmonary inflammation accompanied by a decline in pulmonary function and histopathological changes to the respiratory system indicative of emphysema. These physiological effects were supported by the molecular data, which showed major changes in multiple networks identified at the protein, RNA, and lipid levels. CS exposure also resulted in increased atherosclerotic plaque development relative to sham-exposed animals, which was maintained throughout the 8-month exposure period, together with alterations of the plasma lipoprotein profile. Alternatively, exposure to an aerosol from THS2.2 resulted in very little difference in all measured parameters when compared with sham-exposed animals. This was highlighted by the absence of pulmonary inflammation (FLC infiltration into the lungs) and minimal effects on pulmonary histopathological observations relating to infiltration or emphysema development.

The biological responses of both cessation or of switching to THS2.2 aerosol following 2 months of CS exposure were very similar between the two conditions across the spectrum of endpoints assessed, and showed a

generally positive effect. While some parameters (pulmonary inflammation) demonstrated a complete recovery back to sham-exposure levels, often after only 1 month of switching/cessation, others (lung function and aortic arch plaque area) showed a partial recovery that did not quite return to sham levels. Importantly, in each case, the biological effects of cessation were consistently similar to those of switching to THS2.2.

15 Archiving

All study records (study plan, raw data from test facility, copy of raw data from test sites, statistical reports, final report, and phase reports), histological slides, paraffin blocks of respiratory tract organs, fixed blood smears and bone marrow slides, and samples of reference and test items will be archived at the test facility for up to 2 years, followed by transfer to Iron Mountain Deutschland GmbH (Harpener Hellweg, 44805 Bochum, Germany) according to WKI ‘Manage Archiving’ and ‘Archive test item’. A representative sample of the test and reference items will be stored at the test facility under appropriate storage conditions according to the ‘manage archiving’ WKI. Animal data in In-Vivo LIMS (Provantis) will be electronically archived after the study is completed. Please refer to WKI ‘‘Manage Archiving’ for further details.

Spare biological materials at the test facility such as histological specimens, serum, plasma, urine, and BALF will not be archived but will be disposed of upon phase completion because of limited sample stability.

16 References

Arunachalam G, Sundar IK, Hwang JW, Yao H, Rahman I (2010) Emphysema is associated with increased inflammation in lungs of atherosclerosis-prone mice by cigarette smoke: implications in comorbidities of COPD. *J Inflamm (Lond)* **7:** 34

Berges A. (2011) Study 13882. Impact of smoking cessation and switching to NGP in ApoE-/- mice. Philip Morris Research Laboratories, BVBA.

Boue S, De Leon H, Schlage WK, Peck MJ, Weiler H, Berges A, Vuillaume G, Martin F, Friedrichs B, Lebrun S, Meurrens K, Schracke N, Moehring M, Steffen Y, Schueller J, Vanscheeuwijck P, Peitsch MC, Hoeng J (2013) Cigarette smoke induces molecular responses in respiratory tissues of ApoE(-/-) mice that are progressively deactivated upon cessation. *Toxicology* **314:** 112-124

Boue S, Tarasov K, Janis M, Lebrun S, Hurme R, Schlage W, Lietz M, Vuillaume G, Ekroos K, Steffen Y, Peitsch MC, Laaksonen R, JHoeng J (2012) Modulation of atherogenic lipidome by cigarette smoke in apolipoprotein E-deficient mice. *Atherosclerosis* **225:** 328-334.

Carmella SG, Chen M, Han S, Briggs A, Jensen J, Hatsukami DK, Hecht SS (2009) Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. *Chem Res Toxicol* **22:** 734-741

Chan JR, vuillaume G, Bever C, Lebrun S, Lietz M, Yvonne S, Stolle K, Wahba K, Wang X, Moodie S, Hoeng J, Peitsch M, Powell LM (2012) The ApoE-/- mouse PhysioLab platform: A validated physiologically-based mathematical model of atherosclerotic plaque progression in the ApoE-/- mouse. *Biodiscovery* **3:** 1-13

Dhariwal J, Tennant RC, Hansell DM, Westwick J, Walker C, Ward SP, Pride N, Barnes PJ, Kon OM, Hansel TT (2014) Smoking cessation in COPD causes a transient improvement in spirometry and decreases micronodules on high-resolution CT imaging. *Chest* **145:** 1006-1015

Doruk S, Ozyurt H, Inonu H, Erkorkmaz U, Saylan O, Seyfikli Z (2011) Oxidative status in the lungs associated with tobacco smoke exposure. *Clinical chemistry and laboratory medicine : CCLM / FESCC* **49:** 2007-2012

Foronjy R, Nkyimbeng T, Wallace A, Thankachen J, Okada Y, Lemaitre V, D'Armiento J (2008) Transgenic expression of matrix metalloproteinase-9 causes adult-onset emphysema in mice associated with the loss of alveolar elastin. *Am J Physiol Lung Cell Mol Physiol* **294:** L1149-1157

Gepner AD, Piper ME, Johnson HM, Fiore MC, Baker TB, Stein JH (2011) Effects of smoking and smoking cessation on lipids and lipoproteins: outcomes from a randomized clinical trial. *American heart journal* **161:** 145-151

Han SG, Howatt DA, Daugherty A, Gairola CG (2012) Atherogenic and pulmonary responses of ApoE- and LDL receptor-deficient mice to sidestream cigarette smoke. *Toxicology* **299:** 133-138

Lietz M, Berges A, Lebrun S, Meurrens K, Steffen Y, Stolle K, Schueller J, Boue S, Vuillaume G, Vanscheeuwijck P, Moehring M, Schlage W, De Leon H, Hoeng J, Peitsch M (2013) Cigarette-smoke-induced atherogenic lipid profiles in plasma and vascular tissue of apolipoprotein E-deficient mice are attenuated by smoking cessation. *Atherosclerosis* **229:** 86-93

Moghadasian MH, McManus BM, Nguyen LB, Shefer S, Nadji M, Godin DV, Green TJ, Hill J, Yang Y, Scudamore CH, Frohlich JJ (2001) Pathophysiology of apolipoprotein E deficiency in mice: relevance to apo E-related disorders in humans. *FASEB J* **15:** 2623-2630

Mol MJ, de Rijke YB, Demacker PN, Stalenhoef AF (1997) Plasma levels of lipid and cholesterol oxidation products and cytokines in diabetes mellitus and cigarette smoking: effects of vitamin E treatment. *Atherosclerosis* **129:** 169-176

NACLAR (2004) *National Advisory Committee for Laboratory Animal Research, guidelines on the care and use of animals for scientific purposes*, Singapore.

NTP. (2000) Specification for conduct of studies to evaluate the toxic and carcinogenic potential of chemical, biological, and physical agents in laboratory animals for the national toxicology program. National toxicology program, Research triangle park, NC.

Ochs M (2014) Estimating structural alterations in animal models of lung emphysema. Is there a gold standard? *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft* **196:** 26-33

Pauwels RA, Rabe KF (2004) Burden and clinical features of chronic obstructive pulmonary disease (COPD). *Lancet* **364:** 613-620

Phillips BW. (2013) Study 15001. COPD progression in C57Bl/6 mice in response to chronic exposure to conventional cigarette smoke, and the impact of cessation or switching to aerosol from a next generation product. A 7 month inhalation study on C57Bl/6 mice. Philip Morris International Research Laboratories.

Price JF, Mowbray PI, Lee AJ, Rumley A, Lowe GD, Fowkes FG (1999) Relationship between smoking and cardiovascular risk factors in the development of peripheral arterial disease and coronary artery disease: Edinburgh Artery Study. *European heart journal* **20:** 344-353

Scherle W (1970) A simple method for volumetry of organs in quantitative stereology. *Mikroskopie* **26:** 57-60

Seet RC, Lee CY, Loke WM, Huang SH, Huang H, Looi WF, Chew ES, Quek AM, Lim EC, Halliwell B (2011) Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress? *Free Radic Biol Med* **50:** 1787-1793

Vanoirbeek JA, Rinaldi M, De Vooght V, Haenen S, Bobic S, Gayan-Ramirez G, Hoet PH, Verbeken E, Decramer M, Nemery B, Janssens W (2010) Noninvasive and invasive pulmonary function in mouse models of obstructive and restrictive respiratory diseases. *Am J Respir Cell Mol Biol* **42:** 96-104

Veniant MM, Withycombe S, Young SG (2001) Lipoprotein size and atherosclerosis susceptibility in Apoe(-/-) and Ldlr(-/-) mice. *Arterioscler Thromb Vasc Biol* **21:** 1567-1570

Wong E. (2014) Study 15006, 90 day OECD inhalation study on Sprague-Dawley rats to characterize toxicity of mainstream aerosol from zurich 4.2 cigarettes and comparison to 3R4F mainstream aerosol toxicity., Philip Morris International research Laboratories.

Yi S. (2012) Explore the impact of 3R4F mainstream smoke exposure and smoking cessation on the development of COPD in C57Bl/6 mice A 7-month inhalation study on c57Bl/6 mice. Philip Morris International Research Laboratories, Singapore.

17 Abbreviations

Abbreviation	
AB-PAS	Alcian Blue Periodic Acid-Schiff
BA	Bronchioalveolar Attachments
BALF	Broncho-Alveolar Lavage Fluid
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
DI	Destructive Index
DNA	Deoxyribonucleic Acid
DNPH	2,4-dinitrophenylhydrazine
DUC	Document Unique Code
FLC	Free Lung Cell
GLP	Good Laboratory Practice
H&E	Haematoxylin and Eosin
HbCO	Carboxyhaemoglobin
HPLC	High-Performance Liquid Chromatography
MMP	Matrix Metalloproteinase
N/A	Not Applicable
NACLAR	National Advisory Committee for Laboratory Animal Research
NTP	National Toxicology Program
P-value (P)	Probability of receiving the value of the test statistic, or a more extreme value of the test statistic if the null hypothesis holds
PMIRL-S	Philip Morris International Research Lab in Singapore
PMRL	Philip Morris Research Laboratories
PV	Pressure Volume
PUC	Project Unique Code
QMS	Quality Management System

RBM	Rules-Based Medicine
RNA	Ribonucleic Acid
SOP	Standard Operating Procedure
TBD	To Be Determined
TPM	Total Particulate Matter
WKI	Work Instruction

For complete definitions, refer to PMI OPS Glossary and PMI RD Glossary.

18 Tables and figures

Table 18. Body weight (g)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
-12	Sham	216	16.40	19.25	20.00	20.80	22.90	19.96	1.20	0.08	6.02	19.80	20.12			
-12	3R4F	214	17.00	19.10	20.10	20.80	22.90	19.97	1.16	0.08	5.82	19.82	20.13	-		
-12	THS2.2	214	17.40	19.20	20.00	20.70	22.80	19.97	1.14	0.08	5.69	19.81	20.12	-	-	
-12	Cess	130	16.60	19.20	19.95	20.90	22.30	19.96	1.16	0.10	5.84	19.75	20.16	-	-	
-12	Switch	130	16.80	19.20	20.05	20.80	22.60	19.98	1.20	0.11	6.02	19.77	20.19	-	-	-
-5	Sham	216	18.00	19.50	20.25	20.95	22.40	20.21	1.05	0.07	5.18	20.07	20.35			
-5	3R4F	214	17.90	19.40	20.20	20.90	22.40	20.18	1.06	0.07	5.26	20.04	20.32	-		
-5	THS2.2	214	17.90	19.40	20.20	21.00	22.30	20.18	1.05	0.07	5.20	20.04	20.33	-	-	
-5	Cess	130	17.90	19.40	20.20	20.90	22.30	20.17	1.02	0.09	5.07	19.99	20.35	-	-	
-5	Switch	130	17.40	19.40	20.20	21.00	22.50	20.16	1.09	0.10	5.40	19.97	20.35	-	-	-
1	Sham	216	18.30	19.70	20.35	21.20	23.20	20.48	1.04	0.07	5.09	20.34	20.62			
1	3R4F	214	17.90	19.80	20.50	21.10	23.20	20.51	1.02	0.07	4.95	20.37	20.65	-		
1	THS2.2	214	17.90	19.90	20.65	21.50	24.30	20.66	1.08	0.07	5.25	20.51	20.81	-	-	
1	Cess	130	18.60	20.00	20.85	21.40	23.50	20.76	1.02	0.09	4.91	20.58	20.93	*	*	
1	Switch	130	17.40	19.40	20.30	20.90	22.80	20.30	1.02	0.09	5.04	20.12	20.47	-	-	***
9	Sham	216	18.90	20.60	21.30	22.20	23.80	21.35	1.06	0.07	4.97	21.20	21.49			
9	3R4F	214	19.00	20.90	22.00	22.90	25.30	21.92	1.35	0.09	6.15	21.74	22.10	***		
9	THS2.2	214	18.70	20.50	21.35	22.20	24.10	21.39	1.14	0.08	5.32	21.24	21.54	-	***	
9	Cess	130	17.80	21.20	21.95	22.80	24.70	21.89	1.34	0.12	6.10	21.66	22.12	***	-	
9	Switch	130	17.80	20.70	22.10	22.80	25.50	21.86	1.42	0.12	6.48	21.61	22.11	***	-	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
16	Sham	215	19.30	21.10	21.80	22.50	25.00	21.85	1.11	0.08	5.07	21.70	21.99			
16	3R4F	214	18.90	20.60	21.60	22.60	25.90	21.65	1.39	0.09	6.41	21.46	21.83	-		
16	THS2.2	214	18.70	21.00	21.70	22.50	24.90	21.75	1.16	0.08	5.35	21.59	21.91	-	-	
16	Cess	130	17.30	20.70	21.55	22.60	25.20	21.59	1.44	0.13	6.65	21.34	21.84	-	-	
16	Switch	130	18.20	20.60	21.60	22.60	25.60	21.59	1.40	0.12	6.49	21.34	21.83	-	-	-
23	Sham	216	19.70	21.80	22.50	23.20	25.80	22.53	1.12	0.08	4.97	22.38	22.68			
23	3R4F	214	19.00	21.10	22.00	22.90	26.50	22.01	1.32	0.09	6.00	21.83	22.19	***		
23	THS2.2	214	19.30	21.90	22.55	23.40	25.50	22.58	1.15	0.08	5.10	22.42	22.73	-	***	
23	Cess	130	18.60	21.10	22.00	22.90	25.80	21.95	1.42	0.12	6.46	21.71	22.20	***	-	
23	Switch	130	18.90	21.00	22.00	22.90	25.30	21.90	1.37	0.12	6.27	21.66	22.14	***	-	-
30	Sham	216	18.70	21.80	22.55	23.35	25.10	22.53	1.15	0.08	5.11	22.38	22.69			
30	3R4F	214	19.10	21.40	22.25	23.20	26.40	22.36	1.32	0.09	5.91	22.18	22.54	-		
30	THS2.2	214	19.50	21.90	22.90	23.70	26.00	22.84	1.24	0.08	5.44	22.67	23.01	**	***	
30	Cess	130	18.50	21.20	22.35	23.10	25.90	22.26	1.39	0.12	6.23	22.02	22.50	-	-	
30	Switch	130	18.90	21.30	22.25	23.30	26.30	22.24	1.43	0.13	6.41	21.99	22.49	*	-	-
37	Sham	178	19.10	21.80	22.60	23.20	26.80	22.61	1.18	0.09	5.22	22.44	22.78			
37	3R4F	176	19.50	21.70	22.50	23.40	26.20	22.58	1.30	0.10	5.77	22.38	22.77	-		
37	THS2.2	176	19.70	22.10	22.70	23.60	27.30	22.81	1.22	0.09	5.36	22.63	23.00	-	-	
37	Cess	130	19.40	21.50	22.40	23.40	26.30	22.53	1.44	0.13	6.39	22.28	22.78	-	-	
37	Switch	130	19.10	21.30	22.50	23.30	25.80	22.29	1.42	0.12	6.38	22.04	22.54	*	-	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
44	Sham	178	19.70	22.10	23.00	23.90	26.60	23.02	1.22	0.09	5.31	22.84	23.20			
44	3R4F	176	19.10	21.60	22.40	23.20	26.00	22.51	1.31	0.10	5.83	22.31	22.70	***		
44	THS2.2	176	20.00	22.45	23.30	24.10	27.00	23.30	1.27	0.10	5.45	23.11	23.49	*	***	
44	Cess	130	19.10	21.60	22.50	23.70	26.80	22.60	1.53	0.13	6.78	22.33	22.86	*	-	
44	Switch	130	18.90	21.60	22.60	23.40	26.30	22.55	1.46	0.13	6.48	22.30	22.80	**	-	-
51	Sham	170	20.00	22.60	23.40	24.00	27.00	23.33	1.17	0.09	5.02	23.16	23.51			
51	3R4F	168	19.80	21.60	22.50	23.40	26.20	22.55	1.28	0.10	5.67	22.36	22.75	***		
51	THS2.2	168	20.20	22.60	23.40	24.30	27.40	23.47	1.35	0.10	5.74	23.26	23.67	-	***	
51	Cess	130	18.50	21.50	22.25	23.50	27.30	22.51	1.59	0.14	7.04	22.23	22.78	***	-	
51	Switch	130	19.20	21.50	22.55	23.50	26.80	22.46	1.44	0.13	6.42	22.21	22.71	***	-	-
58	Sham	170	20.60	22.60	23.30	24.40	27.90	23.50	1.32	0.10	5.64	23.30	23.70			
58	3R4F	168	19.90	21.80	22.50	23.50	26.60	22.74	1.34	0.10	5.90	22.53	22.94	***		
58	THS2.2	168	20.60	22.80	23.60	24.50	27.70	23.68	1.36	0.10	5.74	23.47	23.88	-	***	
58	Cess	130	19.30	21.80	22.75	23.50	27.10	22.75	1.50	0.13	6.58	22.49	23.01	***	-	
58	Switch	130	19.10	21.40	22.45	23.50	26.50	22.51	1.48	0.13	6.59	22.25	22.77	***	-	-
65	Sham	132	20.50	22.80	23.55	24.25	26.80	23.57	1.19	0.10	5.07	23.36	23.77			
65	3R4F	130	19.80	22.00	22.80	23.70	26.10	22.80	1.29	0.11	5.68	22.58	23.03	***		
65	THS2.2	130	20.50	23.00	23.85	24.90	27.70	23.90	1.40	0.12	5.87	23.65	24.14	*	***	
65	Cess	130	19.30	22.00	22.70	23.80	27.30	22.91	1.44	0.13	6.27	22.66	23.16	***	-	
65	Switch	130	20.20	21.90	22.80	23.80	26.30	22.91	1.37	0.12	5.99	22.67	23.15	***	-	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
72	Sham	129	20.90	23.20	24.00	24.90	28.20	24.05	1.35	0.12	5.62	23.82	24.29			
72	3R4F	127	20.00	22.00	22.80	23.80	27.00	22.87	1.29	0.11	5.66	22.64	23.09	***		
72	THS2.2	127	20.30	23.10	24.10	25.10	28.90	24.17	1.51	0.13	6.23	23.90	24.43	-	***	
72	Cess	130	19.90	22.50	23.10	24.10	26.90	23.36	1.35	0.12	5.78	23.12	23.59	***	**	
72	Switch	130	19.80	22.50	23.30	24.40	26.40	23.39	1.35	0.12	5.78	23.16	23.63	***	**	-
79	Sham	129	20.20	23.10	24.20	24.90	28.60	24.16	1.45	0.13	5.99	23.91	24.41			
79	3R4F	127	19.90	22.10	22.80	23.90	27.90	23.02	1.37	0.12	5.95	22.78	23.26	***		
79	THS2.2	127	20.20	22.80	24.00	25.30	27.50	24.04	1.51	0.13	6.28	23.78	24.31	-	***	
79	Cess	129	20.20	22.80	23.80	24.50	28.80	23.80	1.51	0.13	6.36	23.53	24.06	-	***	
79	Switch	129	21.00	23.10	23.90	25.00	28.10	24.07	1.40	0.12	5.83	23.83	24.32	-	***	-
86	Sham	129	21.00	23.10	24.10	25.40	27.70	24.19	1.51	0.13	6.26	23.93	24.45			
86	3R4F	127	20.10	22.20	22.90	23.90	28.10	23.11	1.32	0.12	5.72	22.88	23.34	***		
86	THS2.2	127	20.90	23.20	24.30	25.20	29.20	24.36	1.58	0.14	6.48	24.08	24.64	-	***	
86	Cess	130	19.90	22.50	23.45	24.40	27.40	23.57	1.36	0.12	5.77	23.34	23.81	***	**	
86	Switch	130	20.70	22.80	23.70	24.70	28.10	23.78	1.40	0.12	5.88	23.54	24.03	*	***	-
93	Sham	121	21.00	23.50	24.10	25.30	28.60	24.48	1.57	0.14	6.40	24.20	24.76			
93	3R4F	120	19.90	22.30	23.15	24.00	29.10	23.30	1.39	0.13	5.97	23.05	23.55	***		
93	THS2.2	120	20.90	23.70	24.45	25.30	28.50	24.58	1.55	0.14	6.31	24.30	24.86	-	***	
93	Cess	123	20.70	22.70	23.30	24.30	28.10	23.52	1.37	0.12	5.82	23.27	23.76	***	-	
93	Switch	123	20.90	22.80	23.80	24.90	29.20	23.92	1.46	0.13	6.12	23.65	24.18	**	***	*

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
100	Sham	91	21.10	23.70	24.30	25.40	28.70	24.52	1.48	0.15	6.02	24.21	24.83			
100	3R4F	89	20.20	22.50	23.30	24.20	27.30	23.40	1.43	0.15	6.12	23.10	23.70	***		
100	THS2.2	89	21.30	23.60	24.50	25.30	28.30	24.52	1.51	0.16	6.16	24.21	24.84	-	***	
100	Cess	92	21.10	23.00	23.75	24.60	27.80	23.88	1.40	0.15	5.84	23.60	24.17	**	*	
100	Switch	92	20.00	22.80	23.95	24.90	27.20	23.88	1.54	0.16	6.44	23.56	24.19	**	*	-
107	Sham	91	21.30	23.70	24.60	25.50	28.90	24.67	1.62	0.17	6.56	24.33	25.01			
107	3R4F	89	19.90	22.40	23.10	24.10	28.90	23.34	1.45	0.15	6.23	23.03	23.64	***		
107	THS2.2	89	20.90	23.60	24.40	25.60	29.90	24.67	1.72	0.18	6.97	24.31	25.03	-	***	
107	Cess	91	21.20	22.90	23.70	25.10	29.00	23.93	1.56	0.16	6.50	23.61	24.25	**	**	
107	Switch	92	21.60	23.40	24.35	25.45	29.10	24.41	1.49	0.16	6.11	24.10	24.72	-	***	*
114	Sham	85	21.90	23.70	24.90	25.80	29.70	24.92	1.67	0.18	6.71	24.56	25.28			
114	3R4F	83	20.40	22.80	23.30	24.40	29.20	23.56	1.52	0.17	6.46	23.23	23.89	***		
114	THS2.2	83	20.60	23.80	24.80	26.10	29.10	24.99	1.77	0.19	7.08	24.61	25.38	-	***	
114	Cess	84	20.90	23.30	24.20	25.25	28.90	24.38	1.55	0.17	6.35	24.05	24.72	*	***	
114	Switch	84	21.70	23.55	24.40	25.90	28.40	24.70	1.58	0.17	6.39	24.36	25.04	-	***	-
121	Sham	85	22.40	23.90	25.00	26.20	30.80	25.20	1.65	0.18	6.53	24.84	25.55			
121	3R4F	83	19.70	22.30	23.10	24.10	28.00	23.36	1.48	0.16	6.34	23.03	23.68	***		
121	THS2.2	83	21.70	23.90	25.20	26.40	30.50	25.25	1.84	0.20	7.30	24.85	25.66	-	***	
121	Cess	84	21.70	23.70	24.50	25.55	30.40	24.71	1.58	0.17	6.40	24.37	25.05	-	***	
121	Switch	84	21.60	23.35	24.50	25.70	30.80	24.65	1.64	0.18	6.64	24.29	25.00	*	***	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
128	Sham	85	22.40	23.80	25.50	26.60	30.90	25.47	1.83	0.20	7.18	25.07	25.86			
128	3R4F	83	20.10	22.50	23.20	24.20	28.80	23.46	1.48	0.16	6.30	23.14	23.78	***		
128	THS2.2	83	21.00	24.20	25.70	26.80	30.90	25.58	1.89	0.21	7.39	25.17	25.99	-	***	
128	Cess	84	21.30	23.70	24.50	26.05	30.60	24.93	1.86	0.20	7.46	24.53	25.34	-	***	
128	Switch	84	21.40	23.60	25.00	26.25	30.60	24.99	1.87	0.20	7.48	24.58	25.39	-	***	-
135	Sham	85	23.10	24.40	25.70	27.10	30.70	25.85	1.85	0.20	7.14	25.45	26.24			
135	3R4F	83	20.50	22.50	23.30	24.40	29.80	23.52	1.52	0.17	6.47	23.19	23.86	***		
135	THS2.2	83	21.20	24.30	25.30	26.40	30.30	25.56	2.00	0.22	7.83	25.12	26.00	-	***	
135	Cess	84	22.40	23.90	24.50	26.15	31.00	25.03	1.80	0.20	7.19	24.64	25.42	**	***	
135	Switch	84	21.80	23.90	25.00	26.45	32.60	25.28	1.75	0.19	6.93	24.90	25.67	*	***	-
142	Sham	85	22.40	24.60	25.90	26.80	31.80	25.82	1.85	0.20	7.16	25.43	26.22			
142	3R4F	83	20.40	22.70	23.30	24.60	30.50	23.62	1.55	0.17	6.55	23.28	23.96	***		
142	THS2.2	83	21.60	24.30	25.80	26.90	31.20	25.78	1.99	0.22	7.71	25.34	26.21	-	***	
142	Cess	84	21.80	23.75	24.95	25.85	31.40	25.07	1.70	0.19	6.79	24.70	25.44	**	***	
142	Switch	84	21.90	23.90	25.05	26.60	32.60	25.24	1.91	0.21	7.55	24.83	25.66	*	***	-
149	Sham	85	22.00	24.40	25.90	26.90	30.90	25.79	1.88	0.20	7.29	25.38	26.19			
149	3R4F	83	20.50	22.40	23.20	24.20	30.80	23.46	1.58	0.17	6.71	23.12	23.81	***		
149	THS2.2	83	21.30	24.50	25.80	26.80	31.70	25.79	2.16	0.24	8.36	25.32	26.26	-	***	
149	Cess	84	22.40	24.00	24.90	25.80	32.10	25.18	1.70	0.19	6.76	24.81	25.55	*	***	
149	Switch	84	22.00	23.95	25.05	26.05	30.70	25.25	1.85	0.20	7.31	24.85	25.65	-	***	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
156	Sham	84	22.30	24.30	25.95	27.20	31.10	25.94	1.96	0.21	7.55	25.52	26.37			
156	3R4F	83	20.50	22.60	23.30	24.00	28.20	23.55	1.40	0.15	5.94	23.25	23.86	***		
156	THS2.2	83	21.70	24.40	25.80	27.20	31.60	25.89	2.20	0.24	8.48	25.41	26.37	-	***	
156	Cess	84	21.90	24.10	25.15	26.85	30.50	25.41	1.82	0.20	7.16	25.02	25.81	-	***	
156	Switch	84	22.60	24.50	25.50	26.60	32.40	25.64	1.79	0.20	6.98	25.25	26.03	-	***	-
163	Sham	84	22.20	24.60	25.85	27.20	30.70	26.09	1.89	0.21	7.25	25.68	26.50			
163	3R4F	83	20.20	22.80	23.50	24.70	29.00	23.79	1.65	0.18	6.91	23.43	24.15	***		
163	THS2.2	83	21.90	24.70	26.30	27.40	31.30	26.21	2.07	0.23	7.89	25.75	26.66	-	***	
163	Cess	84	22.80	24.55	25.25	26.60	30.80	25.66	1.80	0.20	7.01	25.27	26.05	-	***	
163	Switch	84	22.40	24.50	25.35	27.00	33.40	25.77	2.03	0.22	7.88	25.33	26.21	-	***	-
170	Sham	85	22.20	24.90	26.10	27.40	32.00	26.27	2.04	0.22	7.78	25.83	26.71			
170	3R4F	83	20.90	22.90	23.60	24.60	29.30	23.78	1.50	0.16	6.29	23.45	24.10	***		
170	THS2.2	83	22.30	24.50	26.00	27.70	33.00	26.18	2.24	0.25	8.57	25.69	26.67	-	***	
170	Cess	84	22.10	24.50	25.40	26.85	32.10	25.81	1.96	0.21	7.61	25.38	26.23	-	***	
170	Switch	84	21.30	24.40	25.90	27.20	33.60	25.95	2.02	0.22	7.78	25.51	26.38	-	***	-
177	Sham	84	22.50	24.95	26.25	27.85	32.00	26.45	2.01	0.22	7.59	26.02	26.89			
177	3R4F	83	20.80	22.80	23.80	24.80	31.40	23.95	1.69	0.19	7.06	23.59	24.32	***		
177	THS2.2	83	21.70	25.00	26.80	27.80	33.10	26.84	2.37	0.26	8.84	26.32	27.36	-	***	
177	Cess	84	22.40	24.40	25.90	26.90	32.30	26.04	2.05	0.22	7.85	25.60	26.49	-	***	
177	Switch	84	23.00	24.65	25.90	27.55	31.80	26.14	1.94	0.21	7.43	25.72	26.56	-	***	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
184	Sham	76	22.30	24.80	26.50	28.00	32.20	26.62	2.23	0.26	8.40	26.11	27.13			
184	3R4F	76	20.70	22.80	23.70	24.45	30.10	23.92	1.68	0.19	7.04	23.53	24.30	***		
184	THS2.2	76	22.00	25.05	26.60	28.15	32.90	26.73	2.47	0.28	9.25	26.16	27.29	-	***	
184	Cess	77	22.70	24.80	26.20	27.40	33.20	26.33	2.14	0.24	8.11	25.84	26.81	-	***	
184	Switch	77	22.60	24.80	26.00	27.40	33.90	26.26	2.14	0.24	8.14	25.78	26.75	-	***	-
191	Sham	48	22.50	25.20	27.25	28.20	31.20	26.98	2.18	0.31	8.06	26.34	27.61			
191	3R4F	45	20.90	22.60	23.80	24.40	29.90	23.90	1.92	0.29	8.01	23.32	24.47	***		
191	THS2.2	45	22.00	25.60	27.10	28.00	33.20	27.04	2.52	0.38	9.33	26.28	27.80	-	***	
191	Cess	46	22.70	24.90	25.80	27.40	32.30	26.36	2.13	0.31	8.06	25.73	26.99	-	***	
191	Switch	46	21.90	24.90	27.10	28.70	33.60	27.04	2.40	0.35	8.87	26.33	27.75	-	***	-
198	Sham	47	22.80	25.70	27.40	29.00	32.90	27.44	2.34	0.34	8.54	26.75	28.12			
198	3R4F	45	21.50	23.00	23.80	24.40	31.50	24.09	1.87	0.28	7.76	23.53	24.65	***		
198	THS2.2	45	22.20	26.30	27.50	29.10	32.80	27.54	2.37	0.35	8.59	26.83	28.25	-	***	
198	Cess	46	23.20	24.90	26.25	27.40	33.60	26.54	2.14	0.31	8.05	25.91	27.18	-	***	
198	Switch	46	22.10	25.30	26.30	28.70	34.30	26.93	2.46	0.36	9.15	26.20	27.66	-	***	-
205	Sham	47	22.90	25.90	27.10	28.40	32.80	27.30	2.32	0.34	8.51	26.61	27.98			
205	3R4F	45	20.80	23.10	24.10	25.40	31.60	24.33	1.99	0.30	8.18	23.73	24.92	***		
205	THS2.2	45	21.80	26.30	27.20	28.60	33.70	27.56	2.43	0.36	8.81	26.83	28.29	-	***	
205	Cess	46	23.60	25.50	26.30	27.60	34.30	26.86	2.28	0.34	8.49	26.18	27.53	-	***	
205	Switch	46	21.30	25.50	27.00	28.70	35.60	26.97	2.64	0.39	9.80	26.19	27.76	-	***	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
212	Sham	46	22.70	25.70	27.05	29.60	34.40	27.55	2.70	0.40	9.81	26.75	28.36			
212	3R4F	45	21.30	22.90	24.00	25.00	31.80	24.30	2.11	0.31	8.67	23.67	24.93	***		
212	THS2.2	45	22.50	26.80	27.70	28.80	33.60	27.85	2.39	0.36	8.57	27.13	28.57	-	***	
212	Cess	46	23.30	25.80	27.00	28.00	33.40	27.17	2.12	0.31	7.82	26.54	27.80	-	***	
212	Switch	46	21.30	25.60	27.05	29.30	35.20	27.40	2.66	0.39	9.72	26.61	28.19	-	***	-
219	Sham	45	22.00	26.50	27.90	29.20	33.80	28.09	2.61	0.39	9.28	27.31	28.87			
219	3R4F	45	21.30	23.10	24.00	25.30	32.20	24.35	2.03	0.30	8.35	23.74	24.96	***		
219	THS2.2	45	23.20	26.00	27.40	29.30	35.00	27.75	2.51	0.37	9.05	26.99	28.50	-	***	
219	Cess	46	22.40	25.30	26.50	27.60	33.60	26.80	2.17	0.32	8.09	26.15	27.44	*	***	
219	Switch	46	22.50	26.00	27.35	29.10	35.80	27.64	2.60	0.38	9.41	26.87	28.41	-	***	-
226	Sham	45	23.00	26.40	27.90	29.60	35.00	28.07	2.52	0.38	8.98	27.31	28.83			
226	3R4F	45	21.20	23.00	24.00	24.90	32.70	24.36	2.09	0.31	8.58	23.74	24.99	***		
226	THS2.2	44	23.30	26.30	28.40	29.50	34.80	28.17	2.72	0.41	9.66	27.34	29.00	-	***	
226	Cess	46	23.10	25.70	27.00	28.20	34.60	27.13	2.40	0.35	8.85	26.42	27.84	-	***	
226	Switch	46	22.30	26.10	27.50	29.20	33.40	27.72	2.68	0.39	9.65	26.93	28.52	-	***	-
233	Sham	39	23.30	26.40	28.10	29.40	34.60	28.10	2.75	0.44	9.78	27.21	28.99			
233	3R4F	39	21.20	22.80	24.30	25.40	31.60	24.41	2.10	0.34	8.62	23.72	25.09	***		
233	THS2.2	39	22.60	26.30	28.40	29.90	36.40	28.43	2.75	0.44	9.67	27.54	29.32	-	***	
233	Cess	40	23.30	25.65	26.65	28.65	36.20	27.33	2.77	0.44	10.12	26.45	28.21	-	***	
233	Switch	40	22.50	25.90	28.10	30.50	34.60	28.10	2.88	0.45	10.24	27.18	29.02	-	***	-

Table 18. (Continued)

Study Day	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
240	Sham	31	24.10	25.70	27.10	29.80	32.90	27.99	2.59	0.46	9.24	27.04	28.94			
240	3R4F	31	20.20	22.90	23.40	25.20	31.80	24.13	2.31	0.41	9.56	23.28	24.97	***		
240	THS2.2	31	23.20	26.90	28.40	29.40	34.70	28.43	2.65	0.48	9.34	27.46	29.40	-	***	
240	Cess	33	23.20	25.50	26.80	28.30	35.40	27.03	2.46	0.43	9.10	26.15	27.90	-	***	
240	Switch	33	23.90	25.60	27.40	29.70	34.10	27.72	2.67	0.46	9.62	26.78	28.67	-	***	-

Table 19. HPMA (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	661.39	939.97	1232.3	1585.4	2137.8	1289.3	485.16	171.53	37.63	883.70	1694.9			
2M	3R4F	9	1924.3	2253.7	2426.5	2479.9	3542.2	2513.3	540.68	180.23	21.51	2097.7	2928.9	***		
2M	THS2.2	7	628.32	882.36	1035.3	1626.7	1872.6	1175.8	437.61	165.40	37.22	771.04	1580.5	-	***	
3M	Sham	8	388.33	2373.3	2683.1	3082.0	3324.0	2498.6	931.99	329.51	37.30	1719.5	3277.8			
3M	3R4F	8	3177.4	4027.9	5284.0	6547.0	7545.7	5305.1	1608.8	568.81	30.33	3960.1	6650.1	**		
3M	THS2.2	8	1252.9	2261.2	2676.0	3693.3	4003.2	2814.7	939.31	332.10	33.37	2029.4	3599.9	-	**	
3M	Cess	8	1048.1	1633.8	2084.7	2599.4	4990.3	2334.3	1208.6	427.29	51.77	1323.9	3344.7	-	**	
3M	Switch	8	1848.9	1941.0	2287.9	3159.0	5087.3	2714.0	1120.0	395.96	41.27	1777.7	3650.3	-	**	(-)
6M	Sham	8	447.16	1377.5	2271.3	2652.1	4082.4	2141.4	1132.9	400.56	52.91	1194.3	3088.6			
6M	3R4F	8	5753.2	5995.6	6479.0	8314.7	11405	7342.1	2045.5	723.18	27.86	5632.1	9052.2	***		
6M	THS2.2	8	1484.5	2193.3	3090.5	4437.7	4927.6	3231.9	1339.8	473.68	41.45	2111.8	4352.0	-	***	
6M	Cess	9	1464.1	1726.2	2567.4	2805.4	3072.2	2323.4	653.88	217.96	28.14	1820.8	2826.0	-	***	
6M	Switch	8	1062.2	1436.2	1917.6	2719.8	2819.6	2003.6	686.25	242.62	34.25	1429.9	2577.4	-	***	(-)
8M	Sham	8	743.56	1168.3	1858.7	2497.0	2792.5	1823.0	770.63	272.46	42.27	1178.8	2467.3			
8M	3R4F	8	1942.0	3633.8	4731.7	4983.4	6075.5	4339.4	1260.5	445.67	29.05	3285.6	5393.3	***		
8M	THS2.2	8	1496.0	1905.9	2670.3	3598.8	5039.6	2860.7	1208.4	427.24	42.24	1850.5	3871.0	-	*	
8M	Cess	8	1365.7	1758.7	1971.6	2709.9	3023.9	2158.8	606.81	214.54	28.11	1651.4	2666.1	-	**	
8M	Switch	8	1320.0	1828.4	2241.6	3112.1	3370.0	2381.8	765.93	270.80	32.16	1741.5	3022.1	-	**	-

Table 20. NNAL (absolute, pg)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.21	0.22	0.26	0.30	0.39	0.27	0.06	0.02	22.79	0.22	0.32			
2M	3R4F	9	55.99	96.64	116.48	137.07	176.64	119.34	37.23	12.41	31.19	90.72	147.95	***		
2M	THS2.2	7	0.21	0.23	0.30	30.41	39.77	10.21	17.21	6.50	168.51	-5.70	26.13	(-)	***	
3M	Sham	8	0.63	0.85	0.91	0.99	1.10	0.90	0.14	0.05	15.54	0.79	1.02			
3M	3R4F	8	47.71	148.77	212.38	300.70	329.25	212.58	96.76	34.21	45.52	131.69	293.48	***		
3M	THS2.2	8	0.52	0.59	0.88	17.97	40.84	10.03	17.22	6.09	171.70	-4.37	24.43	-	***	
3M	Cess	8	0.39	0.60	0.74	0.90	1.10	0.74	0.22	0.08	29.93	0.56	0.93	-	***	
3M	Switch	8	0.50	0.73	0.85	46.32	64.21	20.06	28.04	9.92	139.79	-3.38	43.51	-	***	-
6M	Sham	8	0.17	0.29	0.46	0.73	49.32	6.56	17.28	6.11	263.61	-7.89	21.00			
6M	3R4F	8	298.93	508.54	714.54	912.17	1161.1	716.31	292.37	103.37	40.82	471.88	960.74	+++		
6M	THS2.2	8	25.65	30.64	59.56	102.75	129.64	67.65	41.65	14.72	61.56	32.83	102.47	++	***	
6M	Cess	9	0.39	0.45	0.55	0.71	34.11	7.61	14.09	4.70	185.05	-3.22	18.44	(-)	+++	
6M	Switch	8	0.52	0.62	14.75	46.75	51.77	22.07	22.74	8.04	103.05	3.06	41.08	+	***	(-)
8M	Sham	8	0.43	0.49	0.68	0.73	1.08	0.66	0.21	0.07	31.58	0.49	0.84			
8M	3R4F	8	80.28	129.14	163.91	187.20	197.21	154.75	39.64	14.02	25.62	121.61	187.89	***		
8M	THS2.2	8	0.40	0.53	0.77	1.00	22.35	3.42	7.65	2.71	223.92	-2.98	9.82	(-)	***	
8M	Cess	8	0.34	0.45	0.50	0.59	0.67	0.51	0.10	0.04	20.08	0.42	0.60	-	***	
8M	Switch	8	0.39	0.44	0.53	28.24	106.50	20.67	39.74	14.05	192.31	-12.56	53.89	(-)	***	(-)

Table 21. SPMA (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.49	0.60	0.79	1.11	1.52	0.88	0.35	0.13	40.51	0.58	1.17			
2M	3R4F	9	18.14	27.09	31.92	38.44	70.20	35.02	15.91	5.30	45.43	22.79	47.25	***		
2M	THS2.2	7	0.33	0.42	0.79	1.25	2.49	0.98	0.73	0.28	74.96	0.30	1.66	-	+++	
3M	Sham	8	0.07	0.52	0.92	1.17	1.82	0.89	0.55	0.19	61.35	0.43	1.35			
3M	3R4F	8	1.48	39.92	70.00	90.51	116.89	64.90	39.43	13.94	60.76	31.94	97.87	**		
3M	THS2.2	8	1.17	1.31	1.80	2.82	3.37	2.05	0.88	0.31	43.05	1.31	2.79	**	**	
3M	Cess	8	0.36	0.49	0.72	1.69	2.71	1.11	0.91	0.32	82.21	0.35	1.87	-	**	
3M	Switch	8	1.00	1.54	1.66	2.38	3.60	1.97	0.87	0.31	44.03	1.24	2.70	*	**	(-)
6M	Sham	8	0.26	0.75	0.92	1.17	1.62	0.94	0.40	0.14	42.73	0.61	1.28			
6M	3R4F	8	49.39	76.57	99.67	130.05	191.33	106.66	44.53	15.74	41.75	69.44	143.88	***		
6M	THS2.2	8	1.44	2.02	2.90	3.48	5.26	2.94	1.19	0.42	40.62	1.94	3.93	**	***	
6M	Cess	9	0.53	1.05	1.11	1.42	1.50	1.13	0.34	0.11	30.36	0.86	1.39	-	***	
6M	Switch	8	0.87	1.79	2.28	2.90	3.34	2.27	0.83	0.29	36.52	1.58	2.96	**	***	**
8M	Sham	8	0.45	0.97	1.69	2.22	2.47	1.59	0.73	0.26	46.19	0.97	2.20			
8M	3R4F	8	22.82	47.34	63.37	94.63	135.47	71.12	36.19	12.80	50.89	40.86	101.38	***		
8M	THS2.2	8	1.85	2.59	3.02	4.39	5.82	3.46	1.30	0.46	37.67	2.37	4.55	**	**	
8M	Cess	8	1.44	1.56	1.88	2.25	2.68	1.94	0.44	0.15	22.46	1.57	2.30	-	**	
8M	Switch	8	1.64	2.57	3.29	4.07	4.73	3.28	1.02	0.36	31.12	2.42	4.13	**	**	**

Table 22. CEMA (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.01	0.16	0.30	0.42	0.97	0.34	0.29	0.10	85.33	0.10	0.59			
2M	3R4F	9	69.22	106.47	119.31	149.55	272.56	133.11	62.49	20.83	46.95	85.08	181.15	+++		
2M	THS2.2	7	0.52	0.58	1.20	2.15	3.98	1.61	1.20	0.45	74.62	0.50	2.73	++	+++	
3M	Sham	8	0.02	0.95	1.25	1.50	1.82	1.16	0.56	0.20	48.04	0.69	1.62			
3M	3R4F	8	15.86	163.77	290.62	324.61	401.00	246.86	137.43	48.59	55.67	131.96	361.75	+++		
3M	THS2.2	8	1.36	2.98	4.12	5.75	7.51	4.32	1.98	0.70	45.85	2.66	5.97	**	**	
3M	Cess	8	1.95	2.40	2.87	5.08	11.44	4.26	3.24	1.15	76.12	1.55	6.97	*	**	
3M	Switch	8	4.40	6.42	7.41	7.65	11.89	7.41	2.11	0.75	28.50	5.64	9.17	***	**	+
6M	Sham	8	0.72	1.80	2.98	3.14	3.63	2.52	1.12	0.40	44.51	1.58	3.46			
6M	3R4F	8	183.14	314.80	401.02	512.94	744.14	423.10	170.55	60.30	40.31	280.52	565.68	***		
6M	THS2.2	8	4.39	5.82	7.54	10.37	12.14	8.00	2.79	0.99	34.94	5.66	10.34	***	***	
6M	Cess	9	1.94	2.11	2.73	3.42	5.70	3.00	1.16	0.39	38.72	2.11	3.90	-	***	
6M	Switch	8	1.63	4.19	5.66	8.93	10.17	6.17	2.98	1.05	48.34	3.68	8.66	*	***	*
8M	Sham	8	0.02	0.02	0.02	0.03	0.04	0.02	0.01	0.00	31.58	0.02	0.03			
8M	3R4F	8	57.28	140.30	198.28	298.33	394.61	215.71	110.42	39.04	51.19	123.40	308.03	***		
8M	THS2.2	8	4.31	4.70	5.60	9.03	12.56	6.94	3.21	1.14	46.28	4.26	9.63	+++	**	
8M	Cess	8	0.02	0.02	0.02	1.29	3.62	0.79	1.45	0.51	184.13	-0.42	2.00	(-)	+++	
8M	Switch	8	0.02	4.21	7.36	9.80	10.58	6.67	3.67	1.30	55.06	3.60	9.74	**	**	**

Table 23. 8-OHdG (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	10.76	13.65	16.54	25.07	32.97	19.28	7.96	2.81	41.26	12.63	25.93			
2M	3R4F	9	7.75	19.47	20.64	23.68	33.33	20.79	6.68	2.23	32.12	15.66	25.92	-		
2M	THS2.2	7	8.33	14.91	15.43	24.52	25.60	17.28	6.00	2.27	34.72	11.73	22.83	-	-	
3M	Sham	8	5.09	41.93	44.70	48.77	52.43	41.04	15.05	5.32	36.68	28.46	53.63			
3M	3R4F	8	25.33	36.80	42.80	57.46	97.26	49.59	22.22	7.85	44.80	31.01	68.16	-		
3M	THS2.2	8	24.68	35.22	40.00	55.07	68.36	44.20	14.97	5.29	33.86	31.69	56.72	-	-	
3M	Cess	8	22.16	31.21	41.32	43.14	77.29	41.35	16.50	5.83	39.91	27.55	55.14	-	(-)	
3M	Switch	8	24.76	30.44	40.29	57.62	71.49	44.12	16.77	5.93	38.00	30.10	58.14	-	-	-
6M	Sham	8	9.94	21.78	37.55	45.22	66.48	35.69	17.92	6.33	50.20	20.71	50.67			
6M	3R4F	8	29.79	43.24	51.31	55.56	64.29	49.29	10.92	3.86	22.15	40.16	58.42	-		
6M	THS2.2	8	17.05	24.02	38.62	47.50	60.15	37.19	14.80	5.23	39.81	24.81	49.56	-	-	
6M	Cess	9	25.95	35.62	43.15	45.20	47.26	40.19	7.14	2.38	17.77	34.70	45.68	-	-	
6M	Switch	8	14.54	18.10	25.77	44.00	56.06	30.79	16.41	5.80	53.30	17.07	44.51	-	*	-
8M	Sham	8	19.34	25.69	35.00	43.91	59.36	35.99	13.02	4.60	36.18	25.10	46.87			
8M	3R4F	8	22.23	37.85	43.36	52.40	60.70	43.77	12.16	4.30	27.78	33.61	53.93	-		
8M	THS2.2	8	29.74	30.99	47.44	55.90	70.36	46.09	14.77	5.22	32.04	33.75	58.44	-	-	
8M	Cess	8	27.72	36.61	39.44	53.38	71.57	44.77	14.04	4.96	31.37	33.03	56.51	-	-	
8M	Switch	8	31.81	35.51	41.52	51.65	55.98	43.14	9.13	3.23	21.17	35.51	50.78	-	-	-

Table 24. MDA (absolute, pmol)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	1207.2	1428.1	2014.3	2395.6	2914.5	1974.7	610.84	215.96	30.93	1464.0	2485.4			
2M	3R4F	9	1302.6	1986.6	2475.2	2849.3	3572.2	2443.2	679.40	226.47	27.81	1921.0	2965.5	-		
2M	THS2.2	7	1377.1	1481.9	1636.6	2109.8	2262.9	1726.2	331.31	125.22	19.19	1419.8	2032.6	-	*	
3M	Sham	8	498.88	4220.0	5150.3	5787.4	7126.2	4742.6	1960.3	693.07	41.33	3103.7	6381.4			
3M	3R4F	8	3581.0	4909.3	6459.5	6932.7	13194	6672.3	2899.5	1025.1	43.46	4248.3	9096.4	-		
3M	THS2.2	8	1576.7	3787.5	3833.9	5508.6	6473.2	4288.7	1597.6	564.84	37.25	2953.1	5624.4	-	-	
3M	Cess	8	1516.6	3303.0	4404.0	5271.5	9765.0	4654.9	2448.7	865.73	52.60	2607.7	6702.0	-	(-)	
3M	Switch	8	1905.4	2413.8	3437.3	6504.8	7105.1	4215.3	2164.8	765.38	51.36	2405.5	6025.1	-	-	-
6M	Sham	8	576.42	1983.9	3345.7	4352.3	7472.7	3426.6	2118.0	748.84	61.81	1655.9	5197.3			
6M	3R4F	8	5083.3	5678.8	7381.2	7776.3	11288	7255.6	1959.8	692.88	27.01	5617.2	8894.0	**		
6M	THS2.2	8	1713.4	2552.7	4192.0	5111.9	7073.6	4062.5	1817.6	642.62	44.74	2543.0	5582.1	-	**	
6M	Cess	9	1635.1	2434.3	3033.5	3327.7	5053.5	3124.1	1087.2	362.39	34.80	2288.4	3959.8	-	***	
6M	Switch	8	1571.3	1767.0	2104.4	3553.8	4857.7	2659.9	1222.0	432.05	45.94	1638.3	3681.6	-	+++	-
8M	Sham	8	1572.5	2203.7	2963.7	3873.4	5400.5	3131.8	1272.3	449.81	40.62	2068.2	4195.4			
8M	3R4F	8	2933.3	3781.8	4700.4	6790.1	8201.4	5209.9	1867.6	660.30	35.85	3648.5	6771.3	*		
8M	THS2.2	8	1757.3	2479.6	4033.1	4569.1	6167.4	3761.0	1472.6	520.66	39.16	2529.9	4992.2	-	-	
8M	Cess	8	2071.5	2965.4	3184.9	4773.7	5618.0	3692.2	1245.0	440.18	33.72	2651.3	4733.0	-	-	
8M	Switch	8	1183.0	2383.0	2670.3	4232.0	5588.4	3167.7	1409.2	498.21	44.48	1989.6	4345.8	-	*	-

Table 25. 4-HNE (absolute, pmol)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	13.00	15.05	21.18	38.17	61.67	27.93	17.11	6.05	61.26	13.63	42.24			
2M	3R4F	9	11.87	25.00	31.68	50.18	78.97	39.06	21.36	7.12	54.67	22.65	55.48	(-)		
2M	THS2.2	7	5.79	20.63	26.61	40.33	42.76	28.26	13.60	5.14	48.13	15.68	40.84	-	-	
3M	Sham	8	4.50	24.33	39.38	63.93	81.38	42.64	26.17	9.25	61.36	20.77	64.52			
3M	3R4F	8	1.89	34.29	51.56	65.56	129.04	54.22	37.63	13.31	69.41	22.76	85.68	-		
3M	THS2.2	8	7.71	21.86	32.36	39.77	47.64	30.42	13.21	4.67	43.42	19.38	41.46	-	-	
3M	Cess	8	12.64	25.65	34.22	39.04	51.01	32.68	11.56	4.09	35.36	23.02	42.34	-	-	
3M	Switch	8	4.22	13.08	27.49	44.91	50.87	28.25	18.12	6.41	64.12	13.11	43.40	-	-	-
6M	Sham	8	0.33	0.78	11.54	27.47	36.49	14.55	14.63	5.17	100.58	2.32	26.78			
6M	3R4F	8	1.07	32.32	59.18	85.96	127.85	60.48	39.68	14.03	65.61	27.30	93.66	*		
6M	THS2.2	8	1.09	5.82	22.31	34.75	43.08	21.24	16.00	5.66	75.32	7.87	34.62	-	*	
6M	Cess	9	0.76	7.80	15.83	38.11	63.96	24.97	21.34	7.11	85.46	8.57	41.37	-	*	
6M	Switch	8	0.72	9.23	15.03	28.93	34.45	17.69	11.95	4.23	67.56	7.70	27.69	-	*	-
8M	Sham	8	12.77	17.10	27.41	33.75	42.83	26.52	10.28	3.64	38.79	17.92	35.11			
8M	3R4F	8	24.60	39.47	45.96	76.91	89.32	54.82	23.17	8.19	42.27	35.45	74.20	*		
8M	THS2.2	8	10.45	23.01	39.99	60.08	78.58	41.90	24.63	8.71	58.77	21.31	62.49	-	-	
8M	Cess	8	16.81	24.24	31.48	46.06	63.88	35.53	15.47	5.47	43.54	22.60	48.47	-	-	
8M	Switch	8	7.78	13.06	22.55	29.67	54.81	24.14	15.14	5.35	62.73	11.48	36.80	-	**	-

Table 26. t-PGDM (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	22.79	0.00	0.01			
2M	3R4F	9	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	24.53	0.01	0.01	*		
2M	THS2.2	7	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	21.13	0.00	0.01	-	-	
3M	Sham	8	0.01	0.02	0.02	0.02	0.02	0.02	0.00	0.00	15.54	0.02	0.02			
3M	3R4F	8	0.01	0.02	0.02	6.28	12.56	3.15	5.80	2.05	183.92	-1.70	8.00	+		
3M	THS2.2	8	0.01	0.01	0.02	0.02	21.83	2.74	7.71	2.73	281.34	-3.71	9.19	(-)	(-)	
3M	Cess	8	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	29.93	0.01	0.02	-	++	
3M	Switch	8	0.01	0.01	0.01	0.01	20.23	2.54	7.15	2.53	281.41	-3.43	8.51	+	+	(-)
6M	Sham	8	0.01	0.01	1.07	3.04	4.50	1.59	1.73	0.61	108.76	0.14	3.04			
6M	3R4F	8	0.01	2.28	4.78	8.01	9.72	4.98	3.43	1.21	68.75	2.12	7.85	*		
6M	THS2.2	8	0.01	0.02	2.01	3.73	4.04	1.94	1.79	0.63	91.84	0.45	3.44	(-)	*	
6M	Cess	9	0.01	0.01	0.01	2.87	3.90	1.36	1.66	0.55	121.70	0.09	2.63	(-)	+	
6M	Switch	8	0.01	0.01	0.01	0.63	3.90	0.65	1.38	0.49	211.89	-0.50	1.81	(-)	**	(-)
8M	Sham	8	2.59	3.92	5.59	9.05	23.32	7.88	6.91	2.44	87.77	2.10	13.66			
8M	3R4F	8	0.00	4.67	6.76	10.73	12.34	7.08	4.21	1.49	59.45	3.56	10.60	-		
8M	THS2.2	8	3.73	5.66	8.34	9.65	13.20	8.03	3.04	1.07	37.83	5.49	10.57	-	-	
8M	Cess	8	4.72	6.09	7.89	8.29	10.29	7.44	1.74	0.61	23.34	5.99	8.90	-	-	
8M	Switch	8	0.01	3.29	6.25	8.30	11.10	5.85	3.63	1.28	62.08	2.81	8.89	-	-	-

Table 27. t-PGEM (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.00	0.01	6.79	12.16	17.39	6.91	6.75	2.39	97.66	1.27	12.56			
2M	3R4F	9	0.01	11.66	13.38	16.74	36.93	15.67	10.92	3.64	69.70	7.27	24.06	-		
2M	THS2.2	7	0.00	0.00	0.01	12.00	44.83	9.23	16.42	6.21	177.95	-5.96	24.41	-	(-)	
3M	Sham	8	0.01	0.02	9.09	31.14	40.28	15.10	17.19	6.08	113.84	0.73	29.47			
3M	3R4F	8	0.01	0.02	9.51	32.18	35.53	14.87	16.66	5.89	112.02	0.94	28.79	(-)		
3M	THS2.2	8	0.01	0.01	10.29	26.25	41.91	14.38	16.53	5.84	114.95	0.56	28.19	(-)	(-)	
3M	Cess	8	0.01	0.02	11.14	27.18	46.85	15.44	17.30	6.12	112.01	0.98	29.90	(-)	(-)	
3M	Switch	8	0.01	0.01	0.01	0.01	30.26	3.79	10.69	3.78	281.89	-5.15	12.73	+	++	+
6M	Sham	8	0.00	0.01	0.01	0.01	0.02	0.01	0.00	0.00	49.34	0.01	0.01			
6M	3R4F	8	0.01	0.01	0.02	0.02	0.02	0.02	0.00	0.00	25.50	0.01	0.02	**		
6M	THS2.2	8	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	32.99	0.01	0.02	*	-	
6M	Cess	9	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	20.86	0.01	0.01	-	**	
6M	Switch	8	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	19.31	0.01	0.01	-	**	-
8M	Sham	8	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	31.58	0.01	0.02			
8M	3R4F	8	0.00	0.01	0.01	0.01	2.61	0.33	0.92	0.33	275.12	-0.44	1.10	(-)		
8M	THS2.2	8	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	34.01	0.01	0.02	-	(-)	
8M	Cess	8	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	20.08	0.01	0.01	-	(-)	
8M	Switch	8	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	23.52	0.01	0.01	-	(-)	-

Table 28. 2,3-d-8-i-PGF2a (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.30	0.76	1.24	1.65	2.60	1.28	0.72	0.25	56.42	0.67	1.88			
2M	3R4F	9	0.22	1.54	2.04	2.62	6.15	2.36	1.63	0.54	69.00	1.11	3.61	-		
2M	THS2.2	7	0.26	0.74	0.79	1.66	3.89	1.35	1.21	0.46	89.06	0.24	2.47	-	(-)	
3M	Sham	8	0.51	1.00	1.57	2.07	2.26	1.51	0.67	0.24	44.72	0.94	2.07			
3M	3R4F	8	0.45	1.43	1.90	2.24	3.02	1.83	0.77	0.27	41.99	1.19	2.47	-		
3M	THS2.2	8	1.00	1.33	1.90	2.37	2.50	1.84	0.59	0.21	32.06	1.34	2.33	(-)	-	
3M	Cess	8	1.10	1.18	1.41	1.95	2.61	1.60	0.53	0.19	33.22	1.15	2.04	-	-	
3M	Switch	8	0.52	0.89	1.32	2.55	3.42	1.68	1.12	0.40	66.62	0.74	2.62	-	-	(-)
6M	Sham	8	0.49	1.72	2.94	4.66	5.14	3.03	1.71	0.60	56.33	1.60	4.46			
6M	3R4F	8	2.60	4.37	6.06	7.41	11.04	6.17	2.62	0.93	42.49	3.97	8.36	*		
6M	THS2.2	8	1.93	2.81	3.95	4.70	15.01	4.98	4.20	1.48	84.24	1.47	8.49	-	(-)	
6M	Cess	9	1.90	2.07	2.24	3.90	6.68	3.19	1.59	0.53	49.97	1.96	4.41	-	*	
6M	Switch	8	0.97	2.41	2.56	3.69	4.88	2.90	1.17	0.42	40.54	1.92	3.88	-	**	-
8M	Sham	8	1.57	2.53	3.36	4.26	5.63	3.44	1.30	0.46	37.84	2.35	4.52			
8M	3R4F	8	2.63	3.27	5.26	6.08	6.84	4.84	1.63	0.58	33.77	3.47	6.20	-		
8M	THS2.2	8	3.27	3.95	5.98	8.52	13.08	6.66	3.34	1.18	50.14	3.87	9.45	*	-	
8M	Cess	8	1.40	2.56	3.83	4.11	6.54	3.62	1.57	0.56	43.41	2.30	4.93	-	-	
8M	Switch	8	1.86	2.50	3.75	4.90	6.23	3.80	1.55	0.55	40.75	2.50	5.09	-	-	-

Table 29. 2,3-d-TXB2 (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	9.24	23.88	32.74	39.50	61.10	32.82	15.82	5.59	48.21	19.60	46.05			
2M	3R4F	9	3.71	27.22	33.86	37.95	124.14	44.04	36.33	12.11	82.50	16.11	71.97	-		
2M	THS2.2	7	4.29	14.72	20.47	52.25	123.35	39.73	40.53	15.32	102.02	2.24	77.22	-	(-)	
3M	Sham	8	8.50	39.22	54.99	60.53	65.93	47.99	18.71	6.62	38.99	32.35	63.63			
3M	3R4F	8	20.59	23.39	33.92	35.61	45.25	31.46	8.43	2.98	26.79	24.42	38.51	*		
3M	THS2.2	8	12.06	27.48	41.20	54.77	75.89	41.86	20.22	7.15	48.32	24.95	58.76	-	-	
3M	Cess	8	15.26	22.45	27.00	53.67	60.17	35.21	17.74	6.27	50.40	20.37	50.04	-	-	
3M	Switch	8	20.05	25.15	34.10	61.85	86.29	43.57	26.48	9.36	60.78	21.43	65.71	-	-	(-)
6M	Sham	8	36.52	59.45	90.15	121.13	137.18	89.39	36.98	13.08	41.37	58.48	120.31			
6M	3R4F	8	32.82	37.54	54.07	77.87	97.40	58.65	23.85	8.43	40.66	38.71	78.58	-		
6M	THS2.2	8	25.00	34.35	61.60	83.10	93.43	59.57	26.86	9.50	45.10	37.11	82.02	-	-	
6M	Cess	9	54.43	69.19	86.02	121.46	126.55	91.53	29.89	9.96	32.66	68.56	114.51	-	*	
6M	Switch	8	17.86	37.33	52.52	97.79	112.81	63.24	35.80	12.66	56.61	33.31	93.17	-	-	-
8M	Sham	8	42.20	50.23	68.82	90.97	115.92	72.27	26.51	9.37	36.68	50.11	94.43			
8M	3R4F	8	14.33	25.57	41.60	57.05	67.43	41.28	19.20	6.79	46.53	25.22	57.33	*		
8M	THS2.2	8	55.88	77.72	96.97	137.88	159.92	105.12	36.80	13.01	35.01	74.35	135.88	-	**	
8M	Cess	8	52.75	57.36	77.97	122.26	152.16	90.01	37.42	13.23	41.58	58.72	121.30	-	**	
8M	Switch	8	49.16	51.61	63.85	88.10	128.80	73.13	28.14	9.95	38.47	49.61	96.66	(-)	*	(-)

Table 30. 11-dh-TXB2 (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.17	0.25	0.30	0.37	0.42	0.30	0.09	0.03	28.37	0.23	0.37			
2M	3R4F	9	0.21	0.34	0.39	0.69	1.04	0.51	0.29	0.10	56.76	0.29	0.73	-		
2M	THS2.2	7	0.22	0.26	0.28	0.47	1.05	0.41	0.29	0.11	71.57	0.14	0.68	-	(-)	
3M	Sham	8	0.76	0.83	0.98	1.08	2.22	1.10	0.47	0.17	42.96	0.70	1.49			
3M	3R4F	8	0.76	0.86	1.26	1.67	2.05	1.30	0.47	0.17	36.02	0.91	1.69	-		
3M	THS2.2	8	0.48	0.55	0.75	0.96	1.04	0.76	0.23	0.08	30.11	0.57	0.95	-	*	
3M	Cess	8	0.51	0.60	0.69	1.18	1.74	0.90	0.43	0.15	48.16	0.54	1.26	(-)	-	
3M	Switch	8	0.40	0.66	0.81	1.09	1.40	0.87	0.33	0.12	38.04	0.59	1.14	(-)	-	-
6M	Sham	8	0.11	0.35	0.47	0.59	0.84	0.47	0.22	0.08	47.55	0.28	0.66			
6M	3R4F	8	0.52	0.82	1.01	1.19	1.68	1.03	0.35	0.12	33.73	0.74	1.32	**		
6M	THS2.2	8	0.26	0.56	0.78	0.80	0.90	0.68	0.23	0.08	33.53	0.49	0.87	-	*	
6M	Cess	9	0.25	0.44	0.53	0.62	1.45	0.63	0.38	0.13	59.89	0.34	0.92	-	*	
6M	Switch	8	0.42	0.53	0.85	0.88	0.91	0.73	0.20	0.07	27.60	0.56	0.90	*	-	-
8M	Sham	8	0.20	0.32	0.69	1.23	2.17	0.86	0.69	0.24	80.70	0.28	1.44			
8M	3R4F	8	0.37	0.55	1.11	1.29	1.46	0.97	0.42	0.15	43.92	0.61	1.32	-		
8M	THS2.2	8	0.29	0.47	0.78	0.99	1.27	0.76	0.34	0.12	45.06	0.47	1.04	-	-	
8M	Cess	8	0.58	0.84	1.08	1.41	1.46	1.09	0.32	0.11	29.33	0.82	1.36	-	-	
8M	Switch	8	0.00	0.58	0.77	1.39	2.33	0.98	0.74	0.26	76.11	0.36	1.60	(-)	-	-

Table 31. PGF2a (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	1.05	1.61	2.36	2.86	3.40	2.26	0.80	0.28	35.47	1.59	2.93			
2M	3R4F	9	0.92	2.29	2.91	3.37	7.96	3.45	2.17	0.72	63.05	1.78	5.12	-		
2M	THS2.2	7	0.84	1.71	2.25	3.06	9.11	3.07	2.76	1.04	89.83	0.52	5.62	-	(-)	
3M	Sham	8	1.39	2.86	4.54	4.99	7.22	4.17	1.83	0.65	43.91	2.64	5.71			
3M	3R4F	8	2.05	2.16	3.67	4.84	7.03	3.80	1.77	0.63	46.60	2.32	5.28	-		
3M	THS2.2	8	1.68	2.71	3.24	4.08	6.36	3.51	1.40	0.49	39.74	2.35	4.68	-	-	
3M	Cess	8	1.01	1.58	2.44	4.52	5.71	2.98	1.82	0.64	61.18	1.45	4.50	-	-	
3M	Switch	8	2.56	2.93	3.63	4.20	4.90	3.62	0.80	0.28	22.09	2.95	4.29	-	-	-
6M	Sham	8	0.69	1.00	1.83	2.69	2.92	1.83	0.89	0.31	48.57	1.09	2.57			
6M	3R4F	8	2.17	2.52	3.05	3.61	3.92	3.05	0.67	0.24	21.93	2.49	3.61	**		
6M	THS2.2	8	0.85	1.72	2.33	2.47	2.64	2.06	0.63	0.22	30.45	1.54	2.59	-	**	
6M	Cess	9	1.01	1.55	2.12	3.36	5.32	2.61	1.35	0.45	51.84	1.57	3.64	-	-	
6M	Switch	8	1.00	1.65	2.03	3.09	3.37	2.24	0.86	0.30	38.37	1.52	2.96	-	-	-
8M	Sham	8	0.90	1.29	1.63	1.78	2.33	1.58	0.46	0.16	29.15	1.20	1.97			
8M	3R4F	8	0.66	0.80	1.31	1.56	2.12	1.27	0.50	0.18	39.56	0.85	1.68	-		
8M	THS2.2	8	0.81	1.24	1.95	1.99	2.64	1.73	0.59	0.21	34.16	1.23	2.22	-	-	
8M	Cess	8	0.72	0.93	1.62	2.45	2.68	1.68	0.79	0.28	47.18	1.02	2.34	-	-	
8M	Switch	8	0.69	0.92	1.82	2.07	2.66	1.62	0.70	0.25	43.48	1.03	2.21	-	-	-

Table 32. 8i-PGF2a (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.00	0.00	0.05	0.30	0.41	0.14	0.17	0.06	123.18	-0.00	0.28			
2M	3R4F	9	0.00	0.00	0.05	0.53	1.42	0.30	0.48	0.16	161.38	-0.07	0.67	(-)		
2M	THS2.2	7	0.04	0.09	0.24	0.32	0.36	0.22	0.11	0.04	52.66	0.11	0.32	-	-	
3M	Sham	8	0.00	0.67	0.96	1.05	1.09	0.81	0.39	0.14	47.72	0.49	1.13			
3M	3R4F	8	0.01	0.49	0.80	1.02	2.18	0.85	0.64	0.23	75.46	0.31	1.38	-		
3M	THS2.2	8	0.00	0.00	0.42	0.52	0.83	0.34	0.31	0.11	91.03	0.08	0.60	*	-	
3M	Cess	8	0.00	0.37	0.64	0.98	1.03	0.63	0.38	0.13	59.74	0.31	0.94	-	-	
3M	Switch	8	0.00	0.00	0.18	0.72	0.89	0.34	0.39	0.14	114.54	0.01	0.66	*	-	-
6M	Sham	8	0.00	0.00	0.01	0.25	0.53	0.13	0.21	0.07	160.04	-0.04	0.31			
6M	3R4F	8	0.00	0.01	0.01	0.44	0.80	0.21	0.31	0.11	143.90	-0.04	0.47	(-)		
6M	THS2.2	8	0.00	0.00	0.01	0.05	0.17	0.04	0.06	0.02	158.47	-0.01	0.09	(-)	(-)	
6M	Cess	9	0.00	0.00	0.05	0.18	0.57	0.14	0.19	0.06	134.68	-0.01	0.29	(-)	(-)	
6M	Switch	8	0.00	0.00	0.04	0.10	0.13	0.05	0.06	0.02	106.00	0.01	0.10	(-)	(-)	(-)
8M	Sham	8	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	31.58	0.00	0.01			
8M	3R4F	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.21	0.00	0.00	*		
8M	THS2.2	8	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	34.01	0.00	0.01	-	*	
8M	Cess	8	0.00	0.00	0.00	0.00	0.15	0.02	0.05	0.02	240.03	-0.02	0.07	(-)	(-)	
8M	Switch	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.52	0.00	0.00	-	-	(-)

Table 33. LTE4 (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.02	0.04	0.05	0.08	0.25	0.08	0.07	0.03	98.23	0.01	0.14			
2M	3R4F	9	0.00	0.04	0.06	0.10	0.29	0.09	0.09	0.03	96.52	0.02	0.16	(-)		
2M	THS2.2	7	0.00	0.03	0.05	0.07	0.36	0.09	0.12	0.05	137.89	-0.02	0.20	(-)	(-)	
3M	Sham	8	0.00	0.00	0.00	0.00	0.12	0.02	0.04	0.01	263.80	-0.02	0.05			
3M	3R4F	8	0.00	0.00	0.00	0.05	0.30	0.05	0.11	0.04	209.68	-0.04	0.14	+		
3M	THS2.2	8	0.00	0.00	0.05	0.12	0.26	0.08	0.09	0.03	124.11	-0.00	0.15	(-)	(-)	
3M	Cess	8	0.00	0.00	0.03	0.08	0.18	0.05	0.06	0.02	126.46	-0.00	0.10	(-)	(-)	
3M	Switch	8	0.00	0.00	0.07	0.19	1.21	0.22	0.41	0.15	189.24	-0.13	0.56	(-)	(-)	(-)
6M	Sham	8	0.00	0.02	0.03	0.04	0.08	0.03	0.02	0.01	70.91	0.01	0.05			
6M	3R4F	8	0.00	0.01	0.03	0.05	0.08	0.03	0.03	0.01	84.00	0.01	0.05	-		
6M	THS2.2	8	0.00	0.03	0.07	0.16	0.22	0.09	0.08	0.03	85.43	0.03	0.16	-	-	
6M	Cess	9	0.00	0.01	0.02	0.05	0.10	0.03	0.03	0.01	98.30	0.01	0.06	-	-	
6M	Switch	8	0.01	0.02	0.03	0.05	0.06	0.03	0.02	0.01	47.37	0.02	0.05	-	-	-
8M	Sham	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.58	0.00	0.00			
8M	3R4F	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.21	0.00	0.00	*		
8M	THS2.2	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34.01	0.00	0.00	-	*	
8M	Cess	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.08	0.00	0.00	-	-	
8M	Switch	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.52	0.00	0.00	-	-	-

Table 34. 12-HETE (absolute, ng)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
2M	Sham	8	0.02	0.03	0.05	0.07	0.09	0.05	0.02	0.01	48.78	0.03	0.07			
2M	3R4F	9	0.02	0.03	0.04	0.06	0.10	0.05	0.02	0.01	49.63	0.03	0.07	-		
2M	THS2.2	7	0.01	0.03	0.03	0.05	0.14	0.05	0.04	0.02	94.39	0.01	0.09	-	(-)	
3M	Sham	8	0.04	0.06	0.08	0.09	0.18	0.09	0.04	0.02	49.52	0.05	0.12			
3M	3R4F	8	0.00	0.03	0.08	0.11	0.11	0.07	0.05	0.02	66.60	0.03	0.11	-		
3M	THS2.2	8	0.00	0.03	0.06	0.08	0.13	0.06	0.04	0.01	64.59	0.03	0.09	-	-	
3M	Cess	8	0.00	0.04	0.06	0.07	0.09	0.05	0.03	0.01	50.58	0.03	0.08	-	-	
3M	Switch	8	0.00	0.06	0.07	0.18	0.35	0.12	0.12	0.04	99.26	0.02	0.22	(-)	-	-
6M	Sham	8	0.00	0.01	0.02	0.04	0.09	0.03	0.03	0.01	109.18	0.00	0.05			
6M	3R4F	8	0.00	0.00	0.00	0.07	0.22	0.05	0.08	0.03	174.20	-0.02	0.11	(-)		
6M	THS2.2	8	0.00	0.02	0.04	0.07	0.17	0.05	0.05	0.02	102.86	0.01	0.10	(-)	(-)	
6M	Cess	9	0.00	0.02	0.05	0.07	0.18	0.06	0.06	0.02	99.57	0.01	0.11	(-)	(-)	
6M	Switch	8	0.00	0.01	0.04	0.06	0.09	0.04	0.03	0.01	84.42	0.01	0.06	-	(-)	-
8M	Sham	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.58	0.00	0.00			
8M	3R4F	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.21	0.00	0.00	*		
8M	THS2.2	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34.01	0.00	0.00	-	*	
8M	Cess	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.08	0.00	0.00	-	-	
8M	Switch	8	0.00	0.00	0.00	0.00	0.13	0.02	0.05	0.02	272.57	-0.02	0.06	(-)	(-)	(-)

Table 35. Lung function, Resistance (cmH2O.s/ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.64	0.65	0.71	0.80	0.83	0.72	0.07	0.02	10.11	0.67	0.78			
1M	3R4F	10	0.51	0.64	0.67	0.71	0.77	0.67	0.07	0.02	10.10	0.62	0.71	-		
1M	THS2.2	10	0.58	0.61	0.69	0.71	0.80	0.68	0.08	0.02	11.13	0.63	0.73	-	-	
2M	Sham	10	0.61	0.71	0.80	0.89	0.94	0.80	0.11	0.03	13.77	0.72	0.88			
2M	3R4F	10	0.52	0.56	0.58	0.60	0.72	0.59	0.06	0.02	10.81	0.55	0.64	***		
2M	THS2.2	10	0.58	0.69	0.84	0.91	1.01	0.82	0.15	0.05	18.04	0.71	0.92	-	***	
3M	Sham	10	0.53	0.68	0.70	0.77	0.94	0.72	0.12	0.04	17.25	0.63	0.81			
3M	3R4F	10	0.52	0.58	0.64	0.74	0.82	0.66	0.10	0.03	15.34	0.59	0.73	-		
3M	THS2.2	10	0.60	0.66	0.74	0.78	1.00	0.74	0.12	0.04	15.55	0.66	0.82	-	-	
3M	Cess	10	0.52	0.61	0.67	0.69	0.73	0.65	0.06	0.02	9.15	0.61	0.69	-	-	
3M	Switch	10	0.58	0.63	0.65	0.77	0.90	0.69	0.10	0.03	14.71	0.62	0.76	-	-	-
6M	Sham	9	0.50	0.55	0.59	0.62	0.70	0.59	0.06	0.02	9.89	0.55	0.64			
6M	3R4F	10	0.48	0.50	0.57	0.64	0.67	0.58	0.07	0.02	11.62	0.53	0.62	-		
6M	THS2.2	10	0.51	0.60	0.68	0.71	0.75	0.65	0.08	0.03	12.30	0.60	0.71	-	*	
6M	Cess	10	0.49	0.49	0.55	0.66	0.70	0.58	0.08	0.03	13.87	0.52	0.63	-	-	
6M	Switch	10	0.51	0.56	0.61	0.69	0.84	0.63	0.10	0.03	15.48	0.56	0.70	-	-	-
8M	Sham	10	0.49	0.51	0.59	0.65	0.85	0.60	0.11	0.04	18.56	0.52	0.68			
8M	3R4F	10	0.49	0.54	0.60	0.63	0.75	0.60	0.08	0.02	13.07	0.55	0.66	-		
8M	THS2.2	9	0.39	0.47	0.58	0.66	0.76	0.57	0.12	0.04	21.58	0.47	0.66	-	-	
8M	Cess	10	0.44	0.52	0.54	0.60	0.66	0.55	0.07	0.02	13.24	0.50	0.60	-	-	
8M	Switch	9	0.45	0.47	0.53	0.62	0.83	0.57	0.12	0.04	21.88	0.47	0.66	(-)	-	-

Table 36. Lung function, elastance (cmH2O/ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	28.65	31.68	33.39	36.89	39.51	33.97	3.49	1.10	10.27	31.48	36.47			
1M	3R4F	10	21.82	26.99	29.06	30.89	35.58	28.95	3.70	1.17	12.80	26.30	31.60	**		
1M	THS2.2	10	24.43	27.28	30.21	34.08	40.24	30.88	4.79	1.51	15.50	27.46	34.30	-	-	
2M	Sham	10	28.72	33.18	37.85	45.84	48.04	39.25	6.73	2.13	17.13	34.44	44.07			
2M	3R4F	10	22.98	23.61	25.96	27.52	34.56	27.04	4.06	1.28	15.00	24.14	29.95	***		
2M	THS2.2	10	25.74	33.27	40.94	44.10	50.97	39.05	7.77	2.46	19.90	33.49	44.61	-	***	
3M	Sham	10	23.19	28.70	31.88	39.71	46.52	33.87	7.79	2.46	23.01	28.29	39.45			
3M	3R4F	10	22.37	25.97	29.60	33.26	38.82	29.66	5.59	1.77	18.83	25.66	33.65	-		
3M	THS2.2	10	28.03	29.20	32.91	38.16	49.54	34.67	6.82	2.16	19.68	29.79	39.54	-	-	
3M	Cess	10	24.15	25.99	30.28	30.95	33.72	29.33	3.15	0.99	10.72	27.08	31.58	-	-	
3M	Switch	10	24.87	25.93	26.70	35.70	40.16	29.92	5.62	1.78	18.79	25.90	33.94	(-)	-	-
6M	Sham	9	22.05	24.15	25.81	26.22	36.77	26.59	4.35	1.45	16.36	23.24	29.93			
6M	3R4F	10	21.40	23.10	24.77	27.70	30.98	25.44	3.27	1.03	12.86	23.10	27.78	(-)		
6M	THS2.2	10	21.03	25.84	32.93	34.07	37.20	30.49	5.50	1.74	18.05	26.55	34.42	-	*	
6M	Cess	10	19.84	21.99	25.99	31.71	34.64	26.56	4.96	1.57	18.68	23.01	30.11	-	-	
6M	Switch	10	22.65	25.15	25.45	30.45	37.69	27.67	4.84	1.53	17.48	24.21	31.13	(-)	(-)	-
8M	Sham	10	19.37	24.20	28.49	31.21	36.11	27.71	5.10	1.61	18.42	24.06	31.36			
8M	3R4F	10	19.69	23.18	25.08	28.26	35.14	25.56	4.52	1.43	17.68	22.33	28.80	-		
8M	THS2.2	9	19.47	20.83	23.19	28.89	33.88	24.92	5.32	1.77	21.36	20.83	29.01	-	-	
8M	Cess	10	21.65	22.32	23.45	27.72	33.28	25.34	3.95	1.25	15.60	22.52	28.17	-	-	
8M	Switch	9	20.21	22.05	22.68	26.02	35.76	25.13	5.12	1.71	20.36	21.20	29.06	-	-	(-)

Table 37. Lung function, compliance (ml/cmH2O)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	10.07	0.03	0.03			
1M	3R4F	10	0.03	0.03	0.03	0.04	0.05	0.04	0.00	0.00	13.65	0.03	0.04	**		
1M	THS2.2	10	0.02	0.03	0.03	0.04	0.04	0.03	0.00	0.00	14.86	0.03	0.04	-	-	
2M	Sham	10	0.02	0.02	0.03	0.03	0.04	0.03	0.00	0.00	17.90	0.02	0.03			
2M	3R4F	10	0.03	0.04	0.04	0.04	0.04	0.04	0.00	0.00	12.99	0.03	0.04	***		
2M	THS2.2	10	0.02	0.02	0.02	0.03	0.04	0.03	0.01	0.00	22.58	0.02	0.03	-	***	
3M	Sham	10	0.02	0.03	0.03	0.03	0.04	0.03	0.01	0.00	22.38	0.03	0.04			
3M	3R4F	10	0.03	0.03	0.03	0.04	0.04	0.03	0.01	0.00	18.63	0.03	0.04	-		
3M	THS2.2	10	0.02	0.03	0.03	0.03	0.04	0.03	0.01	0.00	17.33	0.03	0.03	-	-	
3M	Cess	10	0.03	0.03	0.03	0.04	0.04	0.03	0.00	0.00	11.07	0.03	0.04	-	-	
3M	Switch	10	0.03	0.03	0.04	0.04	0.04	0.03	0.01	0.00	16.62	0.03	0.04	-	-	-
6M	Sham	9	0.03	0.04	0.04	0.04	0.05	0.04	0.01	0.00	13.64	0.03	0.04			
6M	3R4F	10	0.03	0.04	0.04	0.04	0.05	0.04	0.00	0.00	12.23	0.04	0.04	-		
6M	THS2.2	10	0.03	0.03	0.03	0.04	0.05	0.03	0.01	0.00	20.30	0.03	0.04	-	*	
6M	Cess	10	0.03	0.03	0.04	0.05	0.05	0.04	0.01	0.00	18.43	0.03	0.04	-	-	
6M	Switch	10	0.03	0.03	0.04	0.04	0.04	0.04	0.01	0.00	15.17	0.03	0.04	(-)	-	-
8M	Sham	10	0.03	0.03	0.04	0.04	0.05	0.04	0.01	0.00	19.72	0.03	0.04			
8M	3R4F	10	0.03	0.04	0.04	0.04	0.05	0.04	0.01	0.00	16.91	0.04	0.05	-		
8M	THS2.2	9	0.03	0.03	0.04	0.05	0.05	0.04	0.01	0.00	19.43	0.04	0.05	-	-	
8M	Cess	10	0.03	0.04	0.04	0.04	0.05	0.04	0.01	0.00	14.11	0.04	0.04	-	-	
8M	Switch	9	0.03	0.04	0.04	0.05	0.05	0.04	0.01	0.00	17.29	0.04	0.05	-	-	(-)

Table 38. Lung function, Newtonian resistance (cmH2O.s/ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.28	0.31	0.35	0.39	0.40	0.35	0.05	0.01	13.07	0.31	0.38			
1M	3R4F	10	0.30	0.32	0.35	0.35	0.46	0.35	0.04	0.01	12.55	0.32	0.38	-		
1M	THS2.2	10	0.31	0.32	0.35	0.36	0.40	0.35	0.03	0.01	8.40	0.33	0.37	-	-	
2M	Sham	10	0.34	0.35	0.36	0.38	0.45	0.37	0.03	0.01	8.54	0.35	0.39			
2M	3R4F	10	0.30	0.32	0.32	0.33	0.35	0.32	0.02	0.01	5.26	0.31	0.34	***		
2M	THS2.2	10	0.30	0.34	0.40	0.43	0.49	0.39	0.06	0.02	14.49	0.35	0.43	-	**	
3M	Sham	10	0.30	0.31	0.33	0.37	0.42	0.34	0.04	0.01	11.16	0.31	0.37			
3M	3R4F	10	0.28	0.29	0.30	0.32	0.34	0.31	0.02	0.01	6.63	0.29	0.32	*		
3M	THS2.2	10	0.31	0.34	0.36	0.37	0.38	0.35	0.02	0.01	6.40	0.34	0.37	-	***	
3M	Cess	10	0.29	0.29	0.31	0.33	0.35	0.31	0.02	0.01	6.97	0.30	0.33	-	(-)	
3M	Switch	10	0.28	0.30	0.32	0.33	0.35	0.32	0.02	0.01	7.06	0.30	0.33	-	-	-
6M	Sham	9	0.26	0.29	0.30	0.31	0.33	0.30	0.02	0.01	6.44	0.29	0.32			
6M	3R4F	10	0.23	0.26	0.28	0.29	0.32	0.28	0.03	0.01	9.35	0.26	0.29	*		
6M	THS2.2	10	0.28	0.29	0.30	0.31	0.31	0.30	0.01	0.00	3.52	0.29	0.31	-	*	
6M	Cess	10	0.26	0.28	0.30	0.31	0.32	0.30	0.02	0.01	6.40	0.28	0.31	-	-	
6M	Switch	10	0.28	0.30	0.31	0.32	0.35	0.31	0.02	0.01	6.38	0.30	0.32	-	**	-
8M	Sham	10	0.19	0.27	0.30	0.32	0.37	0.29	0.05	0.02	16.53	0.26	0.33			
8M	3R4F	10	0.23	0.26	0.27	0.28	0.32	0.27	0.03	0.01	10.27	0.25	0.29	-		
8M	THS2.2	9	0.21	0.26	0.29	0.31	0.37	0.29	0.04	0.01	15.54	0.25	0.32	-	-	
8M	Cess	10	0.24	0.26	0.29	0.31	0.36	0.29	0.04	0.01	12.05	0.27	0.32	-	-	
8M	Switch	9	0.25	0.28	0.31	0.32	0.39	0.30	0.04	0.01	13.36	0.27	0.33	-	-	-

Table 39. Lung function, tissue resistance (cmH2O/ml/s)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	4.60	5.05	5.36	6.00	6.17	5.40	0.54	0.17	9.96	5.01	5.78			
1M	3R4F	10	3.56	4.35	4.49	5.03	5.59	4.58	0.57	0.18	12.39	4.18	4.99	**		
1M	THS2.2	10	3.97	4.36	4.80	5.10	6.11	4.79	0.60	0.19	12.49	4.36	5.22	*	-	
2M	Sham	10	3.58	4.76	5.75	6.86	7.89	5.70	1.33	0.42	23.28	4.75	6.65			
2M	3R4F	10	2.90	3.19	3.57	4.10	4.96	3.75	0.73	0.23	19.39	3.23	4.27	**		
2M	THS2.2	10	4.09	4.79	5.32	6.19	7.99	5.46	1.16	0.37	21.22	4.63	6.29	-	**	
3M	Sham	10	3.66	4.51	5.34	5.81	7.41	5.32	1.09	0.35	20.54	4.54	6.10			
3M	3R4F	10	3.39	4.09	4.80	6.10	6.94	4.99	1.24	0.39	24.84	4.10	5.87	-		
3M	THS2.2	10	3.03	4.19	5.00	5.98	8.00	5.18	1.45	0.46	28.08	4.14	6.22	-	-	
3M	Cess	10	3.84	4.39	4.60	5.56	5.86	4.86	0.72	0.23	14.81	4.34	5.37	-	-	
3M	Switch	10	3.86	4.40	4.73	6.29	7.24	5.16	1.15	0.36	22.27	4.33	5.98	-	-	-
6M	Sham	9	3.16	3.59	3.78	4.06	5.62	4.06	0.80	0.27	19.83	3.44	4.68			
6M	3R4F	10	3.05	3.17	4.40	4.94	5.62	4.27	1.02	0.32	23.92	3.54	5.00	-		
6M	THS2.2	10	3.25	4.53	5.61	5.92	6.11	5.10	1.05	0.33	20.56	4.35	5.85	*	-	
6M	Cess	10	2.84	3.18	4.00	5.38	5.73	4.19	1.13	0.36	26.96	3.38	4.99	-	(-)	
6M	Switch	10	3.29	3.52	4.36	5.65	7.52	4.67	1.35	0.43	28.81	3.71	5.63	(-)	-	-
8M	Sham	10	2.74	3.27	4.24	5.65	6.34	4.44	1.31	0.42	29.60	3.50	5.38			
8M	3R4F	10	2.79	4.29	4.83	5.04	6.67	4.69	1.07	0.34	22.87	3.93	5.46	-		
8M	THS2.2	9	2.54	3.24	4.24	5.37	5.38	4.23	1.06	0.35	25.15	3.41	5.05	-	-	
8M	Cess	10	2.74	3.33	3.66	5.23	5.26	4.03	0.97	0.31	24.08	3.33	4.72	(-)	-	
8M	Switch	9	2.63	3.21	3.27	4.53	6.33	3.87	1.26	0.42	32.55	2.91	4.84	-	-	(-)

Table 40. Lung function, tissue elastance (cmH2O/ml/s)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	27.62	29.12	31.43	33.36	38.98	31.93	3.73	1.18	11.68	29.26	34.59			
1M	3R4F	10	21.94	26.11	27.85	29.46	33.53	27.78	3.06	0.97	11.00	25.60	29.97	*		
1M	THS2.2	10	23.18	26.37	28.18	32.20	37.73	29.35	4.25	1.34	14.47	26.31	32.38	-	-	
2M	Sham	10	28.97	32.67	36.74	43.47	49.12	38.21	6.76	2.14	17.68	33.38	43.04			
2M	3R4F	10	22.38	23.42	25.96	27.76	33.38	26.72	3.77	1.19	14.10	24.02	29.42	***		
2M	THS2.2	10	24.91	32.83	39.07	42.26	51.84	37.85	7.86	2.48	20.76	32.23	43.47	-	**	
3M	Sham	10	22.15	26.54	30.93	38.29	45.14	32.70	8.09	2.56	24.75	26.91	38.49			
3M	3R4F	10	21.32	24.83	27.82	31.54	35.73	28.19	4.90	1.55	17.38	24.68	31.69	-		
3M	THS2.2	10	27.19	28.38	31.26	37.02	47.84	33.43	6.59	2.08	19.71	28.72	38.14	-	-	
3M	Cess	10	22.95	24.49	28.47	30.78	31.71	27.90	3.13	0.99	11.23	25.66	30.14	-	-	
3M	Switch	10	23.76	23.91	25.13	33.74	38.00	28.14	5.50	1.74	19.54	24.21	32.08	(-)	-	-
6M	Sham	9	20.46	23.03	23.63	26.24	36.14	25.48	4.61	1.54	18.08	21.94	29.02			
6M	3R4F	10	20.97	21.42	22.55	26.00	29.56	23.77	2.99	0.95	12.58	21.63	25.91	(-)		
6M	THS2.2	10	19.70	24.81	30.66	32.92	36.27	28.99	5.59	1.77	19.27	25.00	32.99	-	*	
6M	Cess	10	18.18	22.65	25.07	29.91	33.44	25.63	4.54	1.43	17.70	22.39	28.88	-	-	
6M	Switch	10	21.88	22.68	24.89	27.51	35.97	26.13	4.53	1.43	17.34	22.89	29.37	(-)	(-)	-
8M	Sham	10	18.45	22.72	26.98	30.28	34.23	26.68	4.71	1.49	17.64	23.31	30.04			
8M	3R4F	10	18.68	21.15	22.59	25.80	39.19	24.38	5.80	1.83	23.79	20.23	28.53	-		
8M	THS2.2	9	17.40	20.70	21.21	27.70	32.96	23.87	5.31	1.77	22.26	19.79	27.95	-	(-)	
8M	Cess	10	21.37	21.81	23.58	27.17	33.10	25.00	3.90	1.23	15.61	22.21	27.79	-	(-)	
8M	Switch	9	20.29	21.37	23.28	25.14	34.67	24.71	4.60	1.53	18.60	21.17	28.24	-	(-)	(-)

Table 41. Lung function, tissue hypersensitivity (G/H)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.16	0.16	0.17	0.17	0.19	0.17	0.01	0.00	6.27	0.16	0.18			
1M	3R4F	10	0.15	0.16	0.17	0.17	0.18	0.17	0.01	0.00	6.22	0.16	0.17	-		
1M	THS2.2	10	0.15	0.16	0.16	0.17	0.19	0.16	0.01	0.00	6.95	0.16	0.17	-	-	
2M	Sham	10	0.12	0.14	0.15	0.16	0.18	0.15	0.02	0.01	11.13	0.14	0.16			
2M	3R4F	10	0.11	0.12	0.14	0.16	0.16	0.14	0.02	0.01	13.11	0.13	0.15	-		
2M	THS2.2	10	0.13	0.14	0.14	0.15	0.16	0.14	0.01	0.00	8.04	0.14	0.15	-	-	
3M	Sham	10	0.13	0.16	0.16	0.17	0.19	0.17	0.02	0.01	9.75	0.15	0.18			
3M	3R4F	10	0.15	0.16	0.18	0.19	0.20	0.18	0.02	0.01	9.42	0.16	0.19	-		
3M	THS2.2	10	0.11	0.14	0.15	0.18	0.18	0.15	0.02	0.01	14.60	0.14	0.17	-	*	
3M	Cess	10	0.14	0.15	0.19	0.19	0.19	0.17	0.02	0.01	11.51	0.16	0.19	-	(-)	
3M	Switch	10	0.16	0.18	0.19	0.19	0.21	0.18	0.01	0.00	7.95	0.17	0.19	*	-	(-)
6M	Sham	9	0.14	0.15	0.16	0.17	0.18	0.16	0.02	0.01	9.49	0.15	0.17			
6M	3R4F	10	0.13	0.15	0.19	0.20	0.22	0.18	0.03	0.01	17.41	0.16	0.20	-		
6M	THS2.2	10	0.13	0.16	0.17	0.20	0.21	0.18	0.02	0.01	13.53	0.16	0.19	-	-	
6M	Cess	10	0.13	0.14	0.17	0.18	0.20	0.16	0.02	0.01	14.92	0.14	0.18	-	-	
6M	Switch	10	0.14	0.15	0.19	0.21	0.21	0.18	0.03	0.01	16.75	0.16	0.20	-	(-)	(-)
8M	Sham	10	0.12	0.13	0.18	0.18	0.23	0.17	0.03	0.01	20.58	0.14	0.19			
8M	3R4F	10	0.14	0.18	0.20	0.21	0.23	0.19	0.03	0.01	13.43	0.17	0.21	-		
8M	THS2.2	9	0.12	0.16	0.18	0.20	0.23	0.18	0.03	0.01	18.76	0.15	0.20	-	-	
8M	Cess	10	0.13	0.14	0.16	0.18	0.19	0.16	0.02	0.01	13.37	0.14	0.18	-	**	
8M	Switch	9	0.12	0.14	0.14	0.18	0.19	0.15	0.03	0.01	16.25	0.13	0.17	-	**	-

Table 42. Lung function, A (ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.51	0.55	0.58	0.63	0.64	0.58	0.05	0.01	7.96	0.55	0.62			
1M	3R4F	10	0.57	0.66	0.69	0.74	0.85	0.69	0.08	0.02	10.96	0.64	0.75	**		
1M	THS2.2	10	0.49	0.55	0.65	0.69	0.79	0.64	0.09	0.03	13.93	0.57	0.70	-	-	
2M	Sham	10	0.44	0.47	0.54	0.59	0.69	0.54	0.08	0.02	14.21	0.48	0.59			
2M	3R4F	10	0.60	0.68	0.77	0.82	1.01	0.77	0.13	0.04	16.47	0.68	0.86	***		
2M	THS2.2	10	0.42	0.45	0.51	0.59	0.73	0.53	0.10	0.03	19.15	0.46	0.60	-	***	
3M	Sham	10	0.47	0.51	0.65	0.69	0.83	0.62	0.12	0.04	19.17	0.54	0.71			
3M	3R4F	10	0.60	0.70	0.78	0.83	0.95	0.77	0.12	0.04	14.91	0.69	0.86	*		
3M	THS2.2	10	0.44	0.54	0.63	0.69	0.71	0.61	0.09	0.03	14.76	0.54	0.67	-	**	
3M	Cess	10	0.65	0.72	0.75	0.83	0.86	0.76	0.07	0.02	9.85	0.71	0.81	**	-	
3M	Switch	10	0.61	0.70	0.78	0.84	0.85	0.75	0.09	0.03	11.28	0.69	0.82	*	-	-
6M	Sham	9	0.57	0.70	0.76	0.78	0.80	0.73	0.07	0.02	10.14	0.67	0.79			
6M	3R4F	10	0.88	0.88	0.96	1.00	1.07	0.96	0.07	0.02	7.39	0.91	1.01	***		
6M	THS2.2	10	0.54	0.62	0.66	0.74	0.86	0.68	0.09	0.03	13.94	0.61	0.75	-	***	
6M	Cess	10	0.62	0.73	0.78	0.88	1.01	0.80	0.12	0.04	14.60	0.72	0.89	-	**	
6M	Switch	10	0.64	0.69	0.80	0.84	0.86	0.77	0.08	0.03	10.82	0.71	0.83	(-)	***	-
8M	Sham	10	0.59	0.63	0.69	0.76	0.84	0.71	0.09	0.03	12.47	0.65	0.77			
8M	3R4F	10	0.81	0.92	0.94	1.03	1.10	0.97	0.09	0.03	8.93	0.91	1.03	***		
8M	THS2.2	9	0.62	0.73	0.77	0.85	0.94	0.78	0.11	0.04	13.58	0.70	0.86	-	***	
8M	Cess	10	0.69	0.77	0.83	0.86	0.90	0.81	0.07	0.02	8.50	0.76	0.86	*	***	
8M	Switch	9	0.63	0.77	0.83	0.85	0.91	0.80	0.09	0.03	11.18	0.73	0.87	*	***	-

Table 43. Lung function, Salazar-Knowles equation (/cmH2O)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.12	0.12	0.13	0.13	0.14	0.13	0.01	0.00	5.43	0.12	0.13			
1M	3R4F	10	0.12	0.12	0.13	0.14	0.14	0.13	0.01	0.00	5.71	0.12	0.14	-		
1M	THS2.2	10	0.13	0.13	0.13	0.14	0.14	0.13	0.01	0.00	4.07	0.13	0.14	*	-	
2M	Sham	10	0.12	0.12	0.13	0.13	0.14	0.13	0.01	0.00	5.09	0.12	0.13			
2M	3R4F	10	0.13	0.13	0.14	0.14	0.15	0.13	0.01	0.00	4.08	0.13	0.14	*		
2M	THS2.2	10	0.12	0.13	0.13	0.14	0.14	0.13	0.01	0.00	5.62	0.12	0.14	-	-	
3M	Sham	10	0.12	0.13	0.13	0.14	0.14	0.13	0.01	0.00	5.48	0.13	0.14			
3M	3R4F	10	0.12	0.12	0.13	0.13	0.14	0.13	0.01	0.00	5.90	0.12	0.13	-		
3M	THS2.2	10	0.12	0.13	0.13	0.14	0.15	0.13	0.01	0.00	6.42	0.12	0.14	-	-	
3M	Cess	10	0.12	0.13	0.13	0.13	0.13	0.13	0.00	0.00	3.01	0.12	0.13	-	-	
3M	Switch	10	0.12	0.12	0.13	0.13	0.14	0.13	0.01	0.00	5.40	0.12	0.13	-	-	-
6M	Sham	9	0.12	0.14	0.14	0.15	0.15	0.14	0.01	0.00	5.72	0.14	0.15			
6M	3R4F	10	0.11	0.12	0.13	0.14	0.15	0.13	0.01	0.00	8.99	0.12	0.14	*		
6M	THS2.2	10	0.12	0.12	0.13	0.14	0.15	0.13	0.01	0.00	8.06	0.13	0.14	*	-	
6M	Cess	10	0.12	0.12	0.13	0.14	0.15	0.13	0.01	0.00	7.23	0.13	0.14	-	(-)	
6M	Switch	10	0.12	0.13	0.13	0.14	0.15	0.13	0.01	0.00	5.93	0.13	0.14	*	-	-
8M	Sham	10	0.12	0.13	0.13	0.14	0.15	0.13	0.01	0.00	5.72	0.13	0.14			
8M	3R4F	10	0.11	0.12	0.13	0.14	0.15	0.13	0.01	0.00	11.11	0.12	0.14	-		
8M	THS2.2	9	0.12	0.14	0.14	0.15	0.15	0.14	0.01	0.00	7.40	0.13	0.15	-	-	
8M	Cess	10	0.12	0.13	0.14	0.14	0.15	0.14	0.01	0.00	6.01	0.13	0.14	-	-	
8M	Switch	9	0.12	0.13	0.14	0.14	0.16	0.14	0.01	0.00	8.93	0.13	0.15	-	-	-

Table 44. Lung function, quasi-static compliance (ml/cmH2O)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.05	0.05	0.06	0.06	0.07	0.06	0.01	0.00	9.41	0.06	0.06			
1M	3R4F	10	0.05	0.07	0.07	0.08	0.09	0.07	0.01	0.00	15.87	0.06	0.08	**		
1M	THS2.2	10	0.05	0.06	0.07	0.07	0.08	0.07	0.01	0.00	16.10	0.06	0.07	-	-	
2M	Sham	10	0.04	0.05	0.05	0.06	0.08	0.05	0.01	0.00	17.84	0.05	0.06			
2M	3R4F	10	0.06	0.08	0.08	0.09	0.11	0.08	0.01	0.00	17.66	0.07	0.09	***		
2M	THS2.2	10	0.04	0.04	0.05	0.06	0.08	0.05	0.01	0.00	24.39	0.05	0.06	-	***	
3M	Sham	10	0.05	0.05	0.07	0.07	0.09	0.06	0.01	0.00	21.98	0.05	0.07			
3M	3R4F	10	0.06	0.07	0.08	0.09	0.11	0.08	0.02	0.00	19.42	0.07	0.09	*		
3M	THS2.2	10	0.04	0.05	0.07	0.07	0.08	0.06	0.01	0.00	18.50	0.05	0.07	-	*	
3M	Cess	10	0.07	0.07	0.08	0.09	0.09	0.08	0.01	0.00	10.01	0.07	0.08	*	-	
3M	Switch	10	0.06	0.07	0.08	0.08	0.09	0.08	0.01	0.00	13.52	0.07	0.09	*	-	-
6M	Sham	9	0.06	0.08	0.08	0.09	0.09	0.08	0.01	0.00	12.45	0.07	0.09			
6M	3R4F	10	0.08	0.09	0.10	0.11	0.12	0.10	0.01	0.00	12.26	0.09	0.11	***		
6M	THS2.2	10	0.05	0.06	0.07	0.08	0.10	0.07	0.01	0.00	18.47	0.06	0.08	-	***	
6M	Cess	10	0.06	0.07	0.09	0.10	0.11	0.09	0.01	0.00	16.85	0.08	0.10	-	*	
6M	Switch	10	0.06	0.07	0.09	0.09	0.10	0.08	0.01	0.00	12.77	0.07	0.09	-	**	-
8M	Sham	10	0.06	0.07	0.07	0.09	0.09	0.07	0.01	0.00	15.09	0.07	0.08			
8M	3R4F	10	0.08	0.10	0.10	0.11	0.12	0.10	0.01	0.00	13.25	0.09	0.11	***		
8M	THS2.2	9	0.06	0.08	0.08	0.10	0.10	0.08	0.01	0.00	17.31	0.07	0.10	-	*	
8M	Cess	10	0.07	0.08	0.09	0.09	0.10	0.09	0.01	0.00	11.16	0.08	0.09	**	*	
8M	Switch	9	0.06	0.08	0.09	0.09	0.11	0.09	0.02	0.01	17.11	0.08	0.10	*	-	-

Table 45. Lung function, quasi-static elastance (cmH2O/ml).

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	14.89	16.01	16.76	18.84	19.23	17.07	1.61	0.51	9.42	15.92	18.22			
1M	3R4F	10	10.61	13.07	13.94	15.07	18.56	14.23	2.28	0.72	16.02	12.60	15.86	**		
1M	THS2.2	10	11.80	13.62	14.77	17.66	20.13	15.35	2.57	0.81	16.72	13.52	17.19	-	-	
2M	Sham	10	13.27	16.62	18.91	21.18	23.12	18.94	3.07	0.97	16.23	16.74	21.14			
2M	3R4F	10	9.41	11.38	12.13	13.21	16.66	12.52	2.38	0.75	19.02	10.82	14.23	***		
2M	THS2.2	10	12.65	15.51	19.62	23.05	23.89	19.29	4.12	1.30	21.39	16.33	22.24	-	***	
3M	Sham	10	11.23	13.67	15.04	19.43	21.97	16.25	3.75	1.18	23.05	13.57	18.93			
3M	3R4F	10	9.46	11.73	12.60	13.68	17.34	12.85	2.60	0.82	20.27	10.98	14.71	*		
3M	THS2.2	10	12.77	13.40	15.24	18.55	23.31	16.38	3.37	1.06	20.55	13.97	18.79	-	*	
3M	Cess	10	11.25	11.75	12.79	14.18	14.97	12.98	1.34	0.42	10.31	12.02	13.93	*	-	
3M	Switch	10	10.85	11.89	12.37	14.21	17.32	13.18	2.02	0.64	15.30	11.74	14.62	*	(-)	-
6M	Sham	9	11.15	11.73	12.14	13.29	16.95	12.79	1.85	0.62	14.49	11.37	14.22			
6M	3R4F	10	8.32	9.25	9.84	11.11	12.01	10.03	1.27	0.40	12.66	9.12	10.94	**		
6M	THS2.2	10	10.45	12.58	15.09	15.72	19.09	14.51	2.52	0.80	17.36	12.71	16.31	-	***	
6M	Cess	10	8.96	10.43	11.71	13.64	16.20	12.00	2.09	0.66	17.43	10.50	13.49	-	*	
6M	Switch	10	10.46	11.33	11.77	13.87	15.50	12.51	1.71	0.54	13.71	11.28	13.73	(-)	**	-
8M	Sham	10	11.07	11.74	14.11	15.27	17.84	13.87	2.13	0.67	15.35	12.35	15.39			
8M	3R4F	10	8.23	9.39	10.03	10.51	13.21	10.03	1.42	0.45	14.13	9.01	11.04	***		
8M	THS2.2	9	10.05	10.21	12.60	12.90	16.06	12.22	2.25	0.75	18.44	10.49	13.96	-	*	
8M	Cess	10	9.67	10.91	11.24	12.05	14.53	11.57	1.40	0.44	12.06	10.57	12.57	*	++	
8M	Switch	9	9.14	10.77	11.13	11.89	16.17	11.67	2.20	0.73	18.83	9.98	13.36	*	-	(-)

Table 46. Area in PV loops (hysteresis) (cmH2O.ml).

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	1.45	1.55	1.71	1.74	2.05	1.68	0.17	0.05	9.86	1.56	1.80			
1M	3R4F	10	1.58	1.66	1.89	1.97	2.26	1.87	0.22	0.07	11.75	1.71	2.03	*		
1M	THS2.2	10	1.37	1.60	1.92	2.16	2.76	1.92	0.40	0.13	20.95	1.64	2.21	-	-	
2M	Sham	10	1.42	1.73	1.85	2.00	2.23	1.84	0.26	0.08	14.20	1.65	2.03			
2M	3R4F	10	2.19	2.29	2.36	2.51	2.75	2.42	0.18	0.06	7.36	2.29	2.54	***		
2M	THS2.2	10	1.45	1.53	1.74	2.02	2.38	1.82	0.34	0.11	18.48	1.58	2.06	-	***	
3M	Sham	10	1.51	1.74	1.95	2.13	2.60	1.96	0.31	0.10	15.84	1.74	2.19			
3M	3R4F	10	2.06	2.15	2.53	2.63	2.95	2.47	0.31	0.10	12.44	2.25	2.69	**		
3M	THS2.2	10	1.46	1.75	1.95	2.02	2.42	1.95	0.30	0.10	15.54	1.73	2.16	-	**	
3M	Cess	10	2.10	2.16	2.28	2.51	2.85	2.35	0.24	0.08	10.30	2.18	2.52	**	-	
3M	Switch	10	2.27	2.30	2.41	2.45	2.54	2.40	0.10	0.03	4.09	2.33	2.48	**	-	-
6M	Sham	9	1.72	2.03	2.14	2.32	2.48	2.14	0.26	0.09	12.36	1.93	2.34			
6M	3R4F	10	2.56	2.81	3.10	3.38	3.72	3.12	0.39	0.12	12.37	2.85	3.40	***		
6M	THS2.2	10	1.53	1.73	2.05	2.19	2.87	2.03	0.38	0.12	18.46	1.76	2.30	-	***	
6M	Cess	10	1.89	2.09	2.28	2.61	2.97	2.35	0.35	0.11	15.09	2.09	2.60	-	***	
6M	Switch	10	1.75	2.08	2.24	2.47	2.55	2.24	0.27	0.08	11.88	2.05	2.43	-	***	-
8M	Sham	10	1.61	1.81	2.04	2.23	2.81	2.04	0.36	0.11	17.55	1.79	2.30			
8M	3R4F	10	2.61	2.68	3.03	3.58	4.22	3.17	0.52	0.16	16.35	2.80	3.54	***		
8M	THS2.2	9	1.84	2.10	2.39	2.50	2.95	2.37	0.39	0.13	16.58	2.06	2.67	-	**	
8M	Cess	10	1.94	2.15	2.27	2.56	2.67	2.31	0.26	0.08	11.29	2.13	2.50	-	***	
8M	Switch	9	2.01	2.26	2.37	2.56	2.77	2.40	0.26	0.09	11.00	2.20	2.60	*	**	-

Table 47. Lung function, FEV0.1 (ml).

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.56	0.62	0.67	0.71	0.79	0.67	0.07	0.02	10.28	0.63	0.72			
1M	3R4F	10	0.68	0.73	0.79	0.82	1.03	0.80	0.10	0.03	12.40	0.73	0.87	**		
1M	THS2.2	10	0.59	0.63	0.73	0.84	0.97	0.75	0.13	0.04	16.80	0.66	0.83	-	-	
2M	Sham	10	0.38	0.59	0.62	0.70	0.84	0.63	0.13	0.04	20.45	0.54	0.72			
2M	3R4F	10	0.64	0.87	0.92	0.96	1.18	0.91	0.17	0.05	18.66	0.79	1.04	***		
2M	THS2.2	10	0.48	0.56	0.60	0.71	0.88	0.63	0.12	0.04	19.05	0.55	0.72	-	***	
3M	Sham	10	0.46	0.58	0.71	0.82	1.05	0.72	0.19	0.06	25.81	0.59	0.85			
3M	3R4F	10	0.64	0.77	0.92	1.02	1.18	0.91	0.18	0.06	19.73	0.78	1.04	*		
3M	THS2.2	10	0.46	0.63	0.75	0.81	0.83	0.71	0.12	0.04	17.49	0.62	0.80	-	**	
3M	Cess	9	0.60	0.82	0.84	0.95	1.07	0.87	0.13	0.04	15.46	0.77	0.98	-	-	
3M	Switch	10	0.46	0.79	0.90	0.99	1.05	0.86	0.17	0.05	19.55	0.74	0.98	-	-	-
6M	Sham	9	0.53	0.60	0.81	0.87	0.96	0.76	0.15	0.05	19.74	0.65	0.88			
6M	3R4F	10	0.73	1.03	1.08	1.21	1.31	1.08	0.16	0.05	15.10	0.97	1.20	***		
6M	THS2.2	10	0.63	0.72	0.77	0.85	1.03	0.79	0.13	0.04	16.03	0.70	0.88	-	***	
6M	Cess	10	0.61	0.67	0.83	0.92	1.18	0.84	0.18	0.06	21.29	0.71	0.97	-	**	
6M	Switch	10	0.68	0.79	0.89	0.94	1.09	0.88	0.11	0.04	12.97	0.80	0.96	-	**	-
8M	Sham	10	0.50	0.68	0.80	0.90	0.91	0.77	0.14	0.04	18.29	0.67	0.88			
8M	3R4F	10	0.85	1.04	1.06	1.11	1.25	1.07	0.12	0.04	10.88	0.99	1.15	***		
8M	THS2.2	9	0.70	0.82	0.93	0.99	1.08	0.90	0.12	0.04	13.59	0.81	1.00	*	**	
8M	Cess	10	0.31	0.87	0.91	0.95	0.99	0.83	0.21	0.07	24.96	0.69	0.98	(-)	**	
8M	Switch	7	0.61	0.72	0.85	0.90	0.96	0.81	0.12	0.04	14.59	0.70	0.92	-	***	(-)

Table 48. Lung function, FEV0.2 (ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.48	0.52	0.61	0.64	0.69	0.59	0.07	0.02	11.71	0.54	0.64			
1M	3R4F	10	0.58	0.66	0.71	0.79	0.98	0.73	0.11	0.04	15.20	0.65	0.81	**		
1M	THS2.2	10	0.49	0.56	0.65	0.77	0.88	0.66	0.13	0.04	19.10	0.57	0.75	-	-	
2M	Sham	10	0.29	0.55	0.61	0.71	0.85	0.61	0.16	0.05	26.23	0.50	0.73			
2M	3R4F	10	0.59	0.88	0.94	0.99	1.25	0.93	0.21	0.07	22.30	0.78	1.08	**		
2M	THS2.2	10	0.41	0.57	0.61	0.70	0.82	0.62	0.12	0.04	18.93	0.54	0.70	-	**	
3M	Sham	10	0.40	0.56	0.66	0.81	1.05	0.68	0.19	0.06	28.12	0.55	0.82			
3M	3R4F	10	0.58	0.69	0.90	1.04	1.20	0.89	0.22	0.07	24.42	0.74	1.05	*		
3M	THS2.2	10	0.39	0.64	0.69	0.82	0.86	0.68	0.16	0.05	23.04	0.57	0.80	-	*	
3M	Cess	9	0.60	0.76	0.84	0.88	1.07	0.84	0.14	0.05	16.60	0.73	0.95	-	-	
3M	Switch	10	0.46	0.76	0.85	0.94	1.08	0.84	0.18	0.06	21.58	0.71	0.97	-	-	-
6M	Sham	9	0.48	0.56	0.74	0.77	0.89	0.69	0.15	0.05	21.32	0.58	0.81			
6M	3R4F	10	0.72	0.95	1.01	1.16	1.25	1.03	0.15	0.05	14.78	0.92	1.14	***		
6M	THS2.2	10	0.55	0.62	0.70	0.78	0.99	0.72	0.14	0.04	19.55	0.62	0.82	-	***	
6M	Cess	10	0.57	0.63	0.76	0.82	1.13	0.78	0.17	0.05	21.89	0.66	0.90	-	**	
6M	Switch	10	0.59	0.71	0.82	0.87	1.04	0.81	0.13	0.04	15.88	0.71	0.90	-	**	-
8M	Sham	10	0.50	0.63	0.77	0.88	0.91	0.75	0.14	0.05	19.19	0.65	0.85			
8M	3R4F	10	0.95	1.07	1.12	1.18	1.25	1.12	0.09	0.03	8.12	1.05	1.18	***		
8M	THS2.2	9	0.64	0.83	0.89	0.98	1.04	0.89	0.12	0.04	13.86	0.80	0.99	*	***	
8M	Cess	10	0.29	0.82	0.88	0.90	0.94	0.80	0.20	0.06	24.98	0.66	0.94	(-)	***	
8M	Switch	7	0.65	0.66	0.82	0.88	0.88	0.79	0.10	0.04	12.58	0.70	0.88	(-)	***	(-)

Table 49. Lung function, FEV0.1/FVC (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	95.00	97.37	97.71	98.73	99.94	97.74	1.39	0.44	1.42	96.74	98.73			
1M	3R4F	10	97.35	98.77	99.49	99.63	99.96	99.19	0.81	0.26	0.81	98.61	99.76	*		
1M	THS2.2	10	94.37	96.49	99.35	99.72	99.77	98.37	1.94	0.61	1.97	96.99	99.76	-	(-)	
2M	Sham	10	88.79	96.73	97.87	99.18	99.75	97.15	3.15	1.00	3.24	94.90	99.41			
2M	3R4F	10	92.26	93.91	96.90	98.24	99.85	96.32	2.69	0.85	2.79	94.40	98.24	(-)		
2M	THS2.2	10	93.53	97.02	97.72	99.56	99.94	97.72	2.09	0.66	2.14	96.22	99.22	(-)	-	
3M	Sham	10	58.61	96.29	98.18	99.60	99.83	93.99	12.60	3.98	13.41	84.97	103.00			
3M	3R4F	10	96.51	97.11	97.89	98.45	99.32	97.87	0.94	0.30	0.96	97.20	98.54	(-)		
3M	THS2.2	10	90.81	95.27	98.21	98.69	99.70	96.86	2.87	0.91	2.96	94.81	98.91	(-)	-	
3M	Cess	9	68.98	99.06	99.27	99.51	99.68	95.91	10.11	3.37	10.54	88.15	103.68	(-)	+	
3M	Switch	10	64.12	96.98	98.29	99.17	99.92	94.82	10.84	3.43	11.43	87.06	102.57	(-)	(-)	(-)
6M	Sham	9	65.31	93.91	97.28	99.52	99.89	90.83	14.24	4.75	15.68	79.89	101.78			
6M	3R4F	10	65.83	98.80	99.37	99.85	99.96	96.01	10.62	3.36	11.06	88.41	103.60	(-)		
6M	THS2.2	10	95.28	98.63	99.38	99.93	99.95	98.71	1.68	0.53	1.70	97.51	99.91	(-)	(-)	
6M	Cess	10	66.57	95.92	99.42	99.88	99.96	92.61	13.77	4.36	14.87	82.76	102.47	(-)	(-)	
6M	Switch	10	94.77	98.97	99.62	99.83	99.93	98.89	1.67	0.53	1.69	97.69	100.08	(-)	(-)	(-)
8M	Sham	10	66.24	93.98	98.25	98.97	99.91	94.29	10.20	3.23	10.82	86.99	101.59			
8M	3R4F	10	74.02	90.52	96.50	99.61	99.82	93.76	8.07	2.55	8.61	87.98	99.53	(-)		
8M	THS2.2	9	92.34	94.15	98.22	99.75	99.97	97.13	2.93	0.98	3.02	94.87	99.38	(-)	(-)	
8M	Cess	10	33.44	91.95	99.37	99.86	99.95	88.82	22.15	7.00	24.93	72.98	104.66	(-)	(-)	
8M	Switch	7	60.53	93.55	99.75	99.94	99.96	93.06	14.53	5.49	15.62	79.62	106.50	(-)	(-)	(-)

Table 50. Lung function, FEV0.2/FVC (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	82.29	82.69	85.37	86.96	92.49	85.97	3.77	1.19	4.39	83.27	88.67			
1M	3R4F	10	83.45	89.60	90.32	92.93	95.69	90.69	3.35	1.06	3.70	88.29	93.09	**		
1M	THS2.2	10	79.93	83.98	88.35	90.04	90.75	87.15	3.72	1.18	4.27	84.49	89.81	-	*	
2M	Sham	10	68.12	87.75	98.99	99.33	99.55	93.22	10.25	3.24	10.99	85.89	100.55			
2M	3R4F	10	90.82	93.77	99.43	99.85	99.97	97.29	3.85	1.22	3.96	94.53	100.04	(-)		
2M	THS2.2	10	81.53	93.70	98.56	99.30	99.73	96.02	5.71	1.81	5.95	91.94	100.10	(-)	(-)	
3M	Sham	10	61.46	85.93	93.19	99.17	99.28	89.04	12.74	4.03	14.30	79.93	98.15			
3M	3R4F	10	86.98	87.88	98.97	99.69	99.84	95.01	5.89	1.86	6.20	90.79	99.22	(-)		
3M	THS2.2	10	76.23	88.02	98.31	99.45	99.93	92.64	9.22	2.92	9.95	86.05	99.24	(-)	(-)	
3M	Cess	9	68.97	89.67	93.67	98.93	99.71	92.23	9.73	3.24	10.55	84.75	99.71	(-)	(-)	
3M	Switch	10	72.70	88.22	95.65	99.66	99.78	92.84	8.73	2.76	9.40	86.60	99.08	(-)	(-)	(-)
6M	Sham	9	59.16	77.12	87.04	92.15	93.03	82.33	13.33	4.44	16.19	72.09	92.58			
6M	3R4F	10	64.80	91.48	94.84	95.13	96.13	90.92	9.41	2.98	10.35	84.19	97.65	+		
6M	THS2.2	10	81.16	87.03	91.15	92.22	96.05	89.35	4.63	1.46	5.18	86.05	92.66	(-)	(-)	
6M	Cess	10	63.04	88.65	90.20	93.20	95.77	85.86	12.23	3.87	14.24	77.11	94.61	(-)	(-)	
6M	Switch	10	81.69	89.70	91.21	93.72	95.49	90.45	4.17	1.32	4.61	87.47	93.44	(-)	(-)	(-)
8M	Sham	10	66.29	88.07	94.13	97.38	99.81	91.41	9.94	3.14	10.88	84.30	98.53			
8M	3R4F	10	96.29	96.96	97.97	99.06	99.78	98.07	1.15	0.37	1.18	97.25	98.90	-		
8M	THS2.2	9	90.09	93.75	95.11	98.88	99.89	95.57	3.74	1.25	3.92	92.69	98.44	-	-	
8M	Cess	10	30.91	90.64	93.95	94.59	96.71	84.97	21.15	6.69	24.89	69.84	100.10	(-)	+++	
8M	Switch	7	64.68	85.71	95.91	96.50	99.48	90.08	12.05	4.55	13.38	78.93	101.22	(-)	-	(-)

Table 51. Lung function, FVC (ml).

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.58	0.63	0.69	0.71	0.81	0.69	0.07	0.02	10.22	0.64	0.74			
1M	3R4F	10	0.70	0.73	0.79	0.83	1.05	0.80	0.10	0.03	12.40	0.73	0.87	**		
1M	THS2.2	10	0.61	0.65	0.74	0.84	0.97	0.76	0.12	0.04	15.55	0.67	0.84	-	-	
2M	Sham	10	0.43	0.60	0.63	0.71	0.86	0.65	0.12	0.04	18.82	0.56	0.73			
2M	3R4F	10	0.66	0.91	0.95	0.99	1.26	0.95	0.19	0.06	19.66	0.82	1.08	***		
2M	THS2.2	10	0.50	0.58	0.61	0.73	0.88	0.64	0.12	0.04	17.92	0.56	0.73	-	***	
3M	Sham	10	0.54	0.65	0.79	0.83	1.06	0.77	0.16	0.05	20.35	0.65	0.88			
3M	3R4F	10	0.65	0.79	0.94	1.04	1.20	0.93	0.19	0.06	19.83	0.80	1.07	*		
3M	THS2.2	10	0.51	0.64	0.75	0.82	0.88	0.73	0.12	0.04	16.26	0.65	0.82	-	*	
3M	Cess	9	0.82	0.84	0.86	0.96	1.08	0.91	0.09	0.03	10.05	0.84	0.98	*	-	
3M	Switch	10	0.48	0.80	0.91	0.99	1.08	0.88	0.17	0.05	19.52	0.76	1.00	-	-	-
6M	Sham	9	0.63	0.82	0.86	0.89	0.96	0.84	0.09	0.03	11.24	0.77	0.92			
6M	3R4F	10	0.96	1.07	1.11	1.22	1.31	1.13	0.11	0.03	9.42	1.05	1.20	***		
6M	THS2.2	10	0.64	0.73	0.78	0.86	1.03	0.80	0.12	0.04	15.26	0.71	0.89	-	***	
6M	Cess	10	0.64	0.84	0.91	1.00	1.18	0.91	0.14	0.05	15.84	0.81	1.02	-	**	
6M	Switch	10	0.72	0.79	0.89	0.94	1.09	0.89	0.10	0.03	11.82	0.81	0.96	-	***	-
8M	Sham	10	0.68	0.74	0.82	0.91	0.96	0.82	0.10	0.03	12.81	0.74	0.89			
8M	3R4F	10	0.96	1.09	1.13	1.23	1.29	1.14	0.10	0.03	8.67	1.07	1.21	***		
8M	THS2.2	9	0.70	0.87	0.94	1.04	1.08	0.93	0.12	0.04	13.11	0.84	1.03	*	***	
8M	Cess	10	0.88	0.92	0.94	0.96	1.00	0.94	0.04	0.01	4.05	0.91	0.97	**	***	
8M	Switch	7	0.72	0.79	0.88	1.02	1.03	0.88	0.11	0.04	12.80	0.78	0.99	-	***	-

Table 52. Lung function, Vtr end (ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.54	0.57	0.63	0.67	0.68	0.62	0.06	0.02	8.90	0.58	0.66			
1M	3R4F	10	0.59	0.69	0.74	0.81	0.94	0.74	0.10	0.03	12.91	0.67	0.81	**		
1M	THS2.2	10	0.51	0.59	0.70	0.76	0.85	0.68	0.10	0.03	14.90	0.61	0.75	-	-	
2M	Sham	10	0.45	0.53	0.58	0.63	0.72	0.58	0.08	0.02	13.53	0.52	0.63			
2M	3R4F	10	0.64	0.76	0.84	0.90	1.12	0.85	0.15	0.05	17.73	0.74	0.96	***		
2M	THS2.2	10	0.47	0.47	0.53	0.63	0.81	0.57	0.12	0.04	21.24	0.48	0.66	-	***	
3M	Sham	10	0.49	0.56	0.68	0.76	0.92	0.67	0.13	0.04	19.59	0.58	0.77			
3M	3R4F	10	0.68	0.76	0.85	0.91	1.07	0.86	0.13	0.04	15.08	0.76	0.95	**		
3M	THS2.2	10	0.47	0.57	0.69	0.74	0.74	0.65	0.10	0.03	15.00	0.58	0.72	-	***	
3M	Cess	10	0.69	0.76	0.84	0.87	0.94	0.82	0.08	0.03	9.97	0.76	0.88	**	-	
3M	Switch	10	0.66	0.76	0.83	0.90	0.93	0.82	0.09	0.03	10.96	0.76	0.88	*	-	-
6M	Sham	9	0.64	0.77	0.81	0.84	0.87	0.79	0.07	0.02	8.51	0.74	0.85			
6M	3R4F	10	0.99	1.00	1.05	1.20	1.24	1.08	0.10	0.03	9.00	1.01	1.15	***		
6M	THS2.2	10	0.60	0.68	0.74	0.79	0.95	0.75	0.10	0.03	13.89	0.68	0.83	-	***	
6M	Cess	10	0.70	0.79	0.83	0.94	1.10	0.87	0.12	0.04	14.34	0.78	0.96	-	***	
6M	Switch	10	0.69	0.78	0.84	0.90	0.99	0.83	0.09	0.03	10.96	0.77	0.90	-	***	-
8M	Sham	10	0.67	0.72	0.79	0.82	0.91	0.78	0.08	0.02	10.07	0.73	0.84			
8M	3R4F	10	0.97	1.08	1.11	1.21	1.25	1.12	0.08	0.03	7.45	1.06	1.18	***		
8M	THS2.2	9	0.70	0.84	0.92	0.93	1.10	0.88	0.12	0.04	13.56	0.79	0.97	-	***	
8M	Cess	10	0.79	0.84	0.90	0.92	0.94	0.88	0.05	0.02	5.91	0.84	0.92	**	***	
8M	Switch	9	0.74	0.82	0.87	0.90	0.98	0.86	0.08	0.03	9.60	0.80	0.93	*	***	-

Table 53. Erythrocyte count (10^6 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	9.54	9.73	10.01	10.18	10.33	9.94	0.27	0.09	2.77	9.74	10.13			
1M	3R4F	10	10.22	10.72	11.16	11.28	12.33	11.14	0.60	0.19	5.39	10.71	11.56	***		
1M	THS2.2	9	8.94	9.46	9.65	10.21	10.57	9.73	0.56	0.19	5.72	9.30	10.16	-	***	
2M	Sham	10	8.53	8.96	9.05	9.48	9.61	9.12	0.34	0.11	3.73	8.88	9.37			
2M	3R4F	10	9.68	9.84	10.66	10.99	11.35	10.52	0.60	0.19	5.69	10.09	10.95	***		
2M	THS2.2	10	6.04	8.77	9.11	9.55	9.85	8.89	1.07	0.34	12.01	8.12	9.65	-	+++	
3M	Sham	10	8.20	9.40	9.50	9.73	10.12	9.48	0.52	0.16	5.48	9.11	9.85			
3M	3R4F	10	10.33	11.57	11.99	12.17	12.29	11.77	0.59	0.19	5.05	11.34	12.19	+++		
3M	THS2.2	10	8.19	9.08	9.55	9.74	10.35	9.43	0.58	0.18	6.15	9.02	9.85	(-)	+++	
3M	Cess	10	8.50	8.60	9.13	9.18	9.37	8.95	0.32	0.10	3.61	8.72	9.18	*	+++	
3M	Switch	10	8.55	8.84	9.17	9.28	9.78	9.15	0.42	0.13	4.58	8.85	9.45	-	***	-
6M	Sham	9	8.78	9.17	9.53	9.92	10.03	9.49	0.46	0.15	4.90	9.13	9.84			
6M	3R4F	10	10.80	11.19	11.40	11.75	11.82	11.39	0.32	0.10	2.83	11.16	11.62	***		
6M	THS2.2	10	8.61	8.95	9.09	9.34	9.86	9.17	0.36	0.11	3.88	8.91	9.42	-	***	
6M	Cess	9	8.63	9.27	9.51	9.80	10.07	9.46	0.50	0.17	5.28	9.08	9.85	-	***	
6M	Switch	10	9.01	9.16	9.58	9.89	11.01	9.63	0.60	0.19	6.19	9.21	10.06	-	***	-
8M	Sham	10	8.76	8.95	9.45	9.74	10.63	9.51	0.66	0.21	6.99	9.04	9.99			
8M	3R4F	10	9.82	10.74	11.27	12.03	12.52	11.33	0.85	0.27	7.52	10.72	11.94	***		
8M	THS2.2	10	8.21	9.36	9.66	10.03	10.75	9.61	0.82	0.26	8.56	9.02	10.19	-	***	
8M	Cess	10	8.52	9.40	9.96	10.24	10.89	9.89	0.68	0.21	6.84	9.41	10.38	-	***	
8M	Switch	10	8.13	8.82	9.36	9.54	9.79	9.22	0.50	0.16	5.46	8.86	9.58	-	***	*

Table 54. Mean erythrocyte volume (fL)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	47.55	48.00	48.28	48.90	51.15	48.54	1.03	0.33	2.12	47.80	49.28			
1M	3R4F	10	49.25	50.05	50.48	50.80	52.30	50.50	0.86	0.27	1.70	49.88	51.11	+++		
1M	THS2.2	9	47.10	47.45	48.25	48.50	49.30	48.07	0.77	0.26	1.59	47.48	48.65	-	***	
2M	Sham	10	47.25	47.65	48.08	48.60	48.85	48.06	0.57	0.18	1.18	47.66	48.46			
2M	3R4F	10	50.35	50.75	51.10	51.70	52.90	51.33	0.78	0.25	1.52	50.77	51.88	***		
2M	THS2.2	10	46.15	47.45	47.78	48.05	59.85	49.01	3.97	1.26	8.10	46.17	51.85	-	++	
3M	Sham	10	45.80	46.70	47.63	48.70	49.65	47.63	1.18	0.37	2.48	46.78	48.47			
3M	3R4F	10	49.05	49.50	49.90	50.15	52.35	50.06	0.94	0.30	1.87	49.39	50.72	***		
3M	THS2.2	10	45.45	46.65	47.25	47.55	48.55	47.14	0.83	0.26	1.76	46.54	47.73	-	***	
3M	Cess	10	48.45	49.25	49.70	50.00	52.25	49.75	1.06	0.34	2.13	48.99	50.50	***	(-)	
3M	Switch	10	47.55	48.10	48.45	49.15	50.25	48.67	0.82	0.26	1.69	48.08	49.25	*	**	*
6M	Sham	9	45.20	46.30	46.55	47.60	50.75	47.10	1.59	0.53	3.37	45.88	48.32			
6M	3R4F	10	48.90	49.25	49.45	50.05	50.85	49.67	0.63	0.20	1.27	49.22	50.11	++		
6M	THS2.2	10	45.50	45.70	46.28	47.90	49.25	46.83	1.29	0.41	2.75	45.91	47.75	-	+++	
6M	Cess	9	40.80	45.40	45.85	46.75	46.95	45.53	1.90	0.63	4.18	44.07	46.99	-	***	
6M	Switch	10	44.40	45.60	46.23	46.50	48.85	46.19	1.26	0.40	2.74	45.29	47.09	-	***	-
8M	Sham	10	45.60	46.35	46.90	47.30	48.00	46.83	0.82	0.26	1.74	46.24	47.41			
8M	3R4F	10	48.35	49.85	50.63	50.80	51.85	50.31	1.17	0.37	2.32	49.47	51.14	***		
8M	THS2.2	10	44.95	45.75	47.23	48.50	50.85	47.44	1.81	0.57	3.81	46.15	48.73	-	***	
8M	Cess	10	44.80	45.85	46.83	48.65	50.05	47.18	1.73	0.55	3.67	45.94	48.42	-	***	
8M	Switch	10	45.55	46.25	46.78	49.65	50.90	47.72	2.05	0.65	4.30	46.25	49.19	-	**	(-)

Table 55. Hemoglobin (g/l)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	146.50	152.00	155.25	157.50	160.00	154.70	4.41	1.39	2.85	151.54	157.86			
1M	3R4F	10	170.50	173.50	180.25	184.50	199.50	180.45	8.67	2.74	4.81	174.25	186.65	***		
1M	THS2.2	9	136.50	142.00	149.50	159.00	163.50	149.56	9.57	3.19	6.40	142.20	156.91	-	***	
2M	Sham	10	132.50	134.00	139.25	143.50	146.00	139.00	4.94	1.56	3.55	135.47	142.53			
2M	3R4F	10	159.00	165.50	175.25	177.50	185.50	173.25	8.91	2.82	5.14	166.88	179.62	***		
2M	THS2.2	10	106.50	135.00	141.25	144.50	146.50	137.45	11.87	3.75	8.63	128.96	145.94	-	***	
3M	Sham	10	127.50	141.50	146.00	150.50	154.00	144.90	7.90	2.50	5.45	139.25	150.55			
3M	3R4F	10	171.50	184.00	191.75	193.50	195.00	188.20	7.54	2.39	4.01	182.80	193.60	+++		
3M	THS2.2	10	130.50	137.00	143.25	146.50	152.00	141.90	6.97	2.20	4.91	136.91	146.89	-	***	
3M	Cess	10	135.50	139.00	143.75	146.00	150.00	143.15	4.74	1.50	3.31	139.76	146.54	-	***	
3M	Switch	10	131.50	139.50	141.75	146.00	151.00	142.50	5.81	1.84	4.08	138.35	146.65	-	***	-
6M	Sham	9	135.50	140.00	141.50	146.50	149.00	142.67	4.39	1.46	3.08	139.29	146.04			
6M	3R4F	10	171.50	177.50	180.50	182.50	190.00	180.25	5.32	1.68	2.95	176.45	184.05	***		
6M	THS2.2	10	127.00	132.00	138.25	144.50	145.00	138.05	6.39	2.02	4.63	133.48	142.62	-	***	
6M	Cess	9	113.50	139.50	144.00	144.00	147.50	138.89	11.13	3.71	8.01	130.33	147.44	-	***	
6M	Switch	10	134.00	136.50	141.75	147.50	159.50	143.55	8.45	2.67	5.89	137.50	149.60	-	***	-
8M	Sham	10	125.50	129.50	142.25	146.50	156.50	140.50	10.58	3.34	7.53	132.94	148.06			
8M	3R4F	10	153.50	169.50	179.00	187.50	192.00	177.70	12.33	3.90	6.94	168.88	186.52	***		
8M	THS2.2	10	122.00	138.50	148.25	149.00	154.00	144.00	9.84	3.11	6.84	136.96	151.04	-	***	
8M	Cess	10	136.50	145.00	146.50	149.00	165.50	147.70	7.46	2.36	5.05	142.36	153.04	-	***	
8M	Switch	10	129.50	132.00	139.75	143.50	150.50	139.55	7.35	2.33	5.27	134.29	144.81	-	***	*

Table 56. Mean hemoglobin content (g/dL)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	31.65	31.80	32.20	32.30	32.45	32.09	0.30	0.09	0.93	31.87	32.30			
1M	3R4F	10	31.45	31.80	32.15	32.35	32.60	32.12	0.35	0.11	1.10	31.86	32.37	-		
1M	THS2.2	9	31.60	31.70	32.00	32.20	32.40	31.97	0.29	0.10	0.90	31.75	32.19	(-)	-	
2M	Sham	10	31.15	31.45	31.63	31.95	32.15	31.71	0.33	0.10	1.05	31.47	31.94			
2M	3R4F	10	31.85	31.95	32.13	32.15	32.35	32.09	0.16	0.05	0.49	31.98	32.20	**		
2M	THS2.2	10	29.45	31.75	31.90	32.20	32.60	31.78	0.89	0.28	2.79	31.14	32.41	-	-	
3M	Sham	10	31.35	31.75	32.10	32.20	32.95	32.11	0.50	0.16	1.55	31.75	32.47			
3M	3R4F	10	31.65	31.85	32.00	32.05	32.35	31.98	0.21	0.07	0.66	31.82	32.13	-		
3M	THS2.2	10	31.50	31.80	31.88	31.95	32.80	31.94	0.34	0.11	1.06	31.69	32.18	(-)	-	
3M	Cess	10	31.65	31.85	32.20	32.40	32.75	32.15	0.36	0.11	1.12	31.89	32.40	-	-	
3M	Switch	10	31.50	31.90	32.05	32.25	32.35	32.03	0.29	0.09	0.91	31.82	32.23	-	-	-
6M	Sham	9	31.40	31.80	31.90	32.25	32.55	31.97	0.37	0.12	1.17	31.69	32.26			
6M	3R4F	10	31.45	31.60	31.90	32.15	32.15	31.86	0.29	0.09	0.91	31.65	32.07	-		
6M	THS2.2	10	31.90	31.95	32.13	32.25	32.70	32.15	0.24	0.08	0.75	31.97	32.32	-	*	
6M	Cess	9	31.55	32.05	32.30	32.35	32.60	32.19	0.32	0.11	1.00	31.94	32.44	-	*	
6M	Switch	10	32.00	32.05	32.23	32.50	32.90	32.28	0.29	0.09	0.90	32.07	32.49	-	**	-
8M	Sham	10	31.05	31.25	31.53	31.70	32.40	31.54	0.39	0.12	1.22	31.26	31.81			
8M	3R4F	10	30.25	31.05	31.23	31.65	31.70	31.22	0.44	0.14	1.40	30.91	31.53	-		
8M	THS2.2	10	30.65	31.45	31.70	31.95	32.25	31.66	0.46	0.15	1.46	31.32	31.99	-	*	
8M	Cess	10	31.35	31.40	31.75	31.80	32.35	31.71	0.32	0.10	1.01	31.48	31.93	-	*	
8M	Switch	10	31.25	31.55	31.85	32.00	32.15	31.76	0.30	0.10	0.95	31.54	31.98	-	**	-

Table 57. Mean hemoglobin volume (pg)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	15.30	15.35	15.48	15.75	16.20	15.57	0.28	0.09	1.82	15.37	15.77			
1M	3R4F	10	15.65	16.05	16.20	16.40	16.70	16.21	0.30	0.10	1.86	16.00	16.43	***		
1M	THS2.2	9	15.00	15.00	15.45	15.55	15.90	15.37	0.31	0.10	2.02	15.13	15.61	-	***	
2M	Sham	10	14.90	14.95	15.23	15.50	15.70	15.24	0.30	0.09	1.96	15.02	15.45			
2M	3R4F	10	16.10	16.30	16.40	16.60	17.10	16.47	0.28	0.09	1.68	16.27	16.67	***		
2M	THS2.2	10	14.90	15.05	15.33	15.60	17.60	15.55	0.81	0.26	5.19	14.97	16.13	-	++	
3M	Sham	10	14.50	14.95	15.30	15.55	16.05	15.29	0.49	0.16	3.24	14.94	15.64			
3M	3R4F	10	15.70	15.90	15.93	16.05	16.60	16.01	0.26	0.08	1.63	15.82	16.20	**		
3M	THS2.2	10	14.50	14.90	15.05	15.20	15.95	15.07	0.39	0.12	2.59	14.79	15.35	-	+++	
3M	Cess	10	15.45	15.75	15.90	16.15	16.95	15.99	0.42	0.13	2.62	15.69	16.29	**	(-)	
3M	Switch	10	15.15	15.40	15.48	15.85	16.25	15.58	0.32	0.10	2.07	15.35	15.81	-	++	+
6M	Sham	9	14.65	14.75	14.90	15.25	15.95	15.06	0.42	0.14	2.82	14.73	15.38			
6M	3R4F	10	15.50	15.75	15.83	16.00	16.05	15.82	0.19	0.06	1.20	15.68	15.95	***		
6M	THS2.2	10	14.60	14.70	14.83	15.25	15.95	15.05	0.46	0.15	3.09	14.72	15.38	(-)	***	
6M	Cess	9	13.15	14.55	14.80	15.05	15.15	14.65	0.61	0.20	4.13	14.18	15.12	-	+++	
6M	Switch	10	14.50	14.65	14.88	15.10	15.60	14.91	0.32	0.10	2.14	14.68	15.14	-	***	-
8M	Sham	10	14.15	14.50	14.75	15.10	15.35	14.77	0.37	0.12	2.52	14.50	15.03			
8M	3R4F	10	15.05	15.50	15.65	15.95	16.45	15.71	0.39	0.12	2.51	15.43	15.99	***		
8M	THS2.2	10	14.30	14.50	14.98	15.40	16.10	15.02	0.58	0.18	3.84	14.60	15.43	-	**	
8M	Cess	10	14.05	14.35	14.90	15.45	16.00	14.96	0.67	0.21	4.45	14.48	15.44	-	**	
8M	Switch	10	14.20	14.65	14.85	15.85	16.25	15.16	0.69	0.22	4.53	14.66	15.65	-	*	-

Table 58. Hematocrit (L/L)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.45	0.47	0.48	0.49	0.51	0.48	0.02	0.01	3.32	0.47	0.49			
1M	3R4F	10	0.52	0.54	0.56	0.57	0.62	0.56	0.03	0.01	4.89	0.54	0.58	***		
1M	THS2.2	9	0.43	0.45	0.46	0.50	0.52	0.47	0.03	0.01	6.29	0.45	0.49	-	***	
2M	Sham	10	0.42	0.42	0.44	0.46	0.46	0.44	0.02	0.01	3.65	0.43	0.45			
2M	3R4F	10	0.50	0.51	0.55	0.55	0.58	0.54	0.03	0.01	5.23	0.52	0.56	***		
2M	THS2.2	10	0.36	0.42	0.44	0.45	0.46	0.43	0.03	0.01	6.82	0.41	0.45	-	***	
3M	Sham	10	0.40	0.44	0.45	0.47	0.48	0.45	0.02	0.01	4.98	0.44	0.47			
3M	3R4F	10	0.54	0.58	0.60	0.60	0.61	0.59	0.02	0.01	3.73	0.57	0.60	+++		
3M	THS2.2	10	0.40	0.43	0.45	0.46	0.48	0.44	0.03	0.01	5.66	0.43	0.46	-	***	
3M	Cess	10	0.43	0.44	0.45	0.46	0.46	0.45	0.01	0.00	2.69	0.44	0.45	-	***	
3M	Switch	10	0.41	0.44	0.44	0.45	0.48	0.45	0.02	0.01	4.56	0.43	0.46	-	***	-
6M	Sham	9	0.42	0.44	0.45	0.45	0.47	0.45	0.01	0.00	3.28	0.44	0.46			
6M	3R4F	10	0.53	0.55	0.57	0.58	0.59	0.57	0.02	0.01	2.87	0.55	0.58	***		
6M	THS2.2	10	0.40	0.41	0.43	0.45	0.45	0.43	0.02	0.01	4.59	0.42	0.44	*	***	
6M	Cess	9	0.35	0.43	0.44	0.45	0.47	0.43	0.04	0.01	8.36	0.40	0.46	-	***	
6M	Switch	10	0.42	0.43	0.44	0.46	0.49	0.44	0.03	0.01	6.01	0.43	0.46	-	***	-
8M	Sham	10	0.40	0.41	0.45	0.46	0.50	0.45	0.03	0.01	7.18	0.42	0.47			
8M	3R4F	10	0.51	0.54	0.57	0.60	0.62	0.57	0.04	0.01	6.22	0.54	0.59	***		
8M	THS2.2	10	0.40	0.44	0.46	0.47	0.49	0.45	0.03	0.01	6.02	0.44	0.47	-	***	
8M	Cess	10	0.43	0.46	0.46	0.47	0.53	0.47	0.02	0.01	5.36	0.45	0.48	-	***	
8M	Switch	10	0.41	0.42	0.44	0.45	0.47	0.44	0.02	0.01	4.51	0.43	0.45	-	***	*

Table 59. Leucocyte count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	3.09	3.84	4.04	5.73	6.84	4.67	1.23	0.39	26.35	3.79	5.55			
1M	3R4F	10	2.56	4.01	4.99	5.89	6.62	4.92	1.34	0.42	27.24	3.96	5.87	-		
1M	THS2.2	9	2.64	4.25	4.57	6.29	9.64	5.24	2.03	0.68	38.77	3.68	6.80	-	-	
2M	Sham	10	2.11	2.98	3.52	3.86	4.78	3.46	0.73	0.23	21.09	2.94	3.98			
2M	3R4F	10	2.45	3.65	3.96	5.74	12.39	5.05	2.90	0.92	57.51	2.97	7.13	-		
2M	THS2.2	10	1.78	2.41	3.30	3.98	5.95	3.40	1.16	0.37	34.15	2.57	4.22	-	(-)	
3M	Sham	10	1.32	1.87	2.32	3.67	7.70	3.08	1.87	0.59	60.66	1.74	4.42			
3M	3R4F	10	1.33	2.12	2.96	3.74	4.99	2.95	1.11	0.35	37.54	2.16	3.75	-		
3M	THS2.2	10	1.33	2.44	3.54	4.17	5.02	3.29	1.17	0.37	35.48	2.45	4.12	-	-	
3M	Cess	10	1.36	2.57	3.26	3.69	4.99	3.19	1.02	0.32	32.05	2.46	3.93	-	-	
3M	Switch	10	1.35	2.50	4.00	5.37	6.26	3.91	1.59	0.50	40.61	2.78	5.05	-	-	-
6M	Sham	9	0.81	1.26	1.41	2.40	3.25	1.76	0.79	0.26	44.75	1.15	2.37			
6M	3R4F	10	1.36	1.59	1.86	2.24	2.43	1.90	0.38	0.12	20.06	1.62	2.17	-		
6M	THS2.2	10	0.53	0.91	1.46	1.87	4.01	1.69	1.04	0.33	61.62	0.95	2.44	-	-	
6M	Cess	9	0.93	1.66	2.29	2.49	7.73	2.66	1.99	0.66	74.78	1.13	4.18	(-)	-	
6M	Switch	10	0.92	1.95	2.00	2.44	2.77	2.00	0.62	0.20	30.99	1.56	2.44	-	-	-
8M	Sham	10	1.27	2.22	2.44	3.59	3.98	2.75	0.90	0.28	32.70	2.10	3.39			
8M	3R4F	10	1.38	1.89	2.09	2.90	4.75	2.37	0.97	0.31	40.87	1.68	3.07	-		
8M	THS2.2	10	1.78	2.35	3.00	4.61	6.19	3.48	1.58	0.50	45.51	2.34	4.61	-	(-)	
8M	Cess	10	1.41	3.36	3.71	4.11	5.93	3.78	1.26	0.40	33.32	2.88	4.68	*	*	
8M	Switch	10	1.95	2.66	2.76	3.24	8.81	3.55	2.01	0.64	56.59	2.11	4.99	(-)	(-)	(-)

Table 60. Lymphocyte count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	2.40	2.60	2.88	4.24	4.80	3.38	0.96	0.30	28.48	2.69	4.07			
1M	3R4F	10	2.10	2.76	3.81	4.53	5.28	3.71	1.12	0.35	30.17	2.91	4.51	-		
1M	THS2.2	9	1.97	3.01	3.27	3.92	5.08	3.39	0.99	0.33	29.24	2.63	4.15	-	-	
2M	Sham	10	1.68	2.06	2.53	2.89	3.87	2.54	0.63	0.20	24.89	2.09	2.99			
2M	3R4F	10	2.18	2.70	3.16	4.44	9.37	3.94	2.16	0.68	54.73	2.40	5.49	+		
2M	THS2.2	10	1.54	1.96	2.45	2.95	4.93	2.64	0.94	0.30	35.54	1.97	3.31	-	(-)	
3M	Sham	10	0.99	1.42	1.54	3.11	4.32	2.17	1.14	0.36	52.52	1.36	2.99			
3M	3R4F	10	0.88	1.53	1.81	2.84	3.96	2.11	0.91	0.29	43.13	1.46	2.77	(-)		
3M	THS2.2	10	1.04	1.82	2.73	3.25	3.71	2.52	0.89	0.28	35.43	1.88	3.15	-	-	
3M	Cess	10	0.98	2.00	2.49	2.91	3.79	2.42	0.79	0.25	32.68	1.85	2.98	-	-	
3M	Switch	10	0.87	1.85	2.97	4.28	4.87	2.99	1.29	0.41	43.17	2.06	3.91	-	-	-
6M	Sham	9	0.39	0.79	0.97	1.60	2.43	1.17	0.63	0.21	53.83	0.68	1.65			
6M	3R4F	10	0.35	1.09	1.37	1.59	1.73	1.28	0.42	0.13	32.63	0.98	1.58	-		
6M	THS2.2	10	0.27	0.62	1.00	1.40	2.74	1.17	0.75	0.24	64.31	0.63	1.71	-	-	
6M	Cess	9	0.48	1.12	1.68	1.78	2.24	1.46	0.58	0.19	39.95	1.01	1.91	-	-	
6M	Switch	10	0.58	1.27	1.44	1.72	2.12	1.38	0.47	0.15	33.97	1.05	1.72	-	-	-
8M	Sham	10	1.01	1.48	1.85	2.73	2.92	2.02	0.68	0.22	33.75	1.53	2.51			
8M	3R4F	10	0.90	1.28	1.43	2.07	3.26	1.63	0.69	0.22	42.17	1.14	2.12	-		
8M	THS2.2	10	1.23	1.75	2.14	3.50	5.05	2.62	1.40	0.44	53.27	1.62	3.62	-	(-)	
8M	Cess	10	0.92	2.29	2.71	3.10	4.76	2.83	1.12	0.36	39.68	2.03	3.63	-	*	
8M	Switch	10	1.42	1.66	2.06	2.54	4.41	2.39	1.00	0.32	41.87	1.67	3.10	-	+	(-)

Table 61. Neutrophil count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.23	0.28	0.38	0.45	0.51	0.37	0.10	0.03	26.67	0.30	0.44			
1M	3R4F	10	0.26	0.35	0.43	0.52	0.61	0.43	0.11	0.04	26.00	0.35	0.51	-		
1M	THS2.2	9	0.19	0.31	0.36	0.62	4.03	0.84	1.22	0.41	144.94	-0.10	1.78	-	(-)	
2M	Sham	10	0.15	0.30	0.37	0.39	0.67	0.36	0.13	0.04	36.46	0.27	0.46			
2M	3R4F	10	0.19	0.25	0.37	0.47	0.54	0.35	0.12	0.04	35.17	0.26	0.44	-		
2M	THS2.2	10	0.19	0.25	0.33	0.37	0.44	0.31	0.08	0.03	27.09	0.25	0.37	-	-	
3M	Sham	10	0.06	0.22	0.32	0.33	1.83	0.43	0.51	0.16	118.70	0.06	0.79			
3M	3R4F	10	0.16	0.22	0.24	0.36	0.91	0.34	0.24	0.08	70.67	0.17	0.52	(-)		
3M	THS2.2	10	0.16	0.21	0.29	0.40	0.76	0.36	0.20	0.06	55.65	0.22	0.50	(-)	(-)	
3M	Cess	10	0.15	0.23	0.32	0.41	0.60	0.33	0.13	0.04	39.54	0.24	0.42	(-)	(-)	
3M	Switch	10	0.13	0.22	0.32	0.37	0.84	0.34	0.20	0.06	57.40	0.20	0.49	(-)	(-)	(-)
6M	Sham	9	0.19	0.20	0.33	0.53	0.55	0.36	0.15	0.05	42.50	0.24	0.48			
6M	3R4F	10	0.14	0.20	0.28	0.40	0.85	0.33	0.20	0.06	60.86	0.19	0.48	-		
6M	THS2.2	10	0.15	0.17	0.21	0.34	0.68	0.29	0.18	0.06	62.43	0.16	0.43	-	(-)	
6M	Cess	9	0.21	0.29	0.30	0.38	3.24	0.64	0.98	0.33	152.95	-0.11	1.39	(-)	(-)	
6M	Switch	10	0.13	0.26	0.37	0.49	0.58	0.36	0.14	0.05	39.64	0.26	0.46	-	-	(-)
8M	Sham	10	0.12	0.25	0.36	0.51	0.75	0.38	0.18	0.06	46.39	0.25	0.51			
8M	3R4F	10	0.14	0.30	0.32	0.47	0.69	0.36	0.16	0.05	44.79	0.25	0.48	-		
8M	THS2.2	10	0.23	0.39	0.42	0.50	0.52	0.41	0.10	0.03	23.82	0.34	0.48	-	-	
8M	Cess	10	0.26	0.42	0.48	0.62	0.63	0.48	0.12	0.04	25.54	0.40	0.57	-	-	
8M	Switch	10	0.33	0.43	0.44	0.67	2.21	0.65	0.56	0.18	86.88	0.25	1.05	(-)	(-)	(-)

Table 62. Monocyte count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.34	0.64	0.80	0.92	1.33	0.77	0.28	0.09	36.41	0.57	0.98			
1M	3R4F	10	0.08	0.57	0.69	0.89	0.91	0.66	0.25	0.08	38.02	0.48	0.84	-		
1M	THS2.2	9	0.34	0.42	0.68	1.13	2.54	0.92	0.70	0.23	76.39	0.38	1.45	-	-	
2M	Sham	10	0.28	0.44	0.50	0.58	0.59	0.49	0.10	0.03	20.23	0.42	0.56			
2M	3R4F	10	0.02	0.17	0.54	0.67	2.38	0.61	0.68	0.22	111.96	0.12	1.10	-		
2M	THS2.2	10	0.02	0.24	0.45	0.51	0.57	0.37	0.19	0.06	51.20	0.23	0.50	(-)	-	
3M	Sham	10	0.07	0.16	0.29	0.56	1.52	0.44	0.42	0.13	96.23	0.14	0.74			
3M	3R4F	10	0.18	0.30	0.38	0.52	0.96	0.45	0.24	0.08	53.03	0.28	0.63	(-)		
3M	THS2.2	10	0.09	0.26	0.42	0.51	0.60	0.37	0.17	0.05	44.74	0.25	0.49	-	-	
3M	Cess	10	0.22	0.26	0.41	0.49	0.71	0.40	0.15	0.05	38.14	0.29	0.51	(-)	-	
3M	Switch	10	0.28	0.31	0.36	0.68	0.95	0.50	0.25	0.08	49.17	0.33	0.68	(-)	(-)	-
6M	Sham	9	0.08	0.12	0.18	0.22	0.31	0.18	0.08	0.03	43.30	0.12	0.24			
6M	3R4F	10	0.11	0.18	0.23	0.29	0.47	0.25	0.11	0.04	44.87	0.17	0.33	-		
6M	THS2.2	10	0.05	0.14	0.16	0.23	0.43	0.19	0.11	0.03	56.51	0.12	0.27	-	-	
6M	Cess	9	0.16	0.19	0.25	0.35	2.33	0.50	0.70	0.23	140.15	-0.04	1.03	(-)	(-)	
6M	Switch	10	0.07	0.18	0.22	0.31	0.38	0.22	0.09	0.03	42.10	0.15	0.29	-	-	(-)
8M	Sham	10	0.13	0.23	0.29	0.37	0.49	0.30	0.11	0.03	36.40	0.22	0.38			
8M	3R4F	10	0.09	0.24	0.31	0.41	0.73	0.34	0.18	0.06	53.48	0.21	0.47	-		
8M	THS2.2	10	0.14	0.19	0.38	0.48	0.80	0.38	0.21	0.07	54.36	0.23	0.53	-	-	
8M	Cess	10	0.23	0.38	0.42	0.47	0.52	0.40	0.10	0.03	24.60	0.33	0.47	*	-	
8M	Switch	10	0.07	0.20	0.26	0.43	1.80	0.43	0.50	0.16	116.62	0.07	0.79	(-)	(-)	-

Table 63. Basophil count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	0.00	0.00	0.01	0.04	0.01	0.01	0.00	179.16	-0.00	0.01			
1M	3R4F	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	116.53	0.00	0.01	(-)		
1M	THS2.2	9	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	212.13	-0.00	0.00	(-)	(-)	
2M	Sham	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	174.80	-0.00	0.00			
2M	3R4F	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	105.41	0.00	0.00	(-)		
2M	THS2.2	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	316.23	-0.00	0.00	(-)	(-)	
3M	Sham	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	210.82	-0.00	0.00			
3M	3R4F	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	210.82	-0.00	0.00	(-)		
3M	THS2.2	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	316.23	-0.00	0.00	(-)	(-)	
3M	Cess	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	210.82	-0.00	0.00	(-)	(-)	
3M	Switch	10	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	253.86	-0.00	0.01	(-)	(-)	(-)
6M	Sham	9	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	94.87	0.00	0.00			
6M	3R4F	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	174.80	-0.00	0.00	(-)		
6M	THS2.2	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.	.	.	+	(-)	
6M	Cess	9	0.00	0.00	0.00	0.00	0.14	0.02	0.04	0.01	277.48	-0.02	0.05	(-)	(-)	
6M	Switch	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.	.	.	+	(-)	(-)
8M	Sham	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	316.23	-0.00	0.00			
8M	3R4F	10	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	253.86	-0.00	0.01	(-)		
8M	THS2.2	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	210.82	-0.00	0.00	(-)	(-)	
8M	Cess	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	174.80	-0.00	0.00	(-)	(-)	
8M	Switch	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	141.42	-0.00	0.01	(-)	(-)	(-)

Table 64. Eosinophil count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.03	0.09	0.13	0.18	0.27	0.14	0.07	0.02	54.21	0.08	0.19			
1M	3R4F	10	0.04	0.09	0.10	0.15	0.25	0.12	0.06	0.02	48.79	0.08	0.16	-		
1M	THS2.2	9	0.04	0.06	0.11	0.11	0.18	0.10	0.05	0.02	48.60	0.06	0.13	-	-	
2M	Sham	10	0.01	0.04	0.06	0.09	0.12	0.06	0.04	0.01	57.11	0.04	0.09			
2M	3R4F	10	0.05	0.07	0.14	0.21	0.28	0.15	0.08	0.03	55.85	0.09	0.20	*		
2M	THS2.2	10	0.02	0.03	0.09	0.11	0.15	0.07	0.05	0.01	64.01	0.04	0.11	-	*	
3M	Sham	10	0.02	0.03	0.04	0.06	0.09	0.04	0.02	0.01	49.09	0.03	0.06			
3M	3R4F	10	0.01	0.03	0.04	0.05	0.10	0.04	0.02	0.01	59.04	0.02	0.06	-		
3M	THS2.2	10	0.00	0.03	0.04	0.06	0.10	0.04	0.03	0.01	67.07	0.02	0.06	-	-	
3M	Cess	10	0.01	0.01	0.03	0.08	0.11	0.04	0.04	0.01	85.06	0.02	0.07	(-)	-	
3M	Switch	10	0.02	0.04	0.08	0.11	0.18	0.08	0.05	0.02	61.20	0.04	0.11	-	-	-
6M	Sham	9	0.01	0.02	0.05	0.07	0.14	0.05	0.04	0.01	79.34	0.02	0.08			
6M	3R4F	10	0.01	0.02	0.03	0.04	0.11	0.03	0.03	0.01	87.63	0.01	0.06	(-)		
6M	THS2.2	10	0.01	0.01	0.02	0.04	0.16	0.03	0.05	0.01	133.08	0.00	0.07	(-)	(-)	
6M	Cess	9	0.01	0.02	0.03	0.04	0.14	0.04	0.04	0.01	99.22	0.01	0.07	(-)	(-)	
6M	Switch	10	0.01	0.02	0.03	0.04	0.08	0.04	0.02	0.01	65.54	0.02	0.05	(-)	(-)	(-)
8M	Sham	10	0.01	0.02	0.03	0.07	0.11	0.04	0.03	0.01	80.22	0.02	0.07			
8M	3R4F	10	0.01	0.02	0.03	0.05	0.09	0.04	0.02	0.01	68.36	0.02	0.05	(-)		
8M	THS2.2	10	0.03	0.04	0.07	0.08	0.10	0.06	0.03	0.01	43.81	0.04	0.08	-	-	
8M	Cess	10	0.00	0.04	0.07	0.10	0.11	0.06	0.04	0.01	56.79	0.04	0.09	-	-	
8M	Switch	10	0.02	0.03	0.04	0.11	0.38	0.09	0.11	0.03	128.19	0.01	0.16	(-)	(-)	-

Table 65. Platelet count (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	527.50	984.00	1021.0	1176.0	1226.0	1019.3	196.28	62.07	19.26	878.89	1159.7			
1M	3R4F	10	543.50	622.50	923.75	998.50	1038.5	848.45	193.04	61.04	22.75	710.36	986.54	(-)		
1M	THS2.2	9	743.00	887.00	972.50	1025.5	1105.0	950.33	111.16	37.05	11.70	864.89	1035.8	-	(-)	
2M	Sham	10	962.50	1110.5	1180.5	1234.5	1426.0	1174.1	128.75	40.71	10.97	1081.9	1266.2			
2M	3R4F	10	247.00	644.00	911.75	985.00	1151.0	837.80	266.34	84.22	31.79	647.27	1028.3	**		
2M	THS2.2	10	246.00	893.50	1065.5	1118.0	1349.0	963.90	314.96	99.60	32.68	738.59	1189.2	-	(-)	
3M	Sham	10	1071.0	1129.5	1199.3	1343.5	1859.5	1312.8	285.30	90.22	21.73	1108.7	1516.9			
3M	3R4F	10	936.00	1026.0	1055.3	1142.5	1730.0	1126.8	230.06	72.75	20.42	962.18	1291.3	+		
3M	THS2.2	10	992.00	1112.5	1225.8	1612.0	2064.0	1341.4	336.50	106.41	25.09	1100.6	1582.1	(-)	(-)	
3M	Cess	10	755.50	1166.5	1254.8	1629.5	1806.0	1334.4	316.23	100.00	23.70	1108.1	1560.6	-	-	
3M	Switch	10	1002.5	1036.0	1259.5	1469.0	2001.0	1307.3	310.59	98.22	23.76	1085.1	1529.4	(-)	(-)	-
6M	Sham	9	771.50	1282.5	1421.0	1656.5	2301.5	1424.4	448.80	149.60	31.51	1079.4	1769.4			
6M	3R4F	10	1273.5	1362.0	1550.3	2077.5	2846.0	1792.2	531.22	167.99	29.64	1412.1	2172.2	-		
6M	THS2.2	10	810.50	1181.5	1366.0	1733.5	2051.0	1431.9	370.27	117.09	25.86	1167.0	1696.7	-	-	
6M	Cess	9	1155.5	1351.0	1484.0	1902.0	2353.0	1610.4	404.67	134.89	25.13	1299.4	1921.5	-	(-)	
6M	Switch	10	929.50	1419.5	1615.8	1796.0	2492.0	1619.5	408.37	129.14	25.22	1327.4	1911.6	-	-	-
8M	Sham	10	1105.0	1401.5	1535.5	1961.5	2021.0	1611.8	312.66	98.87	19.40	1388.1	1835.5			
8M	3R4F	10	1107.0	1820.0	2165.5	2600.5	2844.0	2142.5	551.95	174.54	25.76	1747.7	2537.3	*		
8M	THS2.2	10	281.50	1242.0	1381.3	1695.0	1860.5	1344.6	454.94	143.87	33.83	1019.2	1670.0	-	**	
8M	Cess	10	1182.0	1363.0	1664.3	1967.5	2240.5	1668.5	372.08	117.66	22.30	1402.3	1934.7	-	*	
8M	Switch	10	987.50	1194.5	1362.0	1689.0	2228.5	1444.6	351.24	111.07	24.31	1193.3	1695.8	-	**	-

Table 66. Lymphocytes, relative (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	64.65	69.65	71.15	77.20	78.80	72.17	4.58	1.45	6.35	68.89	75.45			
1M	3R4F	10	65.95	71.60	75.55	79.40	82.00	74.98	5.40	1.71	7.21	71.11	78.84	-		
1M	THS2.2	9	31.15	69.90	74.80	80.00	81.80	68.70	17.57	5.86	25.57	55.19	82.21	-	(-)	
2M	Sham	10	66.15	69.10	71.98	77.55	80.90	73.26	4.86	1.54	6.63	69.78	76.73			
2M	3R4F	10	68.65	76.40	78.50	82.05	89.20	79.14	5.58	1.77	7.06	75.14	83.14	*		
2M	THS2.2	10	71.35	74.55	77.18	81.40	86.45	78.10	4.57	1.44	5.85	74.84	81.37	*	-	
3M	Sham	10	55.25	62.25	72.98	82.75	88.20	72.29	11.49	3.63	15.90	64.06	80.51			
3M	3R4F	10	49.20	66.65	70.40	79.35	82.60	71.17	9.87	3.12	13.87	64.11	78.23	-		
3M	THS2.2	10	71.95	74.05	75.63	78.00	84.55	76.54	3.57	1.13	4.67	73.98	79.09	-	-	
3M	Cess	10	69.20	71.65	76.45	78.10	79.45	75.44	3.58	1.13	4.74	72.88	78.00	-	-	
3M	Switch	10	64.05	74.05	77.55	79.30	83.75	75.33	6.54	2.07	8.68	70.65	80.00	-	-	(-)
6M	Sham	9	47.55	58.85	64.85	68.60	74.75	64.02	8.58	2.86	13.40	57.42	70.61			
6M	3R4F	10	20.75	67.25	70.60	76.80	79.05	67.16	16.88	5.34	25.14	55.08	79.23	(-)		
6M	THS2.2	10	50.55	63.60	68.55	73.75	75.55	67.25	7.38	2.33	10.97	61.97	72.53	-	(-)	
6M	Cess	9	26.05	51.55	67.30	72.40	81.00	61.83	16.88	5.63	27.31	48.85	74.81	-	(-)	
6M	Switch	10	63.35	65.85	67.38	70.90	76.45	68.59	4.06	1.28	5.92	65.69	71.49	-	-	-
8M	Sham	10	62.80	71.30	75.45	76.60	79.90	73.78	5.33	1.68	7.22	69.96	77.59			
8M	3R4F	10	59.90	65.45	68.98	70.45	76.45	68.47	4.64	1.47	6.77	65.15	71.78	*		
8M	THS2.2	10	65.20	69.00	72.50	77.15	82.25	73.43	5.77	1.82	7.85	69.31	77.56	-	*	
8M	Cess	10	65.05	68.15	73.18	78.40	82.15	73.41	5.77	1.82	7.86	69.28	77.54	-	*	
8M	Switch	10	50.30	62.45	73.00	75.55	78.40	69.88	8.92	2.82	12.76	63.50	76.26	(-)	-	-

Table 67. Neutrophils, relative (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	4.25	6.60	8.15	10.35	12.25	8.31	2.44	0.77	29.36	6.56	10.05			
1M	3R4F	10	7.05	7.30	8.38	9.45	13.05	9.05	2.13	0.67	23.53	7.52	10.57	-		
1M	THS2.2	9	4.85	7.20	7.35	11.60	41.80	13.17	11.86	3.95	90.10	4.05	22.29	-	(-)	
2M	Sham	10	6.25	7.05	10.70	12.45	16.85	10.55	3.27	1.03	30.99	8.21	12.88			
2M	3R4F	10	3.15	5.00	7.80	9.55	13.65	7.97	3.27	1.03	40.97	5.63	10.31	-		
2M	THS2.2	10	7.15	8.00	9.25	10.80	13.85	9.55	1.94	0.61	20.32	8.16	10.93	-	-	
3M	Sham	10	4.35	8.90	12.35	12.80	23.70	11.69	5.32	1.68	45.55	7.88	15.49			
3M	3R4F	10	4.35	6.75	10.68	18.10	24.15	12.08	6.47	2.05	53.62	7.44	16.71	-		
3M	THS2.2	10	6.00	6.95	11.53	14.15	16.90	10.99	3.75	1.18	34.11	8.30	13.67	-	-	
3M	Cess	10	7.15	9.50	10.05	11.85	12.85	10.33	1.67	0.53	16.14	9.13	11.52	-	-	
3M	Switch	10	4.80	5.55	9.20	10.70	18.00	9.24	4.04	1.28	43.77	6.35	12.13	(-)	-	-
6M	Sham	9	14.35	16.35	22.45	24.20	29.75	20.99	5.69	1.90	27.12	16.62	25.37			
6M	3R4F	10	9.00	12.50	14.98	17.45	49.80	17.67	11.67	3.69	66.02	9.33	26.02	(-)		
6M	THS2.2	10	8.50	16.65	18.30	20.25	27.30	18.25	4.87	1.54	26.72	14.76	21.73	-	(-)	
6M	Cess	9	9.90	12.05	15.35	26.95	42.00	20.10	11.04	3.68	54.94	11.61	28.59	-	(-)	
6M	Switch	10	9.80	14.25	20.28	21.15	26.05	18.21	5.16	1.63	28.34	14.51	21.90	-	-	-
8M	Sham	10	9.45	10.30	12.60	16.00	22.50	13.68	4.34	1.37	31.73	10.57	16.79			
8M	3R4F	10	9.15	10.90	16.05	18.15	21.70	15.30	4.18	1.32	27.34	12.30	18.29	-		
8M	THS2.2	10	4.35	10.85	14.38	17.35	21.75	13.63	5.13	1.62	37.63	9.96	17.29	-	-	
8M	Cess	10	10.40	10.75	12.68	15.40	18.55	13.41	3.02	0.96	22.52	11.25	15.57	(-)	-	
8M	Switch	10	8.45	13.20	16.48	23.45	25.90	17.56	5.66	1.79	32.23	13.51	21.60	-	-	-

Table 68. Monocytes, relative (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	10.55	11.30	17.33	19.35	24.30	16.61	4.66	1.47	28.08	13.27	19.95			
1M	3R4F	10	2.95	10.20	13.40	17.75	23.60	13.53	5.91	1.87	43.67	9.30	17.76	-		
1M	THS2.2	9	9.65	10.75	13.50	17.95	29.25	16.24	7.08	2.36	43.58	10.80	21.69	-	-	
2M	Sham	10	11.15	12.15	14.03	15.95	19.30	14.39	2.68	0.85	18.59	12.48	16.30			
2M	3R4F	10	0.60	4.50	10.55	15.50	19.20	9.86	6.18	1.95	62.67	5.44	14.27	-		
2M	THS2.2	10	1.10	7.70	11.08	12.80	16.65	10.31	4.97	1.57	48.18	6.75	13.86	*	-	
3M	Sham	10	1.95	10.65	13.13	19.50	30.35	14.24	8.36	2.64	58.73	8.25	20.22			
3M	3R4F	10	8.40	11.45	15.25	18.55	25.45	15.35	4.98	1.58	32.47	11.78	18.92	-		
3M	THS2.2	10	7.05	10.10	10.48	12.65	15.45	11.03	2.69	0.85	24.38	9.11	12.95	-	*	
3M	Cess	10	8.50	10.90	12.33	14.65	19.15	12.90	3.28	1.04	25.43	10.55	15.25	-	-	
3M	Switch	10	8.55	10.40	12.20	15.05	25.20	13.36	4.92	1.56	36.83	9.84	16.87	-	-	-
6M	Sham	9	4.45	7.50	10.10	12.50	28.05	12.02	7.53	2.51	62.60	6.24	17.81			
6M	3R4F	10	6.55	8.80	12.80	16.30	27.70	13.34	6.15	1.94	46.10	8.94	17.73	(-)		
6M	THS2.2	10	4.95	8.50	13.00	17.30	21.25	12.88	5.59	1.77	43.40	8.88	16.88	-	-	
6M	Cess	9	5.95	10.00	13.85	20.35	30.10	15.99	8.09	2.70	50.61	9.77	22.22	-	-	
6M	Switch	10	5.15	8.10	11.15	12.35	19.15	11.34	4.50	1.42	39.71	8.12	14.56	-	-	-
8M	Sham	10	6.15	9.85	12.08	12.50	13.35	11.00	2.19	0.69	19.96	9.43	12.56			
8M	3R4F	10	4.80	10.50	13.73	17.50	26.45	14.68	6.42	2.03	43.72	10.09	19.26	-		
8M	THS2.2	10	5.80	7.70	10.73	12.85	21.65	11.09	4.75	1.50	42.85	7.69	14.49	-	-	
8M	Cess	10	4.40	9.85	12.00	13.80	16.20	11.50	3.46	1.09	30.08	9.03	13.97	-	-	
8M	Switch	10	2.25	7.75	9.30	12.20	20.25	10.39	4.94	1.56	47.56	6.85	13.93	-	-	-

Table 69. Basophils, relative (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	0.00	0.05	0.15	0.65	0.12	0.20	0.06	166.90	-0.02	0.26			
1M	3R4F	10	0.00	0.00	0.05	0.10	0.40	0.09	0.12	0.04	146.86	-0.00	0.17	(-)		
1M	THS2.2	9	0.00	0.00	0.00	0.00	0.20	0.03	0.07	0.02	240.00	-0.02	0.08	(-)	(-)	
2M	Sham	10	0.00	0.00	0.00	0.15	0.25	0.07	0.11	0.03	166.37	-0.01	0.14			
2M	3R4F	10	0.00	0.00	0.03	0.15	0.20	0.07	0.08	0.02	120.54	0.01	0.12	(-)		
2M	THS2.2	10	0.00	0.00	0.00	0.00	0.15	0.02	0.05	0.02	316.23	-0.02	0.05	(-)	(-)	
3M	Sham	10	0.00	0.00	0.00	0.00	0.20	0.04	0.07	0.02	213.49	-0.02	0.09			
3M	3R4F	10	0.00	0.00	0.00	0.00	0.25	0.04	0.09	0.03	218.90	-0.02	0.10	(-)		
3M	THS2.2	10	0.00	0.00	0.00	0.00	0.15	0.02	0.05	0.02	316.23	-0.02	0.05	(-)	(-)	
3M	Cess	10	0.00	0.00	0.00	0.00	0.40	0.06	0.13	0.04	236.48	-0.04	0.15	(-)	(-)	
3M	Switch	10	0.00	0.00	0.00	0.00	0.35	0.06	0.12	0.04	220.40	-0.03	0.14	(-)	(-)	(-)
6M	Sham	9	0.00	0.00	0.15	0.25	0.70	0.19	0.24	0.08	126.10	0.01	0.37			
6M	3R4F	10	0.00	0.00	0.00	0.20	0.45	0.10	0.16	0.05	172.70	-0.02	0.21	(-)		
6M	THS2.2	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.	.	.	+	(-)	
6M	Cess	9	0.00	0.00	0.00	0.00	5.40	0.61	1.80	0.60	293.91	-0.77	1.99	(-)	(-)	
6M	Switch	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.	.	.	+	(-)	(-)
8M	Sham	10	0.00	0.00	0.00	0.00	0.25	0.03	0.08	0.03	316.23	-0.03	0.08			
8M	3R4F	10	0.00	0.00	0.00	0.00	0.95	0.12	0.30	0.10	251.69	-0.10	0.34	(-)		
8M	THS2.2	10	0.00	0.00	0.00	0.00	0.15	0.03	0.05	0.02	216.02	-0.01	0.06	(-)	(-)	
8M	Cess	10	0.00	0.00	0.00	0.10	0.70	0.09	0.22	0.07	242.59	-0.07	0.25	(-)	(-)	
8M	Switch	10	0.00	0.00	0.00	0.20	0.35	0.08	0.13	0.04	156.46	-0.01	0.17	(-)	(-)	(-)

Table 70. Eosinophils, relative (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.80	1.90	2.38	3.95	4.40	2.80	1.22	0.38	43.50	1.93	3.66			
1M	3R4F	10	1.60	1.90	2.18	2.65	4.25	2.37	0.76	0.24	32.30	1.82	2.91	-		
1M	THS2.2	9	0.65	1.30	2.15	2.40	2.70	1.86	0.73	0.24	39.27	1.30	2.42	-	-	
2M	Sham	10	0.25	0.70	1.83	2.60	2.90	1.75	0.98	0.31	55.94	1.05	2.44			
2M	3R4F	10	1.35	1.85	2.78	4.10	4.85	2.97	1.27	0.40	42.86	2.06	3.88	*		
2M	THS2.2	10	0.65	0.85	2.25	2.65	3.80	2.03	1.04	0.33	51.00	1.29	2.77	-	-	
3M	Sham	10	0.40	0.95	1.55	2.20	4.55	1.76	1.18	0.37	67.08	0.92	2.60			
3M	3R4F	10	0.75	0.80	1.30	1.55	2.65	1.36	0.62	0.19	45.10	0.92	1.81	(-)		
3M	THS2.2	10	0.00	0.70	1.15	2.55	2.95	1.43	1.05	0.33	73.05	0.69	2.18	-	-	
3M	Cess	10	0.15	0.70	0.83	2.35	3.30	1.28	1.07	0.34	83.34	0.52	2.04	(-)	(-)	
3M	Switch	10	0.70	1.35	1.75	2.00	4.85	2.03	1.20	0.38	59.16	1.17	2.88	(-)	(-)	(-)
6M	Sham	9	0.70	1.25	2.75	3.95	5.40	2.78	1.67	0.56	59.98	1.50	4.06			
6M	3R4F	10	0.30	0.80	1.45	1.75	4.90	1.74	1.36	0.43	77.90	0.77	2.71	-		
6M	THS2.2	10	0.50	0.90	1.60	1.90	4.00	1.63	1.00	0.32	61.30	0.91	2.34	-	(-)	
6M	Cess	9	0.40	1.35	1.40	1.75	2.60	1.47	0.67	0.22	45.67	0.95	1.98	-	-	
6M	Switch	10	0.40	1.00	1.58	3.05	4.00	1.87	1.18	0.37	63.14	1.02	2.71	-	(-)	-
8M	Sham	10	0.45	0.80	1.18	1.75	3.85	1.53	1.10	0.35	72.04	0.74	2.31			
8M	3R4F	10	0.30	0.95	1.43	1.50	2.90	1.44	0.73	0.23	50.84	0.92	1.97	-		
8M	THS2.2	10	0.90	1.40	1.60	2.45	3.20	1.83	0.71	0.22	38.73	1.32	2.33	(-)	-	
8M	Cess	10	0.00	1.20	1.63	2.00	3.15	1.59	0.86	0.27	53.98	0.98	2.20	-	-	
8M	Switch	10	0.70	0.90	1.35	4.10	4.30	2.10	1.51	0.48	72.16	1.01	3.18	(-)	-	-

Table 71. Reticulocyte counts (10^3 cells/ul)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.31	0.36	0.45	0.49	0.74	0.45	0.12	0.04	27.40	0.36	0.54			
1M	3R4F	10	0.14	0.15	0.16	0.19	0.29	0.18	0.05	0.01	25.77	0.15	0.22	+++		
1M	THS2.2	9	0.33	0.40	0.43	0.48	0.59	0.44	0.09	0.03	20.80	0.37	0.51	-	+++	
2M	Sham	10	0.33	0.35	0.40	0.44	0.49	0.40	0.06	0.02	13.93	0.36	0.44			
2M	3R4F	10	0.10	0.17	0.18	0.20	0.32	0.19	0.06	0.02	32.28	0.14	0.23	***		
2M	THS2.2	10	0.33	0.39	0.43	0.48	1.56	0.54	0.36	0.12	67.71	0.28	0.80	(-)	+++	
3M	Sham	10	0.22	0.35	0.43	0.50	0.68	0.44	0.14	0.05	33.06	0.33	0.54			
3M	3R4F	10	0.35	0.38	0.52	0.73	0.93	0.58	0.22	0.07	38.53	0.42	0.73	-		
3M	THS2.2	10	0.32	0.43	0.47	0.55	0.62	0.48	0.09	0.03	18.75	0.42	0.55	-	-	
3M	Cess	10	0.42	0.48	0.59	0.63	0.67	0.56	0.09	0.03	16.10	0.50	0.63	*	-	
3M	Switch	10	0.43	0.46	0.49	0.56	0.67	0.52	0.08	0.02	14.95	0.47	0.58	-	(-)	-
6M	Sham	9	0.36	0.41	0.45	0.57	0.69	0.50	0.12	0.04	23.74	0.41	0.60			
6M	3R4F	10	0.31	0.34	0.51	0.67	0.79	0.52	0.19	0.06	36.17	0.39	0.66	(-)		
6M	THS2.2	10	0.36	0.39	0.41	0.44	0.48	0.41	0.04	0.01	9.47	0.39	0.44	-	-	
6M	Cess	9	0.32	0.33	0.36	0.46	0.50	0.39	0.07	0.02	18.61	0.34	0.45	(-)	(-)	
6M	Switch	10	0.34	0.37	0.42	0.47	0.54	0.43	0.06	0.02	14.33	0.38	0.47	-	-	-
8M	Sham	10	0.33	0.37	0.45	0.53	0.55	0.45	0.08	0.03	17.99	0.39	0.51			
8M	3R4F	10	0.39	0.47	0.73	0.84	0.92	0.67	0.21	0.07	31.52	0.52	0.82	+		
8M	THS2.2	10	0.31	0.37	0.47	0.58	1.11	0.52	0.23	0.07	44.17	0.36	0.68	-	-	
8M	Cess	10	0.37	0.46	0.48	0.51	0.65	0.48	0.07	0.02	15.13	0.43	0.54	-	*	
8M	Switch	10	0.34	0.43	0.49	0.54	0.86	0.51	0.14	0.04	27.77	0.41	0.61	-	-	(-)

Table 72. Reticulocytes, relative (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	3.10	3.51	4.41	4.96	7.63	4.53	1.29	0.41	28.54	3.60	5.45			
1M	3R4F	10	1.29	1.34	1.48	1.64	2.78	1.64	0.47	0.15	28.61	1.31	1.98	+++		
1M	THS2.2	9	3.40	4.12	4.39	4.70	6.49	4.55	0.98	0.33	21.50	3.80	5.30	(-)	+++	
2M	Sham	10	3.61	3.70	4.40	4.97	5.40	4.38	0.64	0.20	14.71	3.92	4.84			
2M	3R4F	10	0.93	1.53	1.68	2.03	3.30	1.78	0.65	0.20	36.29	1.32	2.24	***		
2M	THS2.2	10	3.40	4.33	4.61	5.27	25.86	6.74	6.75	2.13	100.09	1.91	11.57	(-)	+++	
3M	Sham	10	2.63	3.68	4.41	4.97	7.18	4.59	1.43	0.45	31.16	3.57	5.61			
3M	3R4F	10	2.86	3.09	4.44	6.30	9.05	4.96	2.14	0.68	43.07	3.44	6.49	-		
3M	THS2.2	10	3.37	4.63	5.08	5.95	6.66	5.15	0.96	0.30	18.69	4.46	5.84	-	-	
3M	Cess	10	4.63	5.20	6.62	7.16	7.49	6.29	1.03	0.32	16.33	5.56	7.03	**	-	
3M	Switch	10	4.96	5.29	5.39	6.10	7.57	5.72	0.80	0.25	13.99	5.15	6.29	*	(-)	-
6M	Sham	9	3.75	4.48	4.68	5.96	7.79	5.33	1.34	0.45	25.13	4.30	6.36			
6M	3R4F	10	2.74	3.06	4.34	6.24	7.11	4.61	1.69	0.54	36.78	3.39	5.82	-		
6M	THS2.2	10	3.66	4.33	4.46	4.92	5.23	4.52	0.49	0.15	10.77	4.17	4.87	-	-	
6M	Cess	9	3.21	3.44	3.66	5.02	5.76	4.19	0.94	0.31	22.35	3.47	4.91	+	(-)	
6M	Switch	10	3.40	3.96	4.51	4.78	5.87	4.45	0.73	0.23	16.46	3.93	4.98	-	-	-
8M	Sham	10	3.42	3.86	4.84	5.55	5.86	4.74	0.87	0.28	18.42	4.12	5.37			
8M	3R4F	10	3.57	4.07	6.85	7.23	7.72	5.90	1.74	0.55	29.44	4.66	7.14	(-)		
8M	THS2.2	10	3.30	3.88	4.57	6.08	13.59	5.56	3.01	0.95	54.11	3.41	7.72	-	-	
8M	Cess	10	3.94	4.36	4.89	5.17	5.99	4.91	0.68	0.22	13.93	4.42	5.40	-	-	
8M	Switch	10	3.48	4.49	5.23	5.77	10.62	5.60	1.95	0.62	34.87	4.20	7.00	-	-	-

Table 73.BALF free lung cells, total cell count (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	1.89	2.14	2.52	3.27	4.81	2.79	0.88	0.28	31.35	2.17	3.42			
1M	3R4F	10	3.85	5.68	14.25	17.87	19.52	12.68	6.18	1.95	48.73	8.26	17.10	***		
1M	THS2.2	10	1.46	2.58	2.92	3.52	4.05	2.83	0.83	0.26	29.32	2.24	3.42	-	***	
2M	Sham	10	1.90	2.33	3.03	3.57	4.10	3.03	0.76	0.24	25.18	2.49	3.58			
2M	3R4F	10	3.73	4.92	9.21	15.16	33.89	11.91	8.88	2.81	74.50	5.57	18.26	*		
2M	THS2.2	10	1.26	1.74	2.03	2.66	4.96	2.49	1.25	0.40	50.35	1.59	3.39	-	+++	
3M	Sham	10	1.67	1.83	2.48	2.82	3.09	2.39	0.53	0.17	22.38	2.00	2.77			
3M	3R4F	10	4.85	12.40	15.24	15.62	19.98	13.67	4.31	1.36	31.51	10.59	16.75	***		
3M	THS2.2	10	0.76	1.34	1.69	2.09	2.30	1.63	0.55	0.17	33.54	1.24	2.02	**	***	
3M	Cess	10	1.55	2.61	3.13	6.26	6.70	3.86	1.91	0.60	49.48	2.49	5.23	*	***	
3M	Switch	10	1.56	2.43	4.54	6.03	6.88	4.41	1.99	0.63	45.09	2.99	5.84	*	***	-
6M	Sham	9	1.31	2.49	2.71	3.32	9.39	3.40	2.35	0.78	69.31	1.59	5.20			
6M	3R4F	10	9.42	12.16	18.73	32.77	37.76	21.00	10.17	3.22	48.44	13.72	28.27	+++		
6M	THS2.2	10	1.28	2.21	3.20	3.46	4.23	2.94	0.85	0.27	28.75	2.34	3.55	-	***	
6M	Cess	10	1.58	1.89	2.81	4.59	6.24	3.27	1.60	0.51	48.79	2.13	4.42	(-)	+++	
6M	Switch	10	1.71	2.28	3.74	5.00	5.30	3.71	1.35	0.43	36.28	2.75	4.68	-	***	-
8M	Sham	10	1.78	1.89	1.95	3.69	4.85	2.56	1.10	0.35	42.90	1.78	3.35			
8M	3R4F	10	8.09	10.93	15.23	17.82	25.49	15.06	5.04	1.59	33.49	11.45	18.66	+++		
8M	THS2.2	10	0.87	1.34	2.34	2.69	3.83	2.22	1.02	0.32	45.86	1.49	2.95	-	***	
8M	Cess	10	1.06	1.99	2.36	2.70	3.48	2.39	0.71	0.22	29.81	1.88	2.89	-	***	
8M	Switch	10	1.23	2.13	2.62	3.26	3.78	2.68	0.76	0.24	28.51	2.13	3.22	-	***	-

Table 74. BALF alveolar macrophages, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	1.59	1.91	2.23	2.95	4.13	2.46	0.76	0.24	30.96	1.91	3.00			
1M	3R4F	10	2.57	4.00	7.58	8.84	9.29	6.40	2.63	0.83	41.16	4.52	8.28	***		
1M	THS2.2	10	1.03	2.06	2.40	2.64	3.58	2.33	0.75	0.24	32.40	1.79	2.87	-	***	
2M	Sham	10	1.72	1.93	2.62	3.19	3.61	2.60	0.65	0.20	24.94	2.13	3.06			
2M	3R4F	10	2.68	2.77	4.81	5.08	9.35	4.78	1.97	0.62	41.18	3.37	6.19	**		
2M	THS2.2	10	1.11	1.39	1.76	2.32	4.08	2.06	0.97	0.31	46.90	1.37	2.75	-	+++	
3M	Sham	10	1.08	1.30	1.77	2.34	2.60	1.82	0.57	0.18	31.45	1.41	2.23			
3M	3R4F	10	2.15	2.52	2.91	3.40	4.36	2.98	0.67	0.21	22.52	2.50	3.46	***		
3M	THS2.2	10	0.59	0.98	1.45	1.73	1.99	1.35	0.49	0.16	36.55	1.00	1.70	-	***	
3M	Cess	10	1.07	1.59	1.84	2.58	3.59	2.09	0.74	0.24	35.62	1.56	2.62	-	*	
3M	Switch	10	1.20	1.73	2.12	3.09	3.71	2.31	0.83	0.26	36.06	1.71	2.90	-	-	-
6M	Sham	9	1.09	1.97	2.43	2.90	4.39	2.53	0.97	0.32	38.14	1.79	3.27			
6M	3R4F	10	2.74	3.35	5.44	7.18	11.48	5.75	2.91	0.92	50.63	3.67	7.83	**		
6M	THS2.2	10	1.13	1.81	2.44	2.99	3.71	2.42	0.76	0.24	31.33	1.87	2.96	-	**	
6M	Cess	10	1.21	1.51	2.46	3.24	4.02	2.47	1.04	0.33	41.93	1.73	3.21	-	**	
6M	Switch	10	1.43	1.94	2.97	3.92	4.35	2.93	1.01	0.32	34.61	2.20	3.66	-	*	-
8M	Sham	10	1.48	1.54	1.63	2.14	4.26	2.06	0.93	0.29	45.26	1.39	2.73			
8M	3R4F	10	1.99	3.23	4.37	4.75	7.34	4.25	1.57	0.50	37.06	3.12	5.37	+++		
8M	THS2.2	10	0.65	0.72	1.85	2.43	2.95	1.77	0.87	0.28	49.29	1.15	2.39	-	***	
8M	Cess	10	0.85	1.57	1.82	2.30	2.53	1.87	0.51	0.16	27.58	1.50	2.23	-	***	
8M	Switch	10	0.98	1.52	1.88	2.82	3.00	2.04	0.70	0.22	34.47	1.54	2.54	(-)	**	-

Table 75. Alveolar dendritic cells, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.00	37.67	0.01	0.02			
1M	3R4F	10	0.05	0.17	0.44	0.54	0.68	0.39	0.23	0.07	58.32	0.23	0.55	***		
1M	THS2.2	10	0.01	0.01	0.02	0.02	0.08	0.02	0.02	0.01	82.97	0.01	0.04	(-)	***	
2M	Sham	10	0.02	0.02	0.03	0.04	0.07	0.04	0.02	0.01	49.67	0.02	0.05			
2M	3R4F	10	0.09	0.20	0.39	0.63	1.18	0.48	0.34	0.11	70.52	0.24	0.72	+++		
2M	THS2.2	10	0.01	0.02	0.02	0.03	0.11	0.03	0.03	0.01	84.59	0.01	0.05	(-)	+++	
3M	Sham	10	0.02	0.02	0.04	0.04	0.09	0.04	0.02	0.01	52.38	0.02	0.05			
3M	3R4F	10	0.15	0.41	0.50	0.65	0.80	0.50	0.19	0.06	38.02	0.37	0.64	***		
3M	THS2.2	10	0.01	0.01	0.02	0.02	0.03	0.02	0.01	0.00	36.64	0.01	0.02	**	***	
3M	Cess	10	0.05	0.06	0.12	0.33	0.45	0.18	0.14	0.04	78.60	0.08	0.28	+++	***	
3M	Switch	10	0.03	0.07	0.23	0.29	0.39	0.21	0.13	0.04	61.72	0.12	0.30	**	***	-
6M	Sham	9	0.02	0.02	0.02	0.04	0.09	0.04	0.03	0.01	72.83	0.02	0.06			
6M	3R4F	10	0.37	0.58	0.68	0.76	1.87	0.79	0.42	0.13	53.73	0.49	1.09	+++		
6M	THS2.2	10	0.01	0.03	0.05	0.07	0.08	0.05	0.02	0.01	51.15	0.03	0.06	-	***	
6M	Cess	10	0.02	0.03	0.05	0.11	0.20	0.08	0.06	0.02	78.91	0.03	0.12	(-)	+++	
6M	Switch	10	0.02	0.03	0.06	0.11	0.12	0.07	0.04	0.01	57.53	0.04	0.10	+	+++	(-)
8M	Sham	10	0.01	0.03	0.03	0.06	0.08	0.04	0.02	0.01	53.59	0.02	0.05			
8M	3R4F	10	0.30	0.47	0.54	0.66	1.00	0.59	0.21	0.07	36.06	0.44	0.74	***		
8M	THS2.2	10	0.01	0.02	0.03	0.05	0.09	0.04	0.03	0.01	83.38	0.02	0.06	(-)	+++	
8M	Cess	10	0.01	0.03	0.04	0.06	0.08	0.04	0.02	0.01	51.69	0.03	0.06	-	***	
8M	Switch	10	0.02	0.04	0.05	0.07	0.12	0.06	0.03	0.01	52.91	0.03	0.08	-	***	-

Table 76. BALF neutrophils, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	46.11	0.00	0.01			
1M	3R4F	10	0.21	0.81	4.39	5.28	5.99	3.35	2.33	0.74	69.42	1.69	5.02	**		
1M	THS2.2	10	0.00	0.00	0.01	0.01	0.11	0.02	0.03	0.01	186.53	-0.01	0.04	(-)	+++	
2M	Sham	10	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	59.13	0.00	0.01			
2M	3R4F	10	0.34	0.86	1.69	4.21	16.92	3.73	4.97	1.57	133.38	0.17	7.28	+++		
2M	THS2.2	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	62.80	0.00	0.01	(-)	+++	
3M	Sham	10	0.01	0.01	0.02	0.02	0.10	0.03	0.03	0.01	102.73	0.01	0.04			
3M	3R4F	10	1.39	3.03	4.06	7.00	8.32	4.47	2.31	0.73	51.75	2.81	6.12	+++		
3M	THS2.2	10	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	39.07	0.01	0.01	+++	***	
3M	Cess	10	0.02	0.05	0.19	0.43	0.64	0.24	0.21	0.07	88.66	0.09	0.39	+++	***	
3M	Switch	10	0.04	0.08	0.40	0.71	0.80	0.40	0.31	0.10	77.19	0.18	0.62	+++	***	-
6M	Sham	9	0.00	0.00	0.00	0.01	0.05	0.01	0.02	0.01	143.18	-0.00	0.03			
6M	3R4F	10	1.99	2.47	4.19	6.47	13.01	5.07	3.44	1.09	67.80	2.61	7.53	+++		
6M	THS2.2	10	0.00	0.00	0.01	0.03	0.04	0.02	0.01	0.00	91.73	0.01	0.03	(-)	+++	
6M	Cess	10	0.00	0.01	0.02	0.05	0.08	0.03	0.03	0.01	96.58	0.01	0.04	(-)	+++	
6M	Switch	10	0.00	0.01	0.01	0.04	0.06	0.02	0.02	0.01	88.74	0.01	0.03	(-)	+++	(-)
8M	Sham	10	0.00	0.00	0.00	0.01	0.04	0.01	0.01	0.00	125.79	0.00	0.02			
8M	3R4F	10	2.14	2.34	2.84	4.52	5.81	3.41	1.29	0.41	37.76	2.49	4.33	+++		
8M	THS2.2	10	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.00	81.08	0.00	0.01	(-)	+++	
8M	Cess	10	0.00	0.00	0.01	0.01	0.03	0.01	0.01	0.00	92.53	0.00	0.01	(-)	+++	
8M	Switch	10	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.00	90.93	0.00	0.01	(-)	+++	(-)

Table 77. BALF lymphocytes, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.02	0.02	0.03	0.03	0.06	0.03	0.01	0.00	42.20	0.02	0.04			
1M	3R4F	10	0.09	0.25	0.43	0.56	0.90	0.42	0.25	0.08	59.47	0.24	0.60	+++		
1M	THS2.2	10	0.02	0.03	0.03	0.04	0.05	0.03	0.01	0.00	30.97	0.03	0.04	(-)	***	
2M	Sham	10	0.05	0.07	0.09	0.13	0.44	0.13	0.11	0.04	90.57	0.04	0.21			
2M	3R4F	10	0.21	0.52	0.74	1.64	1.85	1.01	0.64	0.20	63.39	0.55	1.46	+++		
2M	THS2.2	10	0.05	0.07	0.08	0.10	0.91	0.16	0.26	0.08	161.96	-0.03	0.35	(-)	+++	
3M	Sham	10	0.04	0.05	0.07	0.07	0.12	0.07	0.02	0.01	34.71	0.05	0.09			
3M	3R4F	10	0.26	0.98	1.31	1.91	2.46	1.38	0.64	0.20	46.43	0.92	1.84	***		
3M	THS2.2	10	0.02	0.03	0.04	0.05	0.06	0.04	0.01	0.00	36.21	0.03	0.05	++	***	
3M	Cess	10	0.05	0.10	0.15	0.23	0.35	0.16	0.09	0.03	57.72	0.09	0.22	++	***	
3M	Switch	10	0.02	0.10	0.18	0.27	0.40	0.19	0.12	0.04	61.92	0.10	0.27	*	***	-
6M	Sham	9	0.02	0.02	0.02	0.03	0.18	0.04	0.05	0.02	126.31	0.00	0.08			
6M	3R4F	10	0.74	1.15	1.60	1.72	2.96	1.67	0.71	0.23	42.91	1.15	2.18	+++		
6M	THS2.2	10	0.01	0.02	0.03	0.05	0.06	0.03	0.02	0.00	46.84	0.02	0.04	(-)	***	
6M	Cess	10	0.02	0.02	0.04	0.06	0.21	0.06	0.06	0.02	100.89	0.02	0.10	(-)	+++	
6M	Switch	10	0.02	0.02	0.06	0.07	0.07	0.05	0.02	0.01	50.74	0.03	0.07	-	***	-
8M	Sham	10	0.02	0.02	0.03	0.03	0.04	0.03	0.01	0.00	30.19	0.02	0.03			
8M	3R4F	10	0.65	0.72	0.77	1.04	1.80	0.93	0.34	0.11	36.84	0.68	1.17	***		
8M	THS2.2	10	0.01	0.02	0.02	0.04	0.04	0.02	0.01	0.00	41.27	0.02	0.03	-	+++	
8M	Cess	10	0.01	0.02	0.03	0.04	0.05	0.03	0.01	0.00	38.22	0.02	0.04	-	***	
8M	Switch	10	0.01	0.02	0.03	0.04	0.06	0.03	0.02	0.00	45.67	0.02	0.04	-	+++	-

Table 78. Lymphocyte differentiation assay, CD4+ relative to total lymphocytes (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	11.33	12.94	15.74	22.88	30.99	17.86	6.34	2.00	35.49	13.33	22.40			
1M	3R4F	10	21.74	25.93	27.02	31.25	51.21	29.64	8.42	2.66	28.41	23.62	35.66	++		
1M	THS2.2	10	11.59	13.64	20.14	29.92	58.54	24.89	14.93	4.72	59.98	14.21	35.56	(-)	(-)	
2M	Sham	10	11.31	13.18	14.34	16.14	39.32	18.05	9.26	2.93	51.31	11.42	24.67			
2M	3R4F	10	19.13	22.40	24.02	27.20	30.06	24.42	3.34	1.06	13.67	22.03	26.81	+		
2M	THS2.2	10	9.61	13.63	16.20	25.42	27.24	17.74	6.46	2.04	36.41	13.12	22.36	(-)	*	
3M	Sham	10	6.09	11.05	15.39	17.78	45.53	17.59	11.05	3.49	62.81	9.69	25.49			
3M	3R4F	10	17.43	20.13	25.28	27.14	33.65	24.61	5.42	1.71	22.01	20.74	28.49	-		
3M	THS2.2	8	9.09	9.28	11.03	13.01	23.10	12.35	4.65	1.64	37.65	8.46	16.24	(-)	***	
3M	Cess	10	15.24	16.13	18.41	24.25	26.16	19.66	4.16	1.31	21.13	16.69	22.63	(-)	*	
3M	Switch	10	16.32	17.13	20.47	23.36	28.92	21.15	4.55	1.44	21.50	17.90	24.40	(-)	-	(-)
6M	Sham	9	5.44	9.44	13.43	16.31	29.69	14.72	7.40	2.47	50.26	9.03	20.40			
6M	3R4F	10	19.61	23.35	24.50	27.48	28.84	24.89	2.89	0.91	11.61	22.83	26.96	**		
6M	THS2.2	10	7.07	7.73	16.27	27.23	37.82	18.67	10.77	3.41	57.69	10.96	26.37	-	-	
6M	Cess	10	11.69	16.28	20.53	23.35	36.90	20.81	7.17	2.27	34.45	15.68	25.94	-	-	
6M	Switch	10	11.91	14.36	15.94	21.21	30.67	18.31	5.69	1.80	31.09	14.24	22.38	(-)	**	-
8M	Sham	10	7.57	17.97	20.30	28.46	42.05	22.32	9.33	2.95	41.80	15.65	29.00			
8M	3R4F	10	17.07	21.25	22.93	24.49	29.07	23.08	3.41	1.08	14.75	20.65	25.52	-		
8M	THS2.2	8	6.38	14.21	22.02	24.05	24.40	18.92	7.12	2.52	37.61	12.97	24.87	-	-	
8M	Cess	9	10.01	16.42	18.36	20.26	35.23	20.05	7.42	2.47	37.00	14.35	25.75	(-)	-	
8M	Switch	9	15.79	22.38	23.61	29.55	52.39	27.08	10.61	3.54	39.20	18.92	35.24	(-)	-	(-)

Table 79. Lymphocyte differentiation assay, CD8+ relative to total lymphocytes (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.46	1.03	2.57	2.80	3.13	2.02	1.05	0.33	52.13	1.27	2.77			
1M	3R4F	10	1.88	3.67	5.15	7.49	17.13	6.08	4.26	1.35	70.19	3.02	9.13	*		
1M	THS2.2	10	0.00	1.93	2.74	3.91	12.50	3.33	3.53	1.12	105.79	0.81	5.86	-	+	
2M	Sham	10	1.36	2.24	3.43	3.86	10.77	3.82	2.65	0.84	69.45	1.92	5.72			
2M	3R4F	10	4.83	6.48	8.31	14.14	17.75	9.98	4.33	1.37	43.40	6.88	13.08	+++		
2M	THS2.2	10	1.43	2.50	3.51	5.83	8.61	4.30	2.48	0.78	57.59	2.53	6.07	(-)	++	
3M	Sham	10	1.00	1.55	2.62	4.49	7.27	3.16	2.02	0.64	63.89	1.71	4.60			
3M	3R4F	10	1.73	5.59	9.18	11.32	13.62	8.39	3.62	1.14	43.13	5.80	10.97	**		
3M	THS2.2	8	1.14	1.26	1.53	3.17	5.60	2.33	1.58	0.56	67.76	1.01	3.65	(-)	***	
3M	Cess	10	1.28	3.40	4.90	7.05	13.87	5.67	3.60	1.14	63.47	3.10	8.24	(-)	-	
3M	Switch	10	2.35	3.04	4.83	6.84	7.43	4.82	1.89	0.60	39.24	3.47	6.18	-	*	-
6M	Sham	9	1.00	1.37	3.58	4.55	5.57	3.20	1.74	0.58	54.37	1.86	4.54			
6M	3R4F	10	8.56	11.22	13.25	13.99	15.36	12.44	2.23	0.71	17.92	10.85	14.04	***		
6M	THS2.2	10	1.66	2.12	3.28	5.03	8.71	3.79	2.21	0.70	58.28	2.21	5.37	-	***	
6M	Cess	10	3.01	5.05	6.02	7.82	9.76	6.30	2.08	0.66	33.03	4.81	7.79	**	***	
6M	Switch	10	2.61	3.75	6.36	8.38	9.13	6.07	2.28	0.72	37.51	4.44	7.70	**	***	-
8M	Sham	10	3.24	3.85	5.93	6.56	10.02	5.96	2.26	0.72	37.95	4.34	7.58			
8M	3R4F	10	10.41	13.86	15.96	17.41	19.20	15.29	2.74	0.87	17.91	13.33	17.25	***		
8M	THS2.2	8	2.01	3.39	7.28	11.13	13.36	7.37	4.32	1.53	58.59	3.76	10.98	-	***	
8M	Cess	9	1.07	2.29	6.54	8.00	12.69	6.06	4.19	1.40	69.17	2.84	9.28	-	***	
8M	Switch	9	2.43	7.16	8.24	10.45	12.35	8.34	3.08	1.03	36.89	5.97	10.70	-	***	-

Table 80. Lymphocyte differentiation assay, B cells relative to total lymphocytes (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.63	0.69	0.96	2.82	3.86	1.60	1.17	0.37	73.17	0.76	2.43			
1M	3R4F	10	1.83	3.96	6.55	8.99	17.36	7.44	5.10	1.61	68.50	3.80	11.09	+++		
1M	THS2.2	10	0.00	0.33	0.85	4.72	24.62	5.37	9.13	2.89	169.89	-1.16	11.90	(-)	(-)	
2M	Sham	10	0.12	0.52	0.69	1.50	2.81	1.06	0.91	0.29	86.18	0.41	1.71			
2M	3R4F	10	2.55	4.42	6.12	13.99	22.27	8.71	6.18	1.95	70.93	4.29	13.13	+++		
2M	THS2.2	10	0.21	0.39	1.06	2.38	26.91	3.82	8.18	2.59	214.11	-2.03	9.67	(-)	++	
3M	Sham	10	0.38	0.56	0.68	1.28	18.71	3.40	6.10	1.93	179.35	-0.96	7.76			
3M	3R4F	10	3.06	5.14	8.08	8.73	10.56	7.26	2.53	0.80	34.88	5.45	9.07	-		
3M	THS2.2	8	0.05	0.25	0.56	1.41	8.81	1.66	2.94	1.04	176.79	-0.79	4.12	(-)	***	
3M	Cess	10	4.51	6.66	9.57	19.07	22.72	11.95	6.48	2.05	54.19	7.32	16.59	++	-	
3M	Switch	10	1.41	5.39	8.14	19.23	23.76	11.19	7.70	2.44	68.84	5.68	16.70	++	-	(-)
6M	Sham	9	0.52	1.01	1.50	4.48	5.89	2.52	2.10	0.70	83.33	0.91	4.14			
6M	3R4F	10	5.02	7.61	12.25	14.98	21.13	11.60	5.08	1.61	43.75	7.97	15.23	***		
6M	THS2.2	10	0.46	2.03	2.79	5.28	9.03	3.55	2.79	0.88	78.54	1.56	5.55	(-)	***	
6M	Cess	10	2.28	3.17	6.12	11.44	15.30	7.40	4.74	1.50	64.12	4.00	10.79	++	-	
6M	Switch	10	2.51	4.32	7.27	9.10	14.93	7.37	3.84	1.21	52.12	4.62	10.12	**	-	-
8M	Sham	10	0.00	0.82	2.96	6.28	9.52	3.54	3.26	1.03	92.02	1.21	5.87			
8M	3R4F	10	4.09	10.10	11.64	13.19	19.13	11.32	4.05	1.28	35.76	8.43	14.22	***		
8M	THS2.2	8	0.67	3.52	5.28	8.17	16.42	6.38	4.84	1.71	75.94	2.33	10.43	-	*	
8M	Cess	9	0.57	1.37	5.96	7.19	12.28	5.38	4.53	1.51	84.25	1.90	8.87	(-)	**	
8M	Switch	9	3.27	5.97	7.60	10.31	20.61	9.06	5.49	1.83	60.63	4.84	13.28	*	-	-

Table 81. Lymphocyte differentiation assay, lymphocytes, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.02	0.03	0.03	0.04	0.05	0.04	0.01	0.00	31.60	0.03	0.04			
1M	3R4F	10	0.21	0.31	1.14	1.68	1.94	1.07	0.66	0.21	61.83	0.59	1.54	***		
1M	THS2.2	10	0.02	0.03	0.05	0.10	0.58	0.13	0.18	0.06	141.01	-0.00	0.25	(-)	**	
2M	Sham	10	0.04	0.06	0.08	0.12	0.45	0.12	0.12	0.04	99.16	0.04	0.21			
2M	3R4F	10	0.19	0.64	1.32	2.61	2.76	1.53	1.00	0.32	65.37	0.82	2.25	+++		
2M	THS2.2	10	0.04	0.04	0.06	0.10	1.12	0.17	0.33	0.11	198.77	-0.07	0.41	(-)	+++	
3M	Sham	10	0.04	0.06	0.07	0.09	1.01	0.17	0.30	0.09	179.65	-0.05	0.38			
3M	3R4F	10	0.47	1.72	2.12	2.93	3.81	2.24	1.04	0.33	46.38	1.50	2.98	+++		
3M	THS2.2	8	0.02	0.03	0.05	0.07	0.08	0.05	0.02	0.01	43.47	0.03	0.07	(-)	***	
3M	Cess	10	0.12	0.19	0.71	1.34	2.35	0.86	0.74	0.23	85.88	0.33	1.39	+++	**	
3M	Switch	10	0.07	0.17	0.95	1.34	1.86	0.89	0.62	0.19	69.02	0.45	1.33	+++	**	-
6M	Sham	9	0.08	0.12	0.15	0.29	0.59	0.22	0.16	0.05	71.96	0.10	0.35			
6M	3R4F	10	2.10	3.10	4.16	5.49	13.34	4.91	3.19	1.01	64.95	2.63	7.19	+++		
6M	THS2.2	10	0.05	0.19	0.23	0.44	0.71	0.29	0.20	0.06	68.39	0.15	0.43	(-)	+++	
6M	Cess	10	0.08	0.16	0.25	0.85	1.29	0.46	0.41	0.13	90.35	0.16	0.76	(-)	+++	
6M	Switch	10	0.11	0.20	0.31	0.61	0.83	0.38	0.25	0.08	66.11	0.20	0.57	(-)	+++	(-)
8M	Sham	10	0.04	0.11	0.13	0.21	1.13	0.23	0.32	0.10	137.56	0.00	0.46			
8M	3R4F	10	2.09	2.32	3.00	3.63	5.53	3.28	1.20	0.38	36.60	2.42	4.14	+++		
8M	THS2.2	8	0.05	0.06	0.19	0.31	0.62	0.22	0.20	0.07	88.05	0.06	0.39	(-)	+++	
8M	Cess	9	0.07	0.13	0.15	0.20	0.72	0.21	0.20	0.07	92.80	0.06	0.37	(-)	+++	
8M	Switch	9	0.14	0.18	0.34	0.46	0.67	0.34	0.18	0.06	52.83	0.20	0.48	+	+++	(-)

Table 82. Lymphocyte differentiation assay, CD4+ cells, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	55.15	0.00	0.01			
1M	3R4F	10	0.07	0.11	0.30	0.40	0.68	0.31	0.20	0.06	66.68	0.16	0.45	+++		
1M	THS2.2	10	0.00	0.00	0.01	0.03	0.34	0.05	0.11	0.03	195.82	-0.02	0.13	(-)	+++	
2M	Sham	10	0.01	0.01	0.01	0.02	0.18	0.03	0.05	0.02	171.63	-0.01	0.07			
2M	3R4F	10	0.04	0.18	0.30	0.59	0.75	0.37	0.24	0.08	65.65	0.20	0.55	+++		
2M	THS2.2	10	0.00	0.01	0.01	0.02	0.29	0.04	0.09	0.03	229.83	-0.02	0.10	(-)	+++	
3M	Sham	10	0.00	0.01	0.01	0.02	0.46	0.05	0.14	0.04	258.24	-0.05	0.16			
3M	3R4F	10	0.08	0.48	0.55	0.74	0.93	0.55	0.25	0.08	45.82	0.37	0.73	+++		
3M	THS2.2	8	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	54.39	0.00	0.01	(-)	***	
3M	Cess	10	0.02	0.03	0.17	0.25	0.38	0.17	0.13	0.04	78.31	0.07	0.26	++	***	
3M	Switch	10	0.02	0.04	0.22	0.23	0.38	0.17	0.12	0.04	66.79	0.09	0.26	++	***	-
6M	Sham	9	0.00	0.01	0.02	0.03	0.17	0.04	0.05	0.02	128.60	0.00	0.08			
6M	3R4F	10	0.55	0.85	1.06	1.14	3.20	1.20	0.76	0.24	62.91	0.66	1.75	+++		
6M	THS2.2	10	0.00	0.02	0.04	0.09	0.27	0.07	0.08	0.03	117.73	0.01	0.13	(-)	+++	
6M	Cess	10	0.01	0.04	0.05	0.20	0.33	0.11	0.11	0.04	103.38	0.03	0.19	(-)	+++	
6M	Switch	10	0.01	0.03	0.05	0.12	0.19	0.08	0.06	0.02	82.14	0.03	0.12	(-)	+++	(-)
8M	Sham	10	0.01	0.02	0.03	0.05	0.48	0.07	0.14	0.05	195.84	-0.03	0.17			
8M	3R4F	10	0.44	0.55	0.69	0.76	1.61	0.77	0.36	0.11	46.97	0.51	1.03	+++		
8M	THS2.2	8	0.00	0.01	0.04	0.07	0.14	0.05	0.05	0.02	95.54	0.01	0.09	(-)	+++	
8M	Cess	9	0.01	0.02	0.03	0.04	0.25	0.05	0.08	0.03	144.38	-0.01	0.11	(-)	+++	
8M	Switch	9	0.02	0.04	0.10	0.12	0.16	0.09	0.05	0.02	54.16	0.05	0.13	(-)	***	-

Table 83. Lymphocyte differentiation assay, CD8+ cells, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	59.55	0.00	0.00			
1M	3R4F	10	0.01	0.03	0.05	0.09	0.13	0.06	0.04	0.01	70.04	0.03	0.08	**		
1M	THS2.2	10	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	117.53	0.00	0.01	-	+++	
2M	Sham	10	0.00	0.00	0.00	0.00	0.05	0.01	0.01	0.00	199.91	-0.00	0.02			
2M	3R4F	10	0.02	0.04	0.12	0.23	0.49	0.17	0.16	0.05	94.59	0.05	0.28	+++		
2M	THS2.2	10	0.00	0.00	0.00	0.00	0.10	0.01	0.03	0.01	252.32	-0.01	0.03	(-)	+++	
3M	Sham	10	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	183.06	-0.00	0.01			
3M	3R4F	10	0.01	0.12	0.22	0.26	0.35	0.19	0.10	0.03	52.65	0.12	0.26	+++		
3M	THS2.2	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	48.42	0.00	0.00	+	***	
3M	Cess	10	0.00	0.01	0.05	0.08	0.12	0.05	0.04	0.01	85.41	0.02	0.08	+++	**	
3M	Switch	10	0.00	0.01	0.04	0.06	0.09	0.04	0.03	0.01	75.47	0.02	0.07	+++	**	-
6M	Sham	9	0.00	0.00	0.00	0.02	0.03	0.01	0.01	0.00	106.46	0.00	0.02			
6M	3R4F	10	0.27	0.31	0.53	0.72	1.78	0.62	0.45	0.14	71.90	0.30	0.94	+++		
6M	THS2.2	10	0.00	0.00	0.01	0.01	0.06	0.01	0.02	0.01	131.99	0.00	0.03	(-)	+++	
6M	Cess	10	0.00	0.01	0.02	0.04	0.13	0.03	0.04	0.01	115.22	0.01	0.06	(-)	+++	
6M	Switch	10	0.00	0.01	0.02	0.04	0.08	0.03	0.02	0.01	88.10	0.01	0.05	(-)	+++	(-)
8M	Sham	10	0.00	0.01	0.01	0.01	0.11	0.02	0.03	0.01	184.51	-0.01	0.04			
8M	3R4F	10	0.33	0.37	0.44	0.58	0.82	0.49	0.15	0.05	31.48	0.38	0.60	+++		
8M	THS2.2	8	0.00	0.00	0.02	0.03	0.08	0.02	0.03	0.01	118.28	0.00	0.04	(-)	+++	
8M	Cess	9	0.00	0.00	0.01	0.01	0.09	0.02	0.03	0.01	155.27	-0.00	0.04	(-)	+++	
8M	Switch	9	0.01	0.02	0.02	0.04	0.07	0.03	0.02	0.01	71.94	0.01	0.05	+	***	(-)

Table 84. Lymphocyte differentiation assay, B cells, absolute (10^5 cells)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	81.53	0.00	0.00			
1M	3R4F	10	0.00	0.02	0.07	0.13	0.26	0.09	0.09	0.03	96.74	0.03	0.16	+++		
1M	THS2.2	10	0.00	0.00	0.00	0.01	0.06	0.01	0.02	0.01	196.17	-0.00	0.02	(-)	+++	
2M	Sham	10	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	179.40	-0.00	0.00			
2M	3R4F	10	0.01	0.04	0.10	0.20	0.37	0.14	0.13	0.04	95.10	0.04	0.24	+++		
2M	THS2.2	10	0.00	0.00	0.00	0.00	0.30	0.03	0.09	0.03	307.17	-0.04	0.10	(-)	+++	
3M	Sham	10	0.00	0.00	0.00	0.00	0.19	0.02	0.06	0.02	298.85	-0.02	0.06			
3M	3R4F	10	0.01	0.11	0.17	0.25	0.33	0.17	0.09	0.03	55.55	0.10	0.24	+++		
3M	THS2.2	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	189.07	-0.00	0.00	(-)	***	
3M	Cess	10	0.01	0.02	0.04	0.22	0.32	0.11	0.12	0.04	105.13	0.03	0.19	+++	-	
3M	Switch	10	0.00	0.03	0.06	0.15	0.26	0.10	0.09	0.03	91.92	0.03	0.17	++	-	(-)
6M	Sham	9	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.00	108.99	0.00	0.01			
6M	3R4F	10	0.12	0.28	0.45	0.87	2.04	0.62	0.57	0.18	91.50	0.22	1.03	+++		
6M	THS2.2	10	0.00	0.00	0.01	0.01	0.06	0.01	0.02	0.01	139.66	0.00	0.03	(-)	+++	
6M	Cess	10	0.00	0.00	0.02	0.10	0.20	0.05	0.06	0.02	128.68	0.00	0.10	+	+++	
6M	Switch	10	0.00	0.01	0.03	0.04	0.10	0.03	0.03	0.01	91.66	0.01	0.06	+	+++	(-)
8M	Sham	10	0.00	0.00	0.00	0.01	0.05	0.01	0.02	0.00	145.76	-0.00	0.02			
8M	3R4F	10	0.09	0.30	0.35	0.38	0.97	0.38	0.23	0.07	60.14	0.22	0.54	+++		
8M	THS2.2	8	0.00	0.00	0.01	0.04	0.06	0.02	0.03	0.01	130.73	-0.00	0.04	(-)	+++	
8M	Cess	9	0.00	0.00	0.01	0.02	0.09	0.02	0.03	0.01	157.97	-0.00	0.04	(-)	+++	
8M	Switch	9	0.00	0.01	0.03	0.04	0.14	0.04	0.04	0.01	111.54	0.01	0.07	+	+++	(-)

Table 85. Macrophage activation assay, CD54 positive (median fluorescent intensity)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	7	3429.0	3703.0	3979.0	4170.0	4293.0	3925.9	298.39	112.78	7.60	3649.9	4201.8			
1M	3R4F	10	12412	14074	17760	20698	21918	17770	3467.2	1096.4	19.51	15290	20250	***		
1M	THS2.2	8	3171.0	4111.0	4376.0	5329.5	6119.0	4615.4	979.09	346.16	21.21	3796.8	5433.9	-	***	
2M	Sham	5	3450.0	3635.0	3896.0	4064.0	4423.0	3893.6	378.47	169.26	9.72	3423.7	4363.5			
2M	3R4F	10	13347	17041	19150	21263	25686	19212	3363.3	1063.6	17.51	16806	21618	***		
2M	THS2.2	2	4359.0	4359.0	5381.0	6403.0	6403.0	5381.0	1445.3	1022.0	26.86	-7605	18367	-	***	
3M	Sham	2	5150.0	5150.0	5316.0	5482.0	5482.0	5316.0	234.76	166.00	4.42	3206.8	7425.2			
3M	3R4F	10	18246	23944	26970	29089	32114	25957	4389.7	1388.1	16.91	22817	29097	***		
3M	Cess	5	10700	11489	12467	13007	17292	12991	2563.1	1146.3	19.73	9808.5	16174	**	***	
3M	Switch	7	7849.0	11144	11498	12383	12425	11241	1585.0	599.06	14.10	9775.3	12707	(-)	+++	-
6M	Sham	5	2140.0	3544.0	3999.0	4526.0	7184.0	4278.6	1850.3	827.47	43.24	1981.2	6576.0			
6M	3R4F	10	14954	16250	20810	26527	28246	21172	5254.7	1661.7	24.82	17413	24931	***		
6M	THS2.2	7	1868.0	2214.0	5081.0	5322.0	7687.0	4374.6	2044.8	772.86	46.74	2483.5	6265.7	-	***	
6M	Cess	7	2432.0	2566.0	6619.0	9704.0	15219	7080.7	4426.3	1673.0	62.51	2987.0	11174	-	***	
6M	Switch	8	2655.0	3152.5	5009.5	6001.5	7483.0	4808.1	1750.9	619.02	36.41	3344.4	6271.9	-	***	-
8M	Sham	10	5224.0	6261.0	6585.0	7762.0	16062	7693.7	3124.1	987.93	40.61	5458.8	9928.6			
8M	3R4F	10	19146	31775	34999	49511	77225	39681	16671	5271.9	42.01	27755	51607	+++		
8M	THS2.2	7	5281.0	6626.0	8102.0	9838.0	12435	8458.0	2373.5	897.11	28.06	6262.9	10653	(-)	***	
8M	Cess	9	5447.0	7267.0	7431.0	9003.0	10659	7991.1	1567.5	522.50	19.62	6786.2	9196.0	(-)	***	
8M	Switch	9	5871.0	7001.0	7652.0	8701.0	13734	8384.8	2310.3	770.09	27.55	6608.9	10161	(-)	+++	-

Table 86. Macrophage activity assay, CD86 positive (median fluorescent intensity)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	7	358.00	564.00	689.00	817.00	1246.0	725.57	276.33	104.44	38.08	470.01	981.13			
1M	3R4F	10	481.00	572.00	757.50	921.00	1100.0	761.40	218.15	68.98	28.65	605.35	917.45	-		
1M	THS2.2	8	242.00	373.00	588.00	975.00	1387.0	687.63	398.12	140.76	57.90	354.79	1020.5	-	-	
2M	Sham	5	736.00	870.00	878.00	1215.0	1440.0	1027.8	290.45	129.89	28.26	667.16	1388.4			
2M	3R4F	10	755.00	913.00	1137.5	1291.0	1551.0	1150.3	273.13	86.37	23.74	954.91	1345.7	-		
2M	THS2.2	2	810.00	810.00	1273.5	1737.0	1737.0	1273.5	655.49	463.50	51.47	-4616	7162.8	-	-	
3M	Sham	2	329.00	329.00	536.50	744.00	744.00	536.50	293.45	207.50	54.70	-2100	3173.0			
3M	3R4F	10	1073.0	1855.0	2321.5	2763.0	3584.0	2278.6	722.40	228.44	31.70	1761.8	2795.4	**		
3M	Cess	5	1416.0	1699.0	1764.0	1923.0	2560.0	1872.4	425.87	190.46	22.74	1343.6	2401.2	*	-	
3M	Switch	7	904.00	1115.0	1497.0	1841.0	1983.0	1441.4	397.59	150.27	27.58	1073.7	1809.1	-	**	-
6M	Sham	5	104.00	242.00	266.00	378.00	526.00	303.20	158.15	70.73	52.16	106.83	499.57			
6M	3R4F	10	836.00	1002.0	1298.5	1906.0	2593.0	1526.7	644.97	203.96	42.25	1065.3	1988.1	***		
6M	THS2.2	7	95.00	106.00	224.00	336.00	582.00	254.86	167.45	63.29	65.71	99.99	409.73	-	+++	
6M	Cess	7	162.00	169.00	280.00	624.00	2637.0	637.57	895.36	338.41	140.43	-190.5	1465.6	(-)	+	
6M	Switch	8	118.00	185.50	404.50	437.50	969.00	392.75	267.82	94.69	68.19	168.85	616.65	-	+++	(-)
8M	Sham	10	-131.0	223.00	318.50	361.00	723.00	287.40	221.79	70.14	77.17	128.74	446.06			
8M	3R4F	10	608.00	900.00	1045.0	1546.0	4720.0	1506.5	1213.2	383.65	80.53	638.62	2374.4	+++		
8M	THS2.2	7	-8.00	53.00	73.00	623.00	669.00	261.14	287.33	108.60	110.03	-4.59	526.88	-	+++	
8M	Cess	9	-117.0	22.00	149.00	469.00	600.00	227.44	261.06	87.02	114.78	26.77	428.11	-	+++	
8M	Switch	9	125.00	167.00	263.00	522.00	1464.0	415.56	421.52	140.51	101.44	91.55	739.56	(-)	+++	(-)

Table 87. Macrophage activity assay, CD11b positive (median fluorescent intensity)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	7	817.00	916.00	1061.0	1171.0	1394.0	1063.0	187.35	70.81	17.62	889.73	1236.3			
1M	3R4F	10	1589.0	1851.0	3491.5	4288.0	6616.0	3398.7	1586.7	501.76	46.69	2263.6	4533.8	**		
1M	THS2.2	8	719.00	892.00	1038.0	1290.5	1474.0	1079.3	254.24	89.89	23.56	866.70	1291.8	-	**	
2M	Sham	5	1110.0	1150.0	1172.0	1272.0	1311.0	1203.0	84.92	37.98	7.06	1097.6	1308.4			
2M	3R4F	10	2269.0	2897.0	4366.0	6079.0	9039.0	4807.9	2265.4	716.38	47.12	3187.3	6428.5	***		
2M	THS2.2	2	1314.0	1314.0	1408.5	1503.0	1503.0	1408.5	133.64	94.50	9.49	207.76	2609.2	-	**	
3M	Sham	2	490.00	490.00	506.00	522.00	522.00	506.00	22.63	16.00	4.47	302.70	709.30			
3M	3R4F	10	2610.0	5501.0	8417.5	9673.0	11111	7673.6	3015.3	953.51	39.29	5516.6	9830.6	***		
3M	Cess	5	2126.0	2264.0	2679.0	3200.0	5565.0	3166.8	1404.3	628.04	44.35	1423.1	4910.5	*	**	
3M	Switch	7	1841.0	1979.0	2777.0	3936.0	4363.0	2957.6	965.25	364.83	32.64	2064.9	3850.3	***	***	-
6M	Sham	5	154.00	239.00	264.00	310.00	465.00	286.40	114.82	51.35	40.09	143.83	428.97			
6M	3R4F	10	2956.0	4027.0	5040.0	6808.0	13933	6474.7	3974.3	1256.8	61.38	3631.6	9317.8	+++		
6M	THS2.2	7	205.00	222.00	337.00	510.00	512.00	352.14	123.09	46.52	34.96	238.30	465.98	-	+++	
6M	Cess	7	87.00	121.00	196.00	482.00	3909.0	741.14	1402.9	530.26	189.29	-556.3	2038.6	(-)	+++	
6M	Switch	8	215.00	266.50	338.50	567.00	830.00	423.63	224.24	79.28	52.93	236.15	611.10	-	+++	(-)
8M	Sham	10	80.00	154.00	280.50	315.00	414.00	254.10	104.94	33.18	41.30	179.03	329.17			
8M	3R4F	10	2800.0	3824.0	5191.0	5674.0	13614	5548.6	3068.2	970.26	55.30	3353.7	7743.5	***		
8M	THS2.2	7	46.00	117.00	254.00	417.00	466.00	246.86	158.56	59.93	64.23	100.22	393.50	-	+++	
8M	Cess	9	68.00	106.00	231.00	273.00	425.00	218.11	122.19	40.73	56.02	124.19	312.03	-	***	
8M	Switch	9	58.00	172.00	214.00	288.00	591.00	250.33	165.91	55.30	66.27	122.81	377.86	-	+++	-

Table 88. BALF MMP activity (mU/ml)

Exposure Duration	Treatment	N	LOQ	N< LOQ	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.15	0	1.16	1.22	1.31	1.58	1.98	1.42	0.27	0.09	19.29	1.23	1.62			
1M	3R4F	10	0.10	0	1.23	1.52	1.64	1.67	2.54	1.67	0.35	0.11	20.84	1.42	1.92	(-)		
1M	THS2.2	10	0.11	0	1.06	1.16	1.28	1.40	1.77	1.30	0.21	0.07	16.22	1.15	1.46	(-)	++	
2M	Sham	10	0.12	0	0.88	0.98	1.02	1.17	1.23	1.05	0.12	0.04	11.60	0.96	1.14			
2M	3R4F	10	0.12	0	1.28	1.31	1.53	1.99	2.16	1.65	0.35	0.11	21.23	1.40	1.90	***		
2M	THS2.2	10	0.12	0	0.99	1.08	1.13	1.32	1.34	1.17	0.13	0.04	11.15	1.08	1.26	*	++	
3M	Sham	10	0.04	0	0.94	1.20	1.28	1.38	1.55	1.28	0.18	0.06	13.96	1.15	1.41			
3M	3R4F	10	0.05	0	1.30	1.98	2.21	2.57	2.81	2.18	0.47	0.15	21.45	1.85	2.52	***		
3M	THS2.2	9	0.05	0	0.95	1.10	1.17	1.30	1.41	1.19	0.15	0.05	12.16	1.08	1.30	-	***	
3M	Cess	10	0.05	0	0.97	1.08	1.09	1.14	1.39	1.13	0.14	0.04	11.94	1.04	1.23	-	***	
3M	Switch	10	0.07	0	0.98	1.13	1.24	1.29	1.48	1.22	0.14	0.04	11.64	1.12	1.32	-	***	-
6M	Sham	9	0.19	0	0.83	1.05	1.09	1.18	1.45	1.13	0.20	0.07	18.06	0.97	1.29			
6M	3R4F	10	0.17	0	1.58	1.79	2.11	2.43	2.74	2.11	0.36	0.12	17.25	1.85	2.37	***		
6M	THS2.2	10	0.18	0	0.98	1.07	1.24	1.48	1.78	1.29	0.28	0.09	21.60	1.09	1.49	-	***	
6M	Cess	10	0.17	0	1.14	1.21	1.46	1.72	1.93	1.48	0.29	0.09	19.33	1.28	1.68	**	***	
6M	Switch	10	0.18	0	0.90	1.19	1.41	1.68	2.08	1.45	0.35	0.11	24.44	1.20	1.70	*	***	-
8M	Sham	10	0.51	0	0.43	0.58	1.26	1.95	2.34	1.32	0.81	0.26	61.39	0.74	1.89			
8M	3R4F	10	0.43	0	1.10	1.28	2.18	3.12	3.99	2.27	1.07	0.34	47.26	1.50	3.04	(-)		
8M	THS2.2	10	0.53	0	0.57	0.72	1.38	2.13	2.46	1.41	0.76	0.24	53.68	0.87	1.95	(-)	(-)	
8M	Cess	10	0.63	0	0.42	0.64	1.24	1.75	2.28	1.24	0.69	0.22	55.29	0.75	1.73	(-)	(-)	
8M	Switch	10	0.71	2	0.52	0.55	1.04	1.75	2.00	1.13	0.59	0.19	51.95	0.71	1.55	(-)	+	(-)

Table 89. BALF MMP-9 protein levels (ng/ml, MAP analysis)

Exposure Duration	Treatment	N	LOQ	N< LOQ	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.14	0	0.27	0.30	0.30	0.37	0.47	0.33	0.06	0.02	18.60	0.29	0.37			
1M	3R4F	10	0.14	0	1.70	5.10	15.50	17.00	24.00	12.74	8.03	2.54	63.01	7.00	18.48	***		
1M	THS2.2	10	0.14	0	0.24	0.27	0.32	0.63	1.30	0.50	0.37	0.12	73.41	0.24	0.76	(-)	***	
2M	Sham	10	0.08	6	0.04	0.04	0.04	0.11	0.14	0.07	0.04	0.01	55.58	0.04	0.10			
2M	3R4F	10	0.08	0	1.40	4.10	6.00	16.00	22.00	9.52	7.22	2.28	75.85	4.35	14.69	+++		
2M	THS2.2	10	0.08	8	0.04	0.04	0.04	0.04	0.79	0.12	0.24	0.07	191.85	-0.05	0.29	(-)	+++	
3M	Sham	10	0.08	1	0.04	0.13	0.27	0.34	0.56	0.26	0.16	0.05	61.87	0.15	0.38			
3M	3R4F	10	0.08	0	5.20	7.80	22.50	28.00	38.00	21.16	11.59	3.66	54.77	12.87	29.45	***		
3M	THS2.2	10	0.08	4	0.04	0.04	0.09	0.12	0.32	0.11	0.09	0.03	79.73	0.05	0.17	*	***	
3M	Cess	10	0.08	0	0.21	0.35	0.88	1.40	2.80	1.07	0.86	0.27	80.21	0.46	1.69	*	***	
3M	Switch	10	0.08	0	0.28	0.68	1.35	2.30	5.80	1.75	1.65	0.52	94.31	0.57	2.93	*	***	(-)
6M	Sham	9	0.08	3	0.04	0.04	0.09	0.15	0.50	0.15	0.15	0.05	102.15	0.03	0.26			
6M	3R4F	10	0.08	0	5.40	7.10	10.50	22.00	31.00	13.95	9.04	2.86	64.78	7.49	20.41	+++		
6M	THS2.2	10	0.08	1	0.04	0.09	0.14	0.25	0.55	0.18	0.15	0.05	81.04	0.08	0.29	(-)	+++	
6M	Cess	10	0.08	1	0.04	0.18	0.41	0.93	1.20	0.53	0.41	0.13	76.51	0.24	0.82	+	+++	
6M	Switch	10	0.08	2	0.04	0.10	0.21	0.32	0.79	0.27	0.24	0.08	89.65	0.10	0.44	(-)	+++	-
8M	Sham	10	0.08	5	0.04	0.04	0.08	0.22	0.56	0.15	0.16	0.05	112.61	0.03	0.26			
8M	3R4F	10	0.08	0	8.90	13.00	15.00	22.00	28.00	17.35	6.52	2.06	37.56	12.69	22.01	+++		
8M	THS2.2	10	0.08	5	0.04	0.04	0.07	0.10	0.26	0.10	0.08	0.02	81.06	0.04	0.15	(-)	+++	
8M	Cess	10	0.08	4	0.04	0.04	0.09	0.21	0.55	0.15	0.16	0.05	106.52	0.04	0.26	(-)	+++	
8M	Switch	10	0.08	4	0.04	0.04	0.09	0.13	0.37	0.12	0.11	0.04	90.24	0.04	0.21	(-)	+++	(-)

Table 90. BALF Timp-1 protein levels (ng/ml, MAP analysis)

Exposure Duration	Treatment	N	LOQ	N< LOQ	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.02	0	0.01	0.02	0.03	0.03	0.04	0.03	0.01	0.00	30.78	0.02	0.03			
1M	3R4F	10	0.02	0	0.04	0.06	0.10	0.11	0.15	0.09	0.03	0.01	36.96	0.07	0.12	***		
1M	THS2.2	10	0.02	1	0.01	0.02	0.03	0.03	0.04	0.03	0.01	0.00	33.30	0.02	0.03	-	***	
2M	Sham	10	0.02	1	0.01	0.02	0.02	0.03	0.05	0.03	0.01	0.00	45.57	0.02	0.03			
2M	3R4F	10	0.02	0	0.04	0.05	0.08	0.10	0.12	0.08	0.03	0.01	36.13	0.06	0.10	***		
2M	THS2.2	10	0.02	1	0.01	0.02	0.03	0.03	0.03	0.02	0.01	0.00	25.72	0.02	0.03	-	***	
3M	Sham	10	0.02	2	0.01	0.02	0.02	0.03	0.03	0.02	0.01	0.00	40.93	0.01	0.03			
3M	3R4F	10	0.02	0	0.05	0.06	0.08	0.08	0.10	0.08	0.01	0.00	18.27	0.07	0.09	***		
3M	THS2.2	10	0.02	3	0.01	0.01	0.02	0.02	0.03	0.02	0.01	0.00	40.21	0.01	0.02	-	***	
3M	Cess	10	0.02	0	0.02	0.03	0.03	0.04	0.04	0.03	0.01	0.00	22.31	0.03	0.04	**	***	
3M	Switch	10	0.02	1	0.01	0.03	0.03	0.04	0.06	0.03	0.01	0.00	45.36	0.02	0.04	*	***	-
6M	Sham	9	0.02	4	0.01	0.01	0.02	0.02	0.05	0.02	0.01	0.00	82.57	0.01	0.03			
6M	3R4F	10	0.02	0	0.03	0.05	0.05	0.06	0.09	0.05	0.02	0.01	29.86	0.04	0.06	***		
6M	THS2.2	10	0.02	5	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.00	49.67	0.01	0.02	(-)	***	
6M	Cess	10	0.02	2	0.01	0.02	0.02	0.03	0.03	0.02	0.01	0.00	37.17	0.01	0.03	-	***	
6M	Switch	10	0.02	4	0.01	0.01	0.02	0.02	0.05	0.02	0.01	0.00	72.58	0.01	0.03	(-)	***	-
8M	Sham	10	0.02	6	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.00	50.15	0.01	0.02			
8M	3R4F	10	0.02	0	0.04	0.07	0.08	0.09	0.10	0.08	0.02	0.01	23.27	0.06	0.09	***		
8M	THS2.2	10	0.02	3	0.01	0.01	0.02	0.02	0.03	0.02	0.01	0.00	46.29	0.01	0.02	-	***	
8M	Cess	10	0.02	2	0.01	0.02	0.02	0.02	0.02	0.02	0.00	0.00	29.28	0.01	0.02	-	+++	
8M	Switch	10	0.02	1	0.01	0.02	0.02	0.02	0.03	0.02	0.00	0.00	27.58	0.01	0.02	*	***	(-)

Table 91. Plasma 4β-hydroxycholesterol (ng/ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
3M	Sham	5	328.71	368.37	386.85	398.92	449.89	386.55	44.28	19.80	11.45	331.57	441.52			
3M	3R4F	5	265.76	396.14	400.81	404.22	415.47	376.48	62.31	27.86	16.55	299.12	453.85	-		
3M	THS2.2	5	387.27	421.75	438.96	480.01	517.00	449.00	50.60	22.63	11.27	386.17	511.83	-	-	
3M	Cess	5	291.14	325.08	340.63	356.83	357.70	334.27	27.58	12.34	8.25	300.02	368.52	-	(-)	
3M	Switch	5	335.72	341.49	387.49	427.12	460.79	390.52	54.07	24.18	13.84	323.39	457.66	-	-	-
8M	Sham	10	158.11	195.14	209.22	246.26	330.02	225.49	56.58	17.89	25.09	185.01	265.97			
8M	3R4F	10	100.04	135.79	165.13	207.27	282.54	178.05	58.32	18.44	32.75	136.33	219.77	-		
8M	THS2.2	10	170.31	197.42	225.67	251.89	308.39	225.64	43.17	13.65	19.13	194.76	256.53	-	-	
8M	Cess	10	135.33	175.69	187.59	206.31	289.39	196.08	42.49	13.44	21.67	165.68	226.47	(-)	-	
8M	Switch	10	100.85	177.29	215.63	257.47	320.08	218.73	64.90	20.52	29.67	172.30	265.15	-	-	-

Table 92. Plasma 7β-hydroxycholesterol (ng/ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	3	27.73	27.73	28.52	35.88	35.88	30.71	4.49	2.59	14.63	19.54	41.87			
1M	3R4F	3	11.18	11.18	11.93	30.85	30.85	17.99	11.14	6.43	61.96	-9.70	45.67	(-)		
1M	THS2.2	3	27.19	27.19	29.30	48.80	48.80	35.09	11.91	6.88	33.94	5.50	64.69	(-)	(-)	
3M	Sham	5	32.53	55.96	58.42	68.94	103.51	63.87	25.84	11.56	40.46	31.78	95.96			
3M	3R4F	5	45.26	52.09	57.37	64.55	67.53	57.36	9.07	4.06	15.81	46.10	68.63	-		
3M	THS2.2	5	51.53	54.44	56.08	68.54	82.89	62.70	13.02	5.82	20.77	46.52	78.87	-	-	
3M	Cess	5	49.72	51.42	54.17	61.16	63.82	56.06	6.16	2.75	10.98	48.41	63.70	-	-	
3M	Switch	5	44.92	52.45	53.21	64.72	84.35	59.93	15.38	6.88	25.66	40.84	79.02	-	-	-
8M	Sham	10	45.83	51.48	57.20	78.85	83.37	62.45	14.59	4.61	23.36	52.02	72.89			
8M	3R4F	10	37.31	47.49	53.91	57.67	96.59	56.13	16.56	5.24	29.50	44.29	67.98	-		
8M	THS2.2	10	38.59	47.18	49.48	52.65	68.96	50.34	7.98	2.52	15.85	44.63	56.05	*	(-)	
8M	Cess	10	46.10	53.42	57.39	62.40	79.66	59.29	9.32	2.95	15.72	52.62	65.96	-	(-)	
8M	Switch	10	22.54	50.25	56.20	66.64	75.13	55.15	14.91	4.72	27.04	44.48	65.82	-	-	-

Table 93. Plasma 25-hydroxycholesterol (ng/ml)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	3	22.17	22.17	25.31	26.51	26.51	24.67	2.24	1.29	9.08	19.10	30.23			
1M	3R4F	3	26.42	26.42	28.23	31.32	31.32	28.66	2.48	1.43	8.66	22.49	34.82	-		
1M	THS2.2	3	21.40	21.40	21.61	23.67	23.67	22.22	1.25	0.72	5.65	19.11	25.34	-	*	
3M	Sham	5	27.07	28.25	33.74	46.46	50.86	37.28	10.81	4.83	28.99	23.86	50.69			
3M	3R4F	5	50.48	52.30	55.96	62.96	63.19	56.98	5.91	2.64	10.37	49.65	64.31	*		
3M	THS2.2	5	29.08	31.45	37.31	38.52	39.43	35.16	4.61	2.06	13.10	29.44	40.88	-	***	
3M	Cess	5	27.83	32.69	36.93	39.24	39.49	35.23	4.96	2.22	14.07	29.08	41.39	-	***	
3M	Switch	5	34.14	35.53	35.56	41.02	57.44	40.74	9.70	4.34	23.81	28.70	52.78	-	*	-
8M	Sham	10	30.36	32.82	37.21	45.80	46.50	38.28	6.14	1.94	16.04	33.89	42.67			
8M	3R4F	10	41.10	43.72	57.95	70.27	89.64	58.78	15.87	5.02	27.00	47.42	70.13	**		
8M	THS2.2	10	29.39	32.93	36.29	37.89	39.43	35.44	3.40	1.07	9.59	33.01	37.87	-	**	
8M	Cess	10	33.65	34.94	37.57	43.99	48.30	39.00	5.01	1.58	12.85	35.41	42.58	-	**	
8M	Switch	10	23.17	34.96	42.17	44.03	45.73	38.93	7.43	2.35	19.08	33.61	44.24	-	**	-

Table 94. Aortic arch plaque area relative to total measured area (%)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	17	1.01	3.47	5.54	7.76	10.78	5.67	3.02	0.73	53.21	4.12	7.22			
1M	3R4F	18	2.13	3.47	5.29	6.98	11.10	5.51	2.34	0.55	42.53	4.35	6.68	-		
1M	THS2.2	17	2.71	4.97	5.70	6.66	10.35	5.95	1.74	0.42	29.26	5.05	6.84	-	-	
2M	Sham	18	1.28	4.61	7.15	11.18	12.36	7.48	3.35	0.79	44.78	5.81	9.14			
2M	3R4F	18	4.65	7.71	10.44	12.41	15.85	9.99	3.05	0.72	30.56	8.47	11.51	*		
2M	THS2.2	18	2.61	4.47	6.16	8.36	15.51	6.79	3.38	0.80	49.78	5.11	8.48	-	**	
3M	Sham	18	4.68	7.39	8.84	10.07	20.46	9.51	3.78	0.89	39.79	7.63	11.39			
3M	3R4F	18	4.91	12.93	14.52	16.73	22.61	14.64	4.18	0.98	28.53	12.56	16.71	***		
3M	THS2.2	18	2.65	6.59	9.53	14.34	19.84	10.60	4.64	1.09	43.80	8.29	12.91	-	**	
3M	Cess	18	6.31	8.49	11.83	14.74	16.69	11.68	3.30	0.78	28.25	10.04	13.32	-	*	
3M	Switch	18	4.45	9.00	11.60	13.46	16.85	11.01	3.55	0.84	32.26	9.24	12.77	-	**	-
6M	Sham	17	13.77	17.82	21.35	23.76	26.90	21.19	4.24	1.03	20.02	19.01	23.37			
6M	3R4F	18	16.28	21.95	26.55	30.47	38.36	27.21	5.75	1.35	21.11	24.35	30.07	**		
6M	THS2.2	18	15.58	21.12	23.81	28.54	36.97	24.81	5.85	1.38	23.59	21.90	27.72	*	-	
6M	Cess	18	17.66	21.92	26.77	29.25	36.66	26.07	5.29	1.25	20.30	23.44	28.71	**	-	
6M	Switch	18	7.91	18.55	25.07	31.15	40.38	24.99	9.13	2.15	36.51	20.46	29.53	-	-	-
8M	Sham	18	17.78	22.70	26.91	30.69	40.62	26.99	5.99	1.41	22.21	24.01	29.97			
8M	3R4F	18	23.88	31.49	37.59	40.88	57.56	37.30	8.30	1.96	22.26	33.17	41.43	***		
8M	THS2.2	18	9.85	20.61	25.81	29.81	44.72	26.79	8.96	2.11	33.43	22.33	31.24	-	***	
8M	Cess	18	16.61	25.48	28.77	34.57	38.53	29.37	5.87	1.38	20.00	26.45	32.30	-	**	
8M	Switch	18	19.75	25.35	29.15	34.70	46.03	30.08	6.95	1.64	23.10	26.63	33.54	-	**	-

Table 95. Clinical chemistry, cholesterol (mmol/l)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	7.78	8.11	8.24	8.55	9.76	8.39	0.54	0.17	6.45	8.00	8.78			
1M	3R4F	10	5.62	8.98	10.64	11.49	13.25	10.22	2.35	0.74	22.99	8.53	11.90	*		
1M	THS2.2	10	7.69	8.26	8.77	9.31	9.66	8.76	0.63	0.20	7.15	8.31	9.20	-	-	
2M	Sham	10	6.62	7.71	9.19	9.44	10.44	8.84	1.22	0.39	13.79	7.97	9.71			
2M	3R4F	10	7.30	8.42	10.75	12.21	18.03	10.89	3.04	0.96	27.91	8.72	13.07	-		
2M	THS2.2	10	7.83	8.40	9.41	9.64	10.98	9.15	0.96	0.30	10.44	8.47	9.84	-	-	
3M	Sham	10	7.73	8.24	8.66	10.14	11.08	9.12	1.13	0.36	12.40	8.31	9.93			
3M	3R4F	10	9.00	9.37	10.65	11.32	12.75	10.51	1.18	0.37	11.19	9.67	11.35	*		
3M	THS2.2	10	7.86	8.99	9.42	9.78	11.95	9.50	1.04	0.33	11.00	8.75	10.25	-	-	
3M	Cess	10	6.56	7.45	8.53	9.07	9.59	8.22	1.07	0.34	13.04	7.46	8.99	-	***	
3M	Switch	10	8.02	8.45	8.67	9.55	12.03	9.12	1.17	0.37	12.78	8.28	9.95	(-)	*	-
6M	Sham	9	6.30	9.14	10.05	10.86	12.32	9.77	1.75	0.58	17.93	8.43	11.12			
6M	3R4F	10	8.74	9.80	11.04	11.41	12.40	10.79	1.14	0.36	10.59	9.97	11.61	-		
6M	THS2.2	10	7.68	9.52	9.91	10.82	12.67	10.15	1.35	0.43	13.27	9.19	11.12	-	-	
6M	Cess	10	7.23	9.32	10.33	11.13	12.08	10.13	1.43	0.45	14.07	9.11	11.15	-	-	
6M	Switch	10	5.17	8.64	9.38	10.39	11.27	9.22	1.71	0.54	18.52	8.00	10.44	-	*	-
8M	Sham	10	7.44	8.28	9.98	10.40	12.57	9.74	1.49	0.47	15.25	8.68	10.80			
8M	3R4F	10	7.04	10.05	11.12	12.62	14.97	11.17	2.17	0.69	19.46	9.62	12.73	-		
8M	THS2.2	10	7.86	8.10	8.82	8.99	11.11	8.89	0.98	0.31	11.01	8.19	9.59	-	*	
8M	Cess	10	7.14	8.42	8.97	9.63	11.50	9.08	1.19	0.38	13.16	8.22	9.93	-	*	
8M	Switch	10	5.86	9.71	10.36	11.12	13.33	10.19	1.90	0.60	18.62	8.83	11.55	-	-	-

Table 96. Clinical chemistry, glucose (mmol/l)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	10.83	11.13	12.01	12.87	13.48	12.06	0.97	0.31	8.08	11.37	12.76			
1M	3R4F	10	7.82	9.36	10.74	11.96	13.42	10.57	1.82	0.58	17.24	9.27	11.87	*		
1M	THS2.2	10	8.28	10.53	11.55	12.18	14.13	11.43	1.76	0.56	15.38	10.17	12.68	-	-	
2M	Sham	10	9.74	10.21	11.88	12.59	13.68	11.60	1.41	0.45	12.20	10.58	12.61			
2M	3R4F	10	7.14	9.64	9.86	10.31	11.24	9.79	1.06	0.33	10.82	9.03	10.55	**		
2M	THS2.2	10	9.20	10.29	11.21	11.76	13.66	11.13	1.36	0.43	12.20	10.16	12.10	-	*	
3M	Sham	10	8.11	10.01	10.28	10.82	13.10	10.34	1.50	0.48	14.55	9.26	11.41			
3M	3R4F	10	4.80	6.38	8.13	9.28	11.81	7.94	2.24	0.71	28.21	6.34	9.54	*		
3M	THS2.2	10	8.09	9.50	10.12	10.45	10.92	9.87	0.84	0.27	8.56	9.26	10.47	-	*	
3M	Cess	10	7.56	8.85	10.27	12.09	13.80	10.42	2.19	0.69	21.01	8.86	11.99	-	*	
3M	Switch	10	6.27	9.19	9.82	11.38	12.78	10.01	1.89	0.60	18.86	8.66	11.36	-	*	-
6M	Sham	9	8.23	8.90	9.71	11.22	11.61	10.01	1.28	0.43	12.81	9.03	11.00			
6M	3R4F	10	7.43	7.88	8.42	8.82	11.02	8.54	1.02	0.32	11.98	7.81	9.27	*		
6M	THS2.2	10	8.81	9.92	10.70	11.57	11.83	10.62	1.06	0.34	10.01	9.86	11.38	-	***	
6M	Cess	10	7.18	8.49	9.48	10.29	11.05	9.36	1.26	0.40	13.45	8.46	10.26	-	-	
6M	Switch	10	8.30	8.85	9.83	10.06	11.50	9.69	0.96	0.30	9.90	9.01	10.38	-	*	-
8M	Sham	9	8.15	9.24	9.95	10.15	13.77	9.88	1.65	0.55	16.66	8.62	11.15			
8M	3R4F	9	3.94	4.64	6.81	8.27	9.07	6.61	1.91	0.64	28.87	5.14	8.07	**		
8M	THS2.2	8	7.54	8.03	9.10	9.67	10.71	8.98	1.07	0.38	11.95	8.08	9.88	-	**	
8M	Cess	9	8.34	8.59	8.88	9.32	9.92	9.00	0.60	0.20	6.69	8.54	9.46	(-)	**	
8M	Switch	9	5.51	9.12	10.03	10.24	13.27	9.78	2.20	0.73	22.52	8.09	11.47	-	**	-

Table 97. Clinical chemistry, high density lipoprotein (HDL, mmol/l)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	2.02	2.17	2.24	2.30	2.52	2.24	0.13	0.04	5.96	2.15	2.34			
1M	3R4F	10	1.77	2.31	2.69	2.91	3.06	2.60	0.42	0.13	16.08	2.30	2.89	*		
1M	THS2.2	10	1.99	2.13	2.16	2.39	2.62	2.24	0.21	0.07	9.19	2.10	2.39	-	*	
2M	Sham	10	2.03	2.10	2.37	2.53	2.82	2.36	0.25	0.08	10.49	2.19	2.54			
2M	3R4F	10	2.13	2.47	2.70	2.91	4.13	2.82	0.59	0.19	20.83	2.40	3.24	*		
2M	THS2.2	10	1.98	2.27	2.31	2.40	2.92	2.34	0.24	0.08	10.31	2.17	2.51	-	+	
3M	Sham	10	2.09	2.16	2.25	2.38	2.68	2.29	0.18	0.06	8.00	2.15	2.42			
3M	3R4F	10	2.31	2.46	2.62	2.80	3.09	2.64	0.25	0.08	9.48	2.46	2.82	**		
3M	THS2.2	10	2.15	2.27	2.33	2.42	2.95	2.39	0.25	0.08	10.28	2.21	2.57	(-)	*	
3M	Cess	10	1.82	1.97	2.20	2.25	2.39	2.13	0.20	0.06	9.22	1.99	2.27	-	***	
3M	Switch	10	1.95	2.12	2.28	2.42	2.67	2.28	0.21	0.07	9.42	2.12	2.43	-	**	-
6M	Sham	9	1.92	2.36	2.42	2.70	2.94	2.50	0.31	0.10	12.57	2.26	2.74			
6M	3R4F	10	2.29	2.47	2.69	2.80	2.99	2.65	0.24	0.07	8.94	2.48	2.82	-		
6M	THS2.2	10	1.93	2.29	2.54	2.71	2.95	2.51	0.30	0.10	12.07	2.30	2.73	-	-	
6M	Cess	10	1.84	2.31	2.53	2.77	2.92	2.51	0.33	0.11	13.29	2.27	2.74	-	-	
6M	Switch	10	1.55	2.22	2.44	2.62	2.79	2.39	0.35	0.11	14.80	2.14	2.65	-	-	-
8M	Sham	10	1.83	2.07	2.25	2.41	3.03	2.28	0.33	0.10	14.36	2.05	2.52			
8M	3R4F	10	1.56	2.19	2.40	2.86	2.91	2.40	0.42	0.13	17.31	2.10	2.70	-		
8M	THS2.2	10	1.84	1.94	2.09	2.24	2.80	2.13	0.28	0.09	13.27	1.93	2.34	(-)	-	
8M	Cess	10	1.65	1.95	2.20	2.22	2.54	2.09	0.27	0.08	12.76	1.90	2.28	-	-	
8M	Switch	10	1.63	2.23	2.37	2.69	3.20	2.41	0.41	0.13	17.05	2.11	2.70	-	-	-

Table 98. Total cholesterol (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	239.00	256.00	283.50	318.00	356.00	288.70	41.89	13.25	14.51	258.73	318.67			
1M	3R4F	10	208.00	341.00	391.50	430.00	477.00	375.10	76.59	24.22	20.42	320.31	429.89	**		
1M	THS2.2	10	176.00	293.00	326.50	353.00	391.00	315.80	59.41	18.79	18.81	273.30	358.30	-	+	
2M	Sham	10	250.00	271.00	286.50	325.00	347.00	295.70	33.00	10.44	11.16	272.09	319.31			
2M	3R4F	10	203.00	340.00	384.50	409.00	468.00	364.80	76.66	24.24	21.01	309.96	419.64	*		
2M	THS2.2	10	194.00	244.00	321.00	346.00	367.00	301.90	58.49	18.50	19.37	260.06	343.74	-	-	
3M	Sham	10	255.00	326.00	372.50	380.00	401.00	348.70	49.39	15.62	14.16	313.37	384.03			
3M	3R4F	10	335.00	349.00	370.50	384.00	394.00	367.90	20.46	6.47	5.56	353.26	382.54	-		
3M	THS2.2	10	271.00	302.00	335.50	385.00	395.00	335.90	44.00	13.91	13.10	304.42	367.38	-	-	
3M	Cess	10	236.00	281.00	348.50	362.00	400.00	328.30	53.73	16.99	16.37	289.86	366.74	-	-	
3M	Switch	10	252.00	280.00	345.00	361.00	431.00	333.00	53.97	17.07	16.21	294.39	371.61	-	-	-
6M	Sham	9	235.00	319.00	345.00	358.00	374.00	332.78	42.66	14.22	12.82	299.99	365.57			
6M	3R4F	10	234.00	382.00	390.50	415.00	448.00	384.90	57.45	18.17	14.93	343.80	426.00	++		
6M	THS2.2	10	258.00	332.00	362.50	376.00	384.00	345.70	43.16	13.65	12.49	314.82	376.58	(-)	++	
6M	Cess	10	232.00	335.00	352.00	364.00	459.00	343.90	60.65	19.18	17.64	300.51	387.29	-	+	
6M	Switch	10	282.00	308.00	353.50	378.00	382.00	343.40	39.58	12.52	11.53	315.08	371.72	(-)	++	-
8M	Sham	10	164.00	295.00	379.00	429.00	468.00	356.60	96.52	30.52	27.07	287.55	425.65			
8M	3R4F	10	232.00	308.00	350.00	407.00	438.00	348.70	65.24	20.63	18.71	302.03	395.37	-		
8M	THS2.2	9	314.00	344.00	361.00	367.00	400.00	358.44	26.49	8.83	7.39	338.08	378.81	-	-	
8M	Cess	10	222.00	286.00	364.00	404.00	473.00	349.00	78.97	24.97	22.63	292.51	405.49	-	-	
8M	Switch	10	149.00	334.00	361.50	422.00	471.00	347.20	98.82	31.25	28.46	276.51	417.89	-	-	-

Table 99. Lipoprotein – HDL (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	19.00	22.00	23.00	25.00	35.00	24.40	4.38	1.38	17.94	21.27	27.53			
1M	3R4F	10	16.00	24.00	27.00	31.00	33.00	26.60	5.54	1.75	20.83	22.64	30.56	-		
1M	THS2.2	10	13.00	23.00	24.00	26.00	30.00	23.60	4.65	1.47	19.69	20.28	26.92	-	-	
2M	Sham	10	10.00	14.00	16.00	19.00	19.00	16.00	2.94	0.93	18.40	13.89	18.11			
2M	3R4F	10	15.00	18.00	19.00	21.00	26.00	19.40	3.13	0.99	16.15	17.16	21.64	*		
2M	THS2.2	10	13.00	15.00	18.00	19.00	30.00	18.60	4.84	1.53	25.99	15.14	22.06	-	(-)	
3M	Sham	10	15.00	18.00	19.00	21.00	24.00	19.20	2.82	0.89	14.69	17.18	21.22			
3M	3R4F	10	17.00	18.00	22.00	22.00	25.00	21.00	2.94	0.93	14.02	18.89	23.11	-		
3M	THS2.2	10	15.00	17.00	18.50	22.00	28.00	19.70	4.35	1.37	22.07	16.59	22.81	-	-	
3M	Cess	10	14.00	15.00	17.50	20.00	29.00	18.50	4.58	1.45	24.74	15.23	21.77	-	-	
3M	Switch	10	11.00	13.00	18.00	21.00	24.00	17.20	4.26	1.35	24.79	14.15	20.25	-	*	-
6M	Sham	9	10.00	15.00	17.00	18.00	26.00	16.44	4.61	1.54	28.05	12.90	19.99			
6M	3R4F	10	14.00	15.00	18.00	21.00	22.00	18.00	2.94	0.93	16.36	15.89	20.11	-		
6M	THS2.2	10	9.00	16.00	17.50	20.00	22.00	17.30	3.71	1.17	21.46	14.64	19.96	-	-	
6M	Cess	10	11.00	13.00	15.50	18.00	20.00	15.60	3.17	1.00	20.32	13.33	17.87	-	-	
6M	Switch	10	11.00	13.00	16.00	19.00	22.00	16.20	3.77	1.19	23.24	13.51	18.89	-	-	-
8M	Sham	10	8.00	14.00	18.00	22.00	24.00	17.30	5.25	1.66	30.35	13.54	21.06			
8M	3R4F	10	14.00	16.00	19.50	21.00	26.00	19.40	4.12	1.30	21.21	16.46	22.34	-		
8M	THS2.2	9	12.00	17.00	18.00	19.00	23.00	17.89	3.44	1.15	19.25	15.24	20.54	-	-	
8M	Cess	10	10.00	19.00	19.50	24.00	31.00	20.00	6.15	1.94	30.73	15.60	24.40	-	-	
8M	Switch	10	11.00	16.00	18.00	22.00	26.00	18.50	4.53	1.43	24.47	15.26	21.74	-	-	-

Table 100. Lipoprotein – Non-HDL (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	211.00	233.00	262.50	296.00	331.00	264.40	40.57	12.83	15.34	235.38	293.42			
1M	3R4F	10	192.00	315.00	365.00	399.00	445.00	348.50	71.40	22.58	20.49	297.42	399.58	**		
1M	THS2.2	10	163.00	270.00	305.00	323.00	365.00	292.00	55.44	17.53	18.99	252.34	331.66	-	+	
2M	Sham	10	236.00	253.00	270.50	307.00	328.00	279.90	32.75	10.36	11.70	256.47	303.33			
2M	3R4F	10	188.00	321.00	362.50	387.00	452.00	345.50	75.68	23.93	21.91	291.36	399.64	*		
2M	THS2.2	10	181.00	229.00	300.50	328.00	337.00	283.20	55.16	17.44	19.48	243.74	322.66	-	-	
3M	Sham	10	241.00	304.00	353.00	361.00	377.00	329.70	47.36	14.98	14.37	295.82	363.58			
3M	3R4F	10	313.00	331.00	350.50	362.00	372.00	347.00	19.70	6.23	5.68	332.91	361.09	-		
3M	THS2.2	10	254.00	280.00	313.00	359.00	376.00	316.10	43.38	13.72	13.72	285.07	347.13	-	-	
3M	Cess	10	222.00	264.00	323.50	345.00	381.00	309.90	52.20	16.51	16.85	272.56	347.24	-	-	
3M	Switch	10	234.00	267.00	325.50	347.00	411.00	315.90	51.88	16.41	16.42	278.79	353.01	-	-	-
6M	Sham	9	220.00	299.00	328.00	339.00	360.00	316.22	42.73	14.24	13.51	283.38	349.06			
6M	3R4F	10	213.00	363.00	375.50	399.00	431.00	367.00	58.44	18.48	15.92	325.20	408.80	++		
6M	THS2.2	10	249.00	317.00	343.50	356.00	368.00	328.40	40.36	12.76	12.29	299.53	357.27	(-)	++	
6M	Cess	10	217.00	316.00	339.50	346.00	445.00	328.10	60.40	19.10	18.41	284.90	371.30	-	+	
6M	Switch	10	270.00	289.00	338.00	357.00	366.00	327.10	37.90	11.98	11.59	299.99	354.21	(-)	++	-
8M	Sham	10	156.00	281.00	362.00	405.00	452.00	339.30	94.62	29.92	27.89	271.61	406.99			
8M	3R4F	10	217.00	288.00	333.50	391.00	412.00	328.90	64.68	20.45	19.66	282.63	375.17	-		
8M	THS2.2	9	300.00	326.00	340.00	349.00	381.00	340.67	26.81	8.94	7.87	320.06	361.27	-	-	
8M	Cess	10	210.00	276.00	339.00	384.00	454.00	329.10	76.33	24.14	23.19	274.50	383.70	-	-	
8M	Switch	10	138.00	318.00	343.00	403.00	447.00	328.60	94.93	30.02	28.89	260.69	396.51	-	-	-

Table 101. Lipoprotein – HDL2 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	7.00	9.00	9.00	11.00	14.00	9.70	1.95	0.62	20.07	8.31	11.09			
1M	3R4F	10	6.00	10.00	10.50	12.00	14.00	10.40	2.22	0.70	21.36	8.81	11.99	-		
1M	THS2.2	10	5.00	8.00	9.00	10.00	11.00	8.80	1.87	0.59	21.29	7.46	10.14	-	-	
2M	Sham	10	3.00	5.00	6.00	7.00	8.00	5.80	1.48	0.47	25.44	4.74	6.86			
2M	3R4F	10	5.00	7.00	8.50	10.00	10.00	8.20	1.75	0.55	21.36	6.95	9.45	**		
2M	THS2.2	10	3.00	7.00	8.00	9.00	11.00	7.70	2.36	0.75	30.64	6.01	9.39	*	-	
3M	Sham	10	5.00	6.00	7.00	7.00	8.00	6.60	1.07	0.34	16.29	5.83	7.37			
3M	3R4F	10	5.00	7.00	7.50	9.00	9.00	7.60	1.43	0.45	18.81	6.58	8.62	-		
3M	THS2.2	10	5.00	6.00	6.00	7.00	11.00	6.80	1.81	0.57	26.67	5.50	8.10	-	-	
3M	Cess	10	4.00	4.00	6.50	7.00	10.00	6.20	1.99	0.63	32.08	4.78	7.62	-	-	
3M	Switch	10	4.00	5.00	5.00	6.00	8.00	5.70	1.34	0.42	23.46	4.74	6.66	-	**	(-)
6M	Sham	9	3.00	4.00	6.00	6.00	7.00	5.33	1.41	0.47	26.52	4.25	6.42			
6M	3R4F	10	5.00	6.00	6.00	7.00	11.00	6.80	1.69	0.53	24.80	5.59	8.01	-		
6M	THS2.2	10	3.00	5.00	6.00	7.00	8.00	5.90	1.60	0.50	27.04	4.76	7.04	-	-	
6M	Cess	10	3.00	4.00	5.50	6.00	8.00	5.40	1.51	0.48	27.88	4.32	6.48	-	-	
6M	Switch	10	4.00	5.00	5.50	7.00	8.00	5.80	1.48	0.47	25.44	4.74	6.86	-	(-)	-
8M	Sham	10	2.00	4.00	6.00	7.00	7.00	5.40	1.65	0.52	30.49	4.22	6.58			
8M	3R4F	10	5.00	7.00	7.00	8.00	10.00	7.40	1.43	0.45	19.32	6.38	8.42	**		
8M	THS2.2	9	5.00	6.00	7.00	7.00	9.00	6.56	1.24	0.41	18.85	5.61	7.51	-	-	
8M	Cess	10	4.00	5.00	7.00	9.00	12.00	7.30	2.36	0.75	32.32	5.61	8.99	-	-	
8M	Switch	10	3.00	5.00	6.00	7.00	7.00	5.70	1.25	0.40	21.96	4.80	6.60	(-)	*	-

Table 102. Lipoprotein – HDL3 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	11.00	14.00	14.00	15.00	21.00	14.80	2.86	0.90	19.32	12.75	16.85			
1M	3R4F	10	10.00	14.00	17.00	19.00	21.00	16.20	3.82	1.21	23.60	13.46	18.94	-		
1M	THS2.2	10	8.00	14.00	15.50	16.00	19.00	14.90	2.88	0.91	19.36	12.84	16.96	-	-	
2M	Sham	10	8.00	8.00	10.50	12.00	12.00	10.10	1.73	0.55	17.12	8.86	11.34			
2M	3R4F	10	7.00	9.00	11.50	13.00	16.00	11.20	2.78	0.88	24.83	9.21	13.19	-		
2M	THS2.2	10	7.00	10.00	10.00	11.00	20.00	11.10	3.48	1.10	31.34	8.61	13.59	-	-	
3M	Sham	10	9.00	12.00	13.00	14.00	16.00	12.60	2.01	0.64	15.96	11.16	14.04			
3M	3R4F	10	10.00	12.00	14.00	15.00	16.00	13.40	2.17	0.69	16.20	11.85	14.95	-		
3M	THS2.2	10	9.00	11.00	13.00	16.00	17.00	13.10	3.00	0.95	22.89	10.96	15.24	-	-	
3M	Cess	10	10.00	10.00	12.00	13.00	19.00	12.30	2.71	0.86	22.03	10.36	14.24	-	-	
3M	Switch	10	8.00	9.00	12.50	13.00	16.00	11.60	2.91	0.92	25.12	9.52	13.68	-	-	-
6M	Sham	9	7.00	10.00	11.00	12.00	19.00	11.22	3.56	1.19	31.75	8.48	13.96			
6M	3R4F	10	9.00	9.00	10.50	13.00	15.00	11.20	2.20	0.70	19.65	9.63	12.77	-		
6M	THS2.2	10	6.00	10.00	12.00	13.00	15.00	11.40	2.50	0.79	21.96	9.61	13.19	-	-	
6M	Cess	10	6.00	9.00	11.00	12.00	13.00	10.50	2.32	0.73	22.11	8.84	12.16	-	-	
6M	Switch	10	6.00	8.00	10.50	13.00	14.00	10.20	2.90	0.92	28.41	8.13	12.27	-	-	-
8M	Sham	10	5.00	9.00	12.00	15.00	17.00	11.70	4.19	1.33	35.82	8.70	14.70			
8M	3R4F	10	8.00	9.00	12.00	15.00	17.00	12.10	2.96	0.94	24.47	9.98	14.22	-		
8M	THS2.2	9	8.00	11.00	11.00	12.00	17.00	11.67	2.45	0.82	21.00	9.78	13.55	-	-	
8M	Cess	10	6.00	11.00	13.00	15.00	19.00	12.70	4.03	1.27	31.72	9.82	15.58	-	-	
8M	Switch	10	7.00	11.00	12.50	15.00	18.00	12.50	3.27	1.04	26.20	10.16	14.84	-	-	-

Table 103. Lipoprotein – LDL (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	67.00	78.00	92.00	104.00	117.00	92.40	16.04	5.07	17.36	80.93	103.87			
1M	3R4F	10	59.00	114.00	123.50	135.00	142.00	117.50	24.97	7.90	21.25	99.64	135.36	*		
1M	THS2.2	10	54.00	102.00	105.50	111.00	116.00	100.10	18.28	5.78	18.27	87.02	113.18	-	+	
2M	Sham	10	84.00	91.00	96.00	110.00	150.00	102.80	18.98	6.00	18.46	89.22	116.38			
2M	3R4F	10	69.00	104.00	112.50	140.00	142.00	115.40	23.12	7.31	20.04	98.86	131.94	-		
2M	THS2.2	10	71.00	80.00	102.50	111.00	120.00	98.40	17.72	5.60	18.01	85.72	111.08	-	-	
3M	Sham	10	82.00	108.00	113.50	118.00	141.00	113.40	15.10	4.78	13.32	102.60	124.20			
3M	3R4F	10	94.00	99.00	119.00	121.00	124.00	111.80	11.93	3.77	10.67	103.26	120.34	-		
3M	THS2.2	10	82.00	88.00	98.50	110.00	132.00	100.30	15.37	4.86	15.32	89.31	111.29	-	-	
3M	Cess	10	83.00	89.00	104.00	126.00	144.00	107.70	21.48	6.79	19.94	92.33	123.07	-	-	
3M	Switch	10	65.00	92.00	112.00	118.00	134.00	106.20	21.52	6.81	20.27	90.80	121.60	-	-	-
6M	Sham	9	68.00	96.00	109.00	116.00	133.00	104.22	18.95	6.32	18.18	89.65	118.79			
6M	3R4F	10	89.00	113.00	122.00	131.00	152.00	122.30	16.28	5.15	13.31	110.65	133.95	*		
6M	THS2.2	10	98.00	106.00	110.50	121.00	132.00	112.60	10.28	3.25	9.13	105.25	119.95	-	-	
6M	Cess	10	71.00	102.00	109.50	114.00	154.00	109.30	23.94	7.57	21.90	92.17	126.43	-	-	
6M	Switch	10	80.00	94.00	105.00	119.00	137.00	106.90	16.85	5.33	15.76	94.85	118.95	-	-	-
8M	Sham	10	44.00	99.00	126.50	142.00	160.00	118.80	34.68	10.97	29.19	93.99	143.61			
8M	3R4F	10	61.00	94.00	115.50	126.00	144.00	111.40	25.35	8.02	22.76	93.26	129.54	-		
8M	THS2.2	9	84.00	107.00	110.00	125.00	127.00	111.11	15.04	5.01	13.53	99.55	122.67	-	-	
8M	Cess	10	79.00	81.00	102.50	133.00	168.00	109.20	30.71	9.71	28.12	87.23	131.17	-	-	
8M	Switch	10	33.00	97.00	115.00	135.00	160.00	109.60	40.80	12.90	37.23	80.41	138.79	-	-	-

Table 104. Lipoprotein – IDL (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	39.00	41.00	51.50	57.00	62.00	50.50	8.13	2.57	16.09	44.69	56.31			
1M	3R4F	10	35.00	64.00	68.00	69.00	76.00	63.40	12.26	3.88	19.33	54.63	72.17	*		
1M	THS2.2	10	33.00	56.00	59.50	61.00	68.00	56.70	9.80	3.10	17.28	49.69	63.71	-	+	
2M	Sham	10	48.00	50.00	55.50	59.00	62.00	54.50	4.77	1.51	8.75	51.09	57.91			
2M	3R4F	10	34.00	61.00	68.50	74.00	84.00	64.50	14.96	4.73	23.20	53.80	75.20	-		
2M	THS2.2	10	40.00	45.00	56.00	63.00	66.00	54.90	9.99	3.16	18.20	47.75	62.05	-	-	
3M	Sham	10	49.00	57.00	65.00	68.00	78.00	64.10	9.54	3.02	14.88	57.28	70.92			
3M	3R4F	10	56.00	56.00	64.00	68.00	69.00	62.80	5.27	1.67	8.39	59.03	66.57	-		
3M	THS2.2	10	43.00	54.00	57.00	64.00	82.00	58.90	11.03	3.49	18.73	51.01	66.79	-	-	
3M	Cess	10	48.00	53.00	60.50	69.00	83.00	62.30	12.27	3.88	19.69	53.53	71.07	-	-	
3M	Switch	10	40.00	55.00	62.00	69.00	78.00	60.90	11.57	3.66	19.00	52.62	69.18	-	-	-
6M	Sham	9	41.00	56.00	60.00	65.00	72.00	58.56	9.17	3.06	15.65	51.51	65.60			
6M	3R4F	10	41.00	64.00	68.50	71.00	74.00	65.80	9.34	2.95	14.20	59.12	72.48	+		
6M	THS2.2	10	52.00	58.00	62.00	65.00	67.00	61.50	4.55	1.44	7.40	58.24	64.76	-	+	
6M	Cess	10	43.00	59.00	62.50	65.00	102.00	63.90	15.63	4.94	24.46	52.72	75.08	-	(-)	
6M	Switch	10	47.00	53.00	59.50	67.00	68.00	59.30	7.92	2.50	13.35	53.64	64.96	-	+	-
8M	Sham	10	29.00	58.00	70.00	78.00	93.00	67.00	18.73	5.92	27.95	53.60	80.40			
8M	3R4F	10	37.00	54.00	62.00	71.00	77.00	60.60	12.23	3.87	20.18	51.85	69.35	-		
8M	THS2.2	9	48.00	56.00	63.00	70.00	71.00	62.56	8.14	2.71	13.01	56.30	68.81	-	-	
8M	Cess	10	40.00	48.00	60.00	74.00	85.00	61.20	14.99	4.74	24.49	50.48	71.92	-	-	
8M	Switch	10	22.00	58.00	67.00	80.00	92.00	63.80	22.30	7.05	34.96	47.84	79.76	-	-	-

Table 105. Lipoprotein – VLDL (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	136.00	149.00	164.50	203.00	227.00	172.10	31.43	9.94	18.26	149.62	194.58			
1M	3R4F	10	133.00	200.00	234.50	257.00	324.00	231.00	51.21	16.19	22.17	194.37	267.63	**		
1M	THS2.2	10	108.00	168.00	199.50	211.00	249.00	191.80	38.58	12.20	20.11	164.20	219.40	-	-	
2M	Sham	10	87.00	162.00	175.00	212.00	224.00	177.10	40.15	12.70	22.67	148.38	205.82			
2M	3R4F	10	119.00	217.00	243.50	247.00	313.00	230.30	55.86	17.66	24.25	190.34	270.26	*		
2M	THS2.2	10	104.00	149.00	197.00	217.00	223.00	184.80	38.84	12.28	21.02	157.02	212.58	(-)	-	
3M	Sham	10	134.00	196.00	236.50	245.00	246.00	216.20	37.31	11.80	17.26	189.51	242.89			
3M	3R4F	10	216.00	222.00	235.50	247.00	253.00	235.20	12.86	4.07	5.47	226.00	244.40	-		
3M	THS2.2	10	172.00	193.00	216.00	244.00	264.00	215.80	29.89	9.45	13.85	194.42	237.18	-	-	
3M	Cess	10	140.00	173.00	219.00	223.00	245.00	202.10	32.83	10.38	16.25	178.61	225.59	(-)	*	
3M	Switch	10	166.00	184.00	207.50	229.00	277.00	209.80	32.97	10.43	15.72	186.21	233.39	-	*	-
6M	Sham	9	152.00	208.00	219.00	225.00	241.00	212.00	25.85	8.62	12.19	192.13	231.87			
6M	3R4F	10	124.00	241.00	252.50	278.00	280.00	244.60	45.45	14.37	18.58	212.08	277.12	++		
6M	THS2.2	10	117.00	214.00	230.00	243.00	246.00	215.60	41.51	13.13	19.25	185.91	245.29	(-)	+	
6M	Cess	10	146.00	202.00	222.50	242.00	291.00	219.20	39.63	12.53	18.08	190.85	247.55	-	(-)	
6M	Switch	10	177.00	191.00	229.50	243.00	249.00	220.30	26.68	8.44	12.11	201.21	239.39	(-)	+	-
8M	Sham	10	102.00	182.00	242.00	270.00	292.00	220.50	66.96	21.17	30.37	172.60	268.40			
8M	3R4F	10	156.00	179.00	224.00	249.00	286.00	217.60	43.37	13.71	19.93	186.58	248.62	-		
8M	THS2.2	9	203.00	216.00	223.00	238.00	269.00	229.67	21.28	7.09	9.27	213.31	246.03	-	-	
8M	Cess	10	131.00	196.00	232.00	239.00	315.00	220.10	52.20	16.51	23.72	182.76	257.44	-	-	
8M	Switch	10	105.00	214.00	231.00	252.00	287.00	219.00	55.01	17.40	25.12	179.65	258.35	(-)	-	-

Table 106. Lipoprotein – VLDL3 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	60.00	62.00	78.00	81.00	101.00	75.90	12.60	3.98	16.60	66.89	84.91			
1M	3R4F	10	58.00	91.00	98.00	110.00	124.00	96.00	20.04	6.34	20.87	81.67	110.33	*		
1M	THS2.2	10	48.00	80.00	83.00	88.00	96.00	80.40	14.21	4.49	17.68	70.23	90.57	-	-	
2M	Sham	10	45.00	70.00	75.50	83.00	87.00	74.10	12.62	3.99	17.03	65.07	83.13			
2M	3R4F	10	54.00	87.00	100.00	112.00	121.00	95.60	20.79	6.57	21.75	80.73	110.47	*		
2M	THS2.2	10	48.00	64.00	81.50	89.00	94.00	77.60	15.46	4.89	19.92	66.54	88.66	(-)	*	
3M	Sham	10	64.00	85.00	98.00	103.00	115.00	93.70	14.91	4.71	15.91	83.04	104.36			
3M	3R4F	10	86.00	88.00	97.50	105.00	112.00	97.30	9.68	3.06	9.95	90.37	104.23	-		
3M	THS2.2	10	64.00	78.00	89.50	104.00	122.00	90.60	18.45	5.84	20.37	77.40	103.80	-	-	
3M	Cess	10	63.00	78.00	89.00	101.00	118.00	89.30	15.87	5.02	17.78	77.94	100.66	-	-	
3M	Switch	10	60.00	84.00	91.00	106.00	116.00	91.90	17.00	5.38	18.50	79.74	104.06	-	-	-
6M	Sham	9	58.00	79.00	87.00	90.00	95.00	83.00	11.00	3.67	13.25	74.54	91.46			
6M	3R4F	10	53.00	91.00	99.50	107.00	123.00	96.10	18.85	5.96	19.61	82.62	109.58	+		
6M	THS2.2	10	58.00	80.00	89.00	97.00	101.00	86.90	13.40	4.24	15.42	77.31	96.49	(-)	-	
6M	Cess	10	66.00	79.00	89.50	96.00	116.00	90.70	15.33	4.85	16.91	79.73	101.67	-	-	
6M	Switch	10	68.00	78.00	89.50	99.00	112.00	89.90	14.43	4.56	16.05	79.58	100.22	-	-	-
8M	Sham	10	43.00	67.00	102.50	105.00	130.00	91.30	27.99	8.85	30.66	71.28	111.32			
8M	3R4F	10	65.00	74.00	87.50	104.00	123.00	89.90	19.60	6.20	21.80	75.88	103.92	-		
8M	THS2.2	9	80.00	88.00	94.00	100.00	109.00	94.00	9.79	3.26	10.41	86.48	101.52	-	-	
8M	Cess	10	56.00	78.00	93.50	101.00	130.00	91.00	22.23	7.03	24.42	75.10	106.90	-	-	
8M	Switch	10	36.00	95.00	98.50	111.00	140.00	95.50	30.74	9.72	32.18	73.51	117.49	-	-	-

Table 107. Lipoprotein – LDL-4 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	2.80	5.10	5.20	5.70	7.80	5.39	1.32	0.42	24.43	4.45	6.33			
1M	3R4F	10	3.50	5.60	6.45	7.60	8.50	6.45	1.51	0.48	23.48	5.37	7.53	-		
1M	THS2.2	10	2.30	3.80	5.10	5.80	6.50	4.79	1.31	0.41	27.25	3.86	5.72	-	*	
2M	Sham	10	2.80	3.00	3.95	4.70	10.20	4.51	2.22	0.70	49.24	2.92	6.10			
2M	3R4F	10	2.60	3.50	4.95	6.60	8.20	5.09	1.91	0.60	37.47	3.73	6.45	-		
2M	THS2.2	10	2.70	3.70	5.20	6.30	7.00	5.00	1.40	0.44	27.95	4.00	6.00	-	-	
3M	Sham	10	2.90	3.30	3.95	4.80	6.80	4.22	1.16	0.37	27.38	3.39	5.05			
3M	3R4F	10	2.00	3.80	5.00	5.80	6.80	4.77	1.51	0.48	31.67	3.69	5.85	-		
3M	THS2.2	10	0.80	2.80	3.90	5.10	6.00	3.72	1.60	0.51	43.01	2.58	4.86	-	-	
3M	Cess	10	1.90	2.10	3.20	4.20	5.00	3.27	1.08	0.34	32.91	2.50	4.04	-	*	
3M	Switch	10	2.10	2.30	3.90	4.60	6.70	3.92	1.64	0.52	41.90	2.75	5.09	-	-	-
6M	Sham	9	2.80	3.80	3.90	4.20	5.20	3.99	0.63	0.21	15.78	3.51	4.47			
6M	3R4F	10	1.80	2.00	4.00	5.60	9.50	4.41	2.35	0.74	53.36	2.73	6.09	-		
6M	THS2.2	10	2.70	3.70	4.20	6.80	7.20	4.92	1.73	0.55	35.14	3.68	6.16	-	-	
6M	Cess	10	0.00	2.60	3.00	4.50	6.20	3.40	1.78	0.56	52.28	2.13	4.67	-	-	
6M	Switch	10	3.20	3.80	4.30	5.30	7.20	4.63	1.19	0.38	25.68	3.78	5.48	-	(-)	-
8M	Sham	10	2.10	3.20	3.95	5.10	6.10	4.07	1.25	0.39	30.62	3.18	4.96			
8M	3R4F	10	2.20	2.80	4.70	6.10	8.30	4.87	2.06	0.65	42.37	3.39	6.35	-		
8M	THS2.2	9	3.10	4.40	4.60	5.10	7.30	4.90	1.24	0.41	25.33	3.95	5.85	-	-	
8M	Cess	10	1.90	3.60	3.90	5.20	7.90	4.47	1.97	0.62	44.13	3.06	5.88	-	-	
8M	Switch	10	2.80	3.00	3.60	4.30	5.30	3.74	0.86	0.27	22.90	3.13	4.35	-	-	(-)

Table 108. Lipoprotein – LDL3 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	4.40	7.20	8.20	9.20	12.80	8.20	2.17	0.69	26.43	6.65	9.75			
1M	3R4F	10	4.50	10.70	11.80	13.60	15.10	11.49	3.06	0.97	26.61	9.30	13.68	*		
1M	THS2.2	10	3.70	8.80	9.55	10.30	11.50	9.02	2.22	0.70	24.64	7.43	10.61	-	+	
2M	Sham	10	7.50	8.20	8.90	11.30	26.10	10.99	5.54	1.75	50.41	7.03	14.95			
2M	3R4F	10	6.30	8.60	9.85	12.50	14.50	10.37	2.60	0.82	25.06	8.51	12.23	(-)		
2M	THS2.2	10	6.50	7.20	8.40	10.30	12.80	8.78	1.96	0.62	22.31	7.38	10.18	(-)	-	
3M	Sham	10	6.20	10.00	10.35	11.60	14.50	10.78	2.35	0.74	21.83	9.10	12.46			
3M	3R4F	10	8.20	9.10	10.00	11.70	14.30	10.50	2.05	0.65	19.52	9.03	11.97	-		
3M	THS2.2	10	7.20	7.20	8.50	10.40	10.80	8.68	1.44	0.46	16.63	7.65	9.71	*	(-)	
3M	Cess	10	7.50	8.10	9.05	12.60	13.60	9.83	2.36	0.75	24.02	8.14	11.52	-	(-)	
3M	Switch	10	5.00	8.10	10.05	12.00	12.60	9.84	2.44	0.77	24.79	8.09	11.59	-	-	-
6M	Sham	9	5.60	8.60	11.10	11.30	14.40	10.19	2.63	0.88	25.83	8.17	12.21			
6M	3R4F	10	9.40	10.80	11.90	14.50	19.80	12.81	3.01	0.95	23.52	10.65	14.97	-		
6M	THS2.2	10	9.20	9.40	10.45	11.60	20.40	11.49	3.38	1.07	29.38	9.08	13.90	-	(-)	
6M	Cess	10	6.10	8.10	9.45	11.00	15.10	9.81	2.89	0.91	29.45	7.74	11.88	-	*	
6M	Switch	10	6.90	8.80	10.40	11.60	15.60	10.55	2.35	0.74	22.31	8.87	12.23	-	-	-
8M	Sham	10	1.60	9.90	11.70	14.80	15.60	11.57	4.19	1.32	36.20	8.57	14.57			
8M	3R4F	10	4.50	9.30	11.45	12.90	16.00	11.00	3.21	1.02	29.20	8.70	13.30	-		
8M	THS2.2	9	7.10	9.00	10.70	12.30	13.40	10.39	2.29	0.76	22.04	8.63	12.15	-	-	
8M	Cess	10	5.60	7.40	9.45	12.30	19.40	10.38	4.01	1.27	38.67	7.51	13.25	-	-	
8M	Switch	10	0.60	8.00	10.50	12.40	16.30	9.80	4.73	1.50	48.25	6.42	13.18	-	-	-

Table 109. Lipoprotein – LDL2 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	0.00	1.50	2.15	2.30	4.70	2.04	1.18	0.37	57.92	1.19	2.89			
1M	3R4F	10	0.00	2.60	3.55	4.90	5.40	3.28	1.77	0.56	54.10	2.01	4.55	-		
1M	THS2.2	10	0.00	1.20	2.25	2.70	3.50	1.98	1.07	0.34	53.86	1.22	2.74	-	-	
2M	Sham	10	1.10	1.60	2.15	3.40	12.60	3.32	3.39	1.07	102.10	0.90	5.74			
2M	3R4F	10	0.50	1.00	2.45	3.60	6.10	2.64	1.84	0.58	69.74	1.32	3.96	(-)		
2M	THS2.2	10	0.30	1.00	2.00	3.30	3.80	2.08	1.18	0.37	56.70	1.24	2.92	(-)	-	
3M	Sham	10	0.70	2.20	2.80	3.60	4.50	2.78	1.21	0.38	43.52	1.91	3.65			
3M	3R4F	10	0.70	2.10	2.55	3.50	6.30	2.92	1.67	0.53	57.16	1.73	4.11	-		
3M	THS2.2	10	0.00	1.30	1.55	2.50	3.40	1.74	1.12	0.35	64.29	0.94	2.54	-	-	
3M	Cess	10	1.10	1.40	2.05	3.40	5.20	2.44	1.37	0.43	56.17	1.46	3.42	-	-	
3M	Switch	10	0.00	1.40	2.30	3.20	3.90	2.21	1.19	0.38	53.90	1.36	3.06	-	-	-
6M	Sham	9	0.00	1.30	3.10	3.40	5.80	2.68	1.69	0.56	63.26	1.38	3.98			
6M	3R4F	10	2.40	2.80	3.00	4.70	6.90	3.72	1.49	0.47	40.15	2.65	4.79	-		
6M	THS2.2	10	0.70	2.10	2.90	3.40	8.70	3.20	2.16	0.68	67.65	1.65	4.75	-	(-)	
6M	Cess	10	0.10	0.60	1.45	3.20	5.80	2.01	1.81	0.57	89.99	0.72	3.30	-	(-)	
6M	Switch	10	1.20	2.10	2.60	2.90	7.20	2.79	1.67	0.53	59.75	1.60	3.98	(-)	+	(-)
8M	Sham	10	0.00	1.30	3.90	4.70	5.30	3.19	2.01	0.64	63.14	1.75	4.63			
8M	3R4F	10	0.00	2.00	3.85	5.10	6.10	3.51	1.90	0.60	54.03	2.15	4.87	-		
8M	THS2.2	9	1.20	1.70	2.90	3.70	4.40	2.73	1.14	0.38	41.75	1.86	3.61	-	-	
8M	Cess	10	0.00	1.00	1.95	4.00	8.20	2.66	2.44	0.77	91.75	0.91	4.41	-	-	
8M	Switch	10	0.00	1.80	2.40	3.50	5.30	2.38	1.62	0.51	67.98	1.22	3.54	-	-	-

Table 110. Lipoprotein - LDL1 (VAP test, Atherotech)

Exposure Duration	Treatment	N	MIN	Q1	MED	Q3	MAX	MEAN	STD	SEM	CV%	95%-LCLM	95%-UCLM	vs. Sham	vs. 3R4F	vs. cess
1M	Sham	10	13.40	16.40	19.10	21.30	27.10	19.39	4.11	1.30	21.18	16.45	22.33			
1M	3R4F	10	11.60	22.40	25.05	27.20	30.30	24.09	5.35	1.69	22.20	20.26	27.92	*		
1M	THS2.2	10	11.70	22.00	22.25	23.20	24.00	21.15	3.71	1.17	17.56	18.49	23.81	-	+	
2M	Sham	10	18.20	20.80	21.90	24.00	32.30	22.92	4.10	1.30	17.89	19.99	25.85			
2M	3R4F	10	12.90	22.20	23.95	31.50	32.00	24.82	6.07	1.92	24.46	20.48	29.16	-		
2M	THS2.2	10	15.80	17.70	21.35	24.30	26.80	21.23	4.00	1.26	18.83	18.37	24.09	-	-	
3M	Sham	10	17.20	23.70	24.95	25.90	31.40	24.77	3.68	1.16	14.85	22.14	27.40			
3M	3R4F	10	20.10	21.40	25.30	27.20	27.80	24.44	2.87	0.91	11.73	22.39	26.49	-		
3M	THS2.2	10	16.20	18.80	21.40	24.20	31.50	21.96	4.37	1.38	19.92	18.83	25.09	-	-	
3M	Cess	10	17.90	20.60	22.05	28.60	35.90	24.69	5.80	1.83	23.50	20.54	28.84	-	-	
3M	Switch	10	14.50	21.00	23.90	27.30	29.70	23.72	4.85	1.53	20.43	20.25	27.19	-	-	-
6M	Sham	9	15.10	20.70	24.00	27.40	32.20	23.83	5.32	1.77	22.33	19.74	27.92			
6M	3R4F	10	15.60	27.30	27.80	30.40	34.50	27.90	4.97	1.57	17.81	24.35	31.45	-		
6M	THS2.2	10	21.40	23.00	25.35	26.50	31.20	25.21	2.80	0.89	11.10	23.21	27.21	-	-	
6M	Cess	10	15.10	22.40	23.95	25.90	38.50	24.82	6.56	2.07	26.43	20.13	29.51	-	-	
6M	Switch	10	17.80	21.40	22.35	26.60	31.00	23.51	4.03	1.28	17.15	20.62	26.40	-	*	-
8M	Sham	10	9.10	21.60	30.40	33.10	37.40	27.41	8.71	2.75	31.76	21.18	33.64			
8M	3R4F	10	12.90	22.30	25.55	30.20	31.70	25.03	5.96	1.89	23.83	20.76	29.30	-		
8M	THS2.2	9	18.00	22.40	24.30	27.60	29.10	24.26	3.93	1.31	16.22	21.23	27.28	-	-	
8M	Cess	10	15.10	17.10	22.55	28.40	38.40	23.88	7.56	2.39	31.65	18.47	29.29	-	-	
8M	Switch	10	5.70	21.00	25.95	29.40	37.60	24.57	10.09	3.19	41.08	17.35	31.79	-	-	-

19 Appendix 1. Amendments and deviations

FORM
STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdus name : PHLRD_FOR_00075

P -/ Short Title Amendment - 1

15015 | ApoE ZRH Switching study

Study Director	Author	Date Submitted
Blaine Phillips	Donital Sharma	30 Aug 2013

Subject

Amend section relevant to in life assessment of "Insulin Resistant" that describes the procedure for collecting blood from anaesthetized mice and perform whole blood glucose determination in ApoE mice.

Reason(s) for Change

Training for the blood collection from facial vein in unanesthetized mice and whole blood glucose determination was concluded after the study plan was finalized.

Change

Please see attachment

Consequences of Change and Measures to be taken

Treatment team and bio-lab team is informed. No impact.

APPROVED **Controlled Copy** Page 1 of 2

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name : PMI_RD_FOR_000175

Remarks

None

Author:	30 Aug 2013 Date/Signature
Study Director:	30 Aug 2013 Date/Signature
TFM:	30 Aug 2013 Date/Signature
Sponsor:	Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [2]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site

[2] If applicable

Controlled Copy

Page **1** of **2**

Study 15015 Apo E ZRH switching study Amendment #1, Attachment 1

Study director: Blaine Phillips, 30 Aug 2013

Blood Glucose determination in Apo E mice (groups 601, 611, 602, 612, 603 and 613).

This amendment describes the procedure for collecting blood from un-anesthetized mice from insulin resistance groups in study 15015 (and is an update of section 4.4.14.1.1 of the study plan).

The blood collection will be performed as follows:

Mice scheduled for blood glucose determination will be fasted for 6 hours

Longitudinal blood collection (fasting glucose) – blood/plasma will be collected for assessment of the endpoints described in Table 1

The collection of the blood will be via facial vein bleeding according to the standard working instructions (PMI_RD_WKI_000375) but with the following differences:

1. As inhaled anesthetics interfere with metabolic parameters, the blood collection will be performed without anesthetics.

2. Use 23G instead of 20G needle to puncture the facial vein for blood collection

3. Analgesics will be used to alleviate pain in the form of topical lidocain gel.

4. The total blood collected will be approximately 150 µl according to the following volumes and applications list:
10 µl Whole blood (facial vein) for fasting glucose
140 µl whole blood (EDTA tube) – ~30 µl plasma fasting insulin
– ~30 µl plasma for fasting HbA1C

Table 1: Endpoints for longitudinal blood analysis (from 6 – 7 hour fasted animals)

Primary Endpoint	Method	Timing of the analysis
Whole blood fasting glucose	Glucometer	Immediate in SG
Plasma fasting insulin	ELISA	Outsourced (TBD)
Plasma fasting HbA1C	ELISA	Outsourced (TBD)

Perform a fasting glucose test using ACCU CHEK Performa glucose meter system as follows

1. Make sure that supplies needed to run the test are ready. Accu-Chek Performa glucose meter(s), Accu-Chek Performa glucose test strips, Accu-Chek control solutions (level 1 and 2). Use record Table 2 sheet to capture the control check results.
2. Perform two levels of control check every start of the test day for each glucose meter.

Page **2** of **2**

2.1 Prepare meters for testing. Always check expiry date printed on the bottle of test strips. Record Asset ID and test strips lot number and expiry date in the instrument logbook.

2.2 Insert the test strip into the meter in the direction of the arrows, and gently squeeze the level 1 control solution to the front edge of the yellow window of the test strip. A measurement appears on the display with control bottle symbol and flashing "L". Press "▶" once for level 1 control. Check if the result is within the permitted range given on the test strip container label. Record result and permitted range. Press "on/off" button to set the level in meter. Remove test strip.

2.3 Repeat step 2.2 for level 2 control solution, press "▶" twice for level 2 control. Check if the result is within the permitted range.

2.4 Insert a fresh test strip into the meter in the direction of the arrows for every sample.

2.5 Appears on the display. Collect the blood sample from mice via facial vein, and gently apply the blood to the front edge of the yellow window of the test strip. flashes when enough blood is applied. Result appears on display in 5 seconds; record the result in the table illustrated in Table 2 on the record sheets and attach these record sheets to PMI_RD_FOR_000118 mentioning the particulars of record sheets .

2.6 Remove the test strip. Repeat step 2.4 and 2.5 for the other animals too.

Table2: Glucose concentration record sheet

Determination of whole blood glucose concentration (ACCU-CHEK-PERFORMA)						
Asset ID:						
Control	Lot Number	Expiry Date	Result (mmol/L)	Acceptable range	Remark	Initials
1						
2						
Sample ID	Time		Result (mmol/L)	Glucometer ID	Remark	Initials

3. The meter measureable range is 0.6 to 33.3 mmol/L. If the display shows "HI" the blood glucose maybe higher than the measuring range of the system or "LO" The blood glucose maybe lower than the measuring range of the system Please record as 'HI' or 'LO', accordingly.
4. The blood glucose determination should be performed immediately after blood collection or no more than 30 minutes.
5. Centrifuge the rest of blood sample at 3000RPM for 10 minutes and collected plasma will be archived in two aliquots for fasting insulin and fasting HbA1C respectively at ≤ -70 ° C.

FORM
STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Amendment 2

P -/	Short Title
15015	ApoE-2RH switch : extend concentration adaptation

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	06 Sept 13.

Subject

Increase aerosol concentration adaptation period from 6 days to 7 days.

Reason(s) for Change

1. prefered to have first day of target concentration on a working day (Monday).
2. ApoE-/- mice are sensitive to 3R4F smoke, so a slightly longer adaptation is preferred.

Change

new schedule:
Day 1-2 : 150µg/l TPM (nicotine equivalent)
Day 3-4 : 300µg/l TPM (nicotine equivalent)
Day 5-7: 450µg/l TPM (nicotine equivalent)
Day 8 onwards: 600µg/l TPM (nicotine equivalent)

Consequences of Change and Measures to be taken

This will have no impact on the study

APPROVED

Controlled Copy

Page 1 of 2

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 20

Remarks

NA

Author:	06 Sept 13 Date/Signature
Study Director:	06 Sept 13 Date/Signature
TFM:	06 Sept 13 Date/Signature
Sponsor:	Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [2]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site

[2] If applicable

Controlled Copy

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdas name : PMI_RD_FOR_000175

Amendment - ⓐ 3

P -/	Short Title
15015	ApoE ZRH Switching study.

Study Director	Author	Date Submitted
Blaine Phillips	Denilal Sharma	16 sep 13

Subject

Amend section "In life assessment of Insulin Resistance" that describes the procedure to determine fasting blood glucose and plasma seperation from collected blood.

Reason(s) for Change

These amendments are proposed based on the experience of whole blood glucose determination activity done on 31 Aug 13 after the finalization of study plan.

Change

Please see attachment

Consequences of Change and Measures to be taken

The concerned treatment and bio analytical team is informed - No impact

ⓐ 19/ 16 sep 13

APPROVED

Controlled Copy

Page 1 of 2

FORM

STUDY PLAN AMENDMENT

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Remarks

None

Author:	16 Sep 13 — Date/Signature
Study Director:	16 Sept 13 — Date/Signature
TFM:	16 Sept 2013 — Date/Signature
Sponsor:	Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [2]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site

[2] If applicable

Controlled Copy

Page 2 of 2

APPROVED

Page 1 of 1

(A) 16 Sep 13

Study 15015 Apo E ZRH switching study Amendment #2, 3 Attachment 1

Study director: Blaine Phillips, 16 September 2013

Blood Glucose determination and plasma collection activity in Apo E mice (groups 601, 611, 602, 612, 603 and 613).

1. Fasting for groups 601(Cage 124 & 125), 602(Cage 129 & 130), 603(Cage 134 & 135) for 6 hours should be done at 8.00 am in morning with a cage change and confirmation of availability of water throughout fasting.

2. Fasting for groups 611(Cage 126 & 127), 612(Cage 131 & 132), 613(Cage 136 & 137) for 6 hours should be done at 9.15 am in morning with a cage change and confirmation of availability of water throughout fasting.

3. Blood collection for groups 601, 602 & 603 will be done in the following order (group (cage)): 601(124), 602(129), 603(134), 601(125), 602(130), 603(135) starting at 2.00pm.

4. Blood collection for groups 611, 612 & 613 will be done in the order (group (cage)): 611(126), 612(131), 613(136), 611(127), 612(132), 613(137) immediately after blood collection for 601, 602 & 603.

5. One cage at a time will be taken for blood collection from animals; the animals from one cage will be distributed amongst personals randomly. Note, blood will be collected away from the animal housing room to reduce animal stress.

6. 1st or 2nd drop of the facial vein blood will be taken for whole blood glucose determination. The rest of the blood will be collected in a K2EDTA microvette up to maximum of 140µl (based on the condition of animals), then immediately inverted 4 times and transferred to either centrifuge at 4° C or the ice tray before sample goes to centrifuge. Ensure effective mixing of the samples in the tube.

7. Centrifugation will be performed for 5-8 samples at a time at 3000 RPM for 10 minutes. All Plasma is collected in aliquot 1, using a 10 µl pipette – ensure that no interface or pellet is transferred. The plasma will then be distributed as follows, depending on the plasma volume:
   - Greater than 40 µl: 20 µl transferred to aliquot '2'
   - Between 20 µl and 40 µl: 20 µl transferred to aliquot '2', rest stays with aliquot '1'
   - Less than 20 µl: No aliquot ' 2'.

8. All aliquots are placed in ice until they are stored at -80° C.

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdus name : PMI_RD_FOR_000175

P -/	Short Title
15015	ApoE - ZRH - Switching - Dissection plan update - Amendment 4

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	01 Oct 2013

Subject

This amendment describes updates to the dissection plan

Reason(s) for Change

see attachment 1

Change

See attachment 1

Consequences of Change and Measures to be taken

See attachment 1.

APPROVED

Controlled Copy

FORM
STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Remarks

NA

Author:	01 Oct 2013 — Date/Signature
Study Director:	01 Oct 2013 — Date/Signature
TFM:	01 Oct 2013 — Date/Signature
Sponsor:	Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [2]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site
[2] If applicable

Controlled Copy

APPROVED

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

Table 1. Number of Animals From Each Exposure Group, and Per Necropsy Phase

Short Name	Group Code	A (BALF)	B (Histopathology)	C (Lung Function)	D (Omics)	E Vascular Inflammation/platelet	F IR	Total mice
Sham_1m	1	10	10	10	8	10		48
Sham_2m	2	10	10	10	8			38
Sham_3m	3	10	10	10	8	8		46
Sham_6m	6	10	10	10	8	8		46
Sham_8m	8	10	10	10	8			38
3R4F_1m	101	10	10	10	8	8		46
3R4F_2m	102	10	10	10	8			38
3R4F_3m	103	10	10	10	8	8		46
3R4F_6m	106	10	10	10	8	8		46
3R4F_8m	108	10	10	10	8			38
ZRH_1m	201	10	10	10	8	8		46
ZRH_2m	202	10	10	10	8			38
ZRH_3m	203	10	10	10	8	8		46
ZRH_6m	206	10	10	10	8	8		46
ZRH_8m	208	10	10	10	8	8		46
Cessation_1m	303	10	10	10	8	8		46
Cessation_4m	306	10	10	10	8			38
Cessation_6m	308	10	10	10	8			38
Switching_1m	403G	10	10	10	8	8		46
Switching_4m	406G	10	10	10	8	8		46
Switching_6m	408G	10	10	10	8			38
Sham_chow	601						10	10
Sham_HFD	611						15	15
3R4F_chow	602						10	10
3R4F_HFD	612						15	15
ZRH_chow	603						10	10
ZRH_HFD	613						15	15
Total		210	210	210	168	106	75	979

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

Reason for change

This is an update to the dissection plan, including several revisions to the endpoints and detailed methods which were unavailable at the time of study plan signing.

Change

The revised dissection plan is as follows (any additions or changes marked in red font):

1.1 Necropsy

The endpoints of the study will be oriented towards the examination of endpoints related to inflammation state, development and progression of emphysema/COPD, atherosclerosis, and IR. This will logistically be performed by splitting the animals into 6 dissection streams (A through F), depicted in Table 1. The details of each dissection stream, and the methods utilized will follow. Note, due to the length of the study, necropsies will be performed as 28 day month cycles, in order to standardize the timing between the dissection endpoints.

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

The endpoint necropsy consists of 6 separate dissection streams. The tissues and methods will be performed as follows:

A) BALF (10 mice per group)

- Free lung cells – Quantification and differential count (section 1.1.2)
- BALF – Inflammatory mediators (study phase 4, Myriad/Rules-based Medicine, RodentMAP analysis)
- BALF – MMP activity
- Aortic arch – Plaque size (section 1.1.7.4)
- Thoracic aorta - Histopathology (paraffin embedding)
- Blood – White blood cell counts (Sysmex)
- Serum – Serum inflammatory mediators (study phase 4, Myriad/Rules-based Medicine, RodentMAP analysis)
  - ELISA (siCAM, sP-selectin)

B) Histopathology (10 mice per group)

- Left lung – Histopathology/stereological assessment/immunohistochemistry (section 1.1.9, 1.1.13)
- Right lung (all lobes) – Histopathology/morphometry back-up
- Liver (left lobe) – Back up (fixed in 4% formaldehyde)
- Nose (3 levels) – Histopathology (H&E, AB-PAS)
- Plasma – Glucose, HDL, LDL, VLDL (Singapore) (section 1.1.7)
- Plasma – Oxysterols (section 1.1.7.1)
- Serum – Proteomics

C) Lung Function (10 mice per group)

- Lung function analysis (section 1.1.8)
- Serum – Proteomics (section 1.1.6)

D) Omics (8 mice per group)

- Aortic arch – Plaque size (section 1.1.7.4)
- Nasal epithelium – Transcriptomics (left and right nostril) (1.1.4)
- Lung (left) – Transcriptomics/genomics
- Lung (right lung c) – Lipidomics
- Lung (right lung a and b) - Proteomics
- Liver (left lobe) – Transcriptomics
- Liver – Genomics

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

- Liver - Proteomics
- Liver (left lobe) – Lipidomics
- Liver (left lobe) – Histopathology – (cryosection Oil red O)
- Liver (left lobe) – Histopathology (fixed in 4% formaldehyde) (H&E, PAS, PASD, IHC (1.1.13))
- Liver (remaining lobes) - back-up tissue (frozen)
- Heart (left ventricle, apex) – Storage (frozen)
- Thoracic aorta – Transcriptomics
- Abdominal aorta – Lipidomics (test site 3)
- Blood – Transcriptomics
- Blood - Genomics
- Plasma – Lipidomics (test site 3)

E) Vascular inflammation and platelet aggregation

- Aortic arch and descending aorta – Cell dissociation/FACS (section 1.1.14)
- Blood – Platelet aggregation/FACS (section 1.1.15)

F) IR endpoints (10 to 15 mice per group)

- Mesenteric fat – Transcriptomics
- Liver (left lobe) –Histopathology (H&E, PAS, PASD)
- Liver (left lobe) – Histopathology (cryosections, oil red O)
- Liver (left lobe) – Transcriptomics
- Liver (left lobe) – Protein (Western blotting for validation of biomarkers)
- Liver (left lobe) - Lipidomics
- Pancreas – Histopathology (H&E)
- Plasma– Glucose, HDL, LDL, VLDL (Singapore)
- Plasma – Lipidomics (Zora Biosciences)
- Serum – Metabolic MAP (study phase 4, Myriad/Rules-based Medicine, Metabolic MAP analysis)
- Muscle (soleus and gastrocnemius as single tissue piece) – Transcriptomics
- Aortic arch – Morphometric evaluation of total plaque area
- Thoracic aorta – Transcriptomics
- Heart (apex) – Transcriptomics

Table 2. Necropsy and endpoint details

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

Tissue	Dissection group	Target	Application	Location
Free lung cells	A	Inflammation	Differential count	Singapore
Free lung cells	A	Inflammation	Viability	Singapore
BALF (cell free)	A	Inflammation	Inflammatory mediators	Myriad/Rules-based Medicine
BALF (cell free)	A	Back-up	Back-up	Back-up
BALF (cell free)	A	Inflammation	MMP activity	Singapore
Aortic arch	A	Lipidomics	Plaque size	Singapore
Thoracic aorta	A	Histopathology	Embedding (back-up)	Singapore
Blood	A	Haematology	complete blood cell counts	Singapore
Serum	A	Inflammation	Inflammatory mediators	Myriad/Rules-based Medicine
Lung (left)	B	Histopathology	Morphometry and histopathology/immunhistochemistry	Singapore/Neuchatel (IHC)
Lung (right)	B	Histopathology	Back-up	Singapore
Liver	B	Histopathology	Back-up (for embedding)	Singapore (IMCB)
Nose (3 levels)	B	Histopathology	Histopathology	Singapore
Plasma	B	Lipidomics	Lipoprotein analysis	Singapore
Plasma	B	Lipidomics	Oxysterols	Neuchatel
Serum	C	Proteomics	Proteomics	Neuchatel
Aortic arch	D	Lipidomics	Plaques size	Singapore
Nasal epithelium (left)	D	Transcriptomics	Transcriptomics	Neuchatel
Nasal epithelium (right)	D	Transcriptomics	Transcriptomics	Neuchatel
Lung (left)	D	Transcriptomics/ genomics	Transcriptomics/genomic	Neuchatel
Lung (right lung a and b)	D	Proteomics	Proteomics	Neuchatel
Lung (right lung c)	D	Lipidomics	Lipidomics	Zora Biosciences
Liver (left lobe 1a)	D	RNA	Transcriptomics	Neuchatel
Liver (left lobe, 1b)	D	DNA	Genomics	Neuchatel
Liver (left lobe, 1c)	D	Protein	Proteomics	Neuchatel
Liver (left lobe 3)	D	Lipidomics	Lipidomics	Zora Biosciences
Liver (left lobe, 2a)	D	Histolopathology	H&E, PAS, PASD (paraffin)	Singapore
Liver (left lobe 2b)	D	Histopathology	Oil Red O (cryosections)	Singapore
Liver (remaining lobes)	D	Back-up	Back-up	Neuchatel
Heart	D	Back-up	Back-up	Neuchatel

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

Tissue	Dissection group	Target	Application	Location
Thoracic aorta	D	Transcriptomics	Transcriptomics	Neuchatel
Abdominal aorta	D	Lipidomics	Lipidomics	Zora Biosciences
Blood	D	Transcriptomics	Transcriptomics	Neuchatel
Blood	D	Genomics	Methylation	Neuchatel
Plasma	D	Lipidomics	Lipidomics	Zora Biosciences
Serum	D	Proteomics	Proteomics	Neuchatel
Aortic arch	E	Inflammation	FACS	Singapore
Blood	E	Platelet aggregation	FACS	Singapore
Mesenteric fat	F	Transcriptomics	Transcriptomics	Neuchatel
Liver (left lobe, 2b)	F	Histopathology	Oil Red O (cryosections)	Singapore/Neuchatel
Liver (left lobe, 2a)	F	Histopathology	H&E, PAS, PASD (paraffin)	Singapore/Neuchatel
Liver (left lobe, 1a)	F	Transcriptomics	Transcriptomics	Neuchatel
Liver (left lobe, 1c)	F	Proteomics	Western blot	Neuchatel
Liver (left lobe, 3)	F	Lipidomics	Lipidomics	Neuchatel
Pancreas	F	Histopathology	H&E (paraffin)	Singapore
Plasma	F	Lipidomics	Glucose, HDL/LDL/VLDL	Singapore
Plasma	F	Lipidomics	Lipidomics	Zora Biosciences
Plasma	F	Proteomics	Rodent Metabolic MAP	Myriad/RBM
Muscle (soleus and gastrocnemius combined)	F	Transcriptomics	Transcriptomics	Neuchatel
Aortic arch	F	Lipidomics (Morphometry)	Plaque size	Singapore
Thoracic aorta	F	Transcriptomics	Transcriptomics	Neuchatel
Heart	F	Transcriptomics	Transcriptomics	Neuchatel

1.1.1 Partitioning of liver and lungs during dissection

As there are multiple uses for both the liver and lung, the organs will be partitioned as described in Figure 1.

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

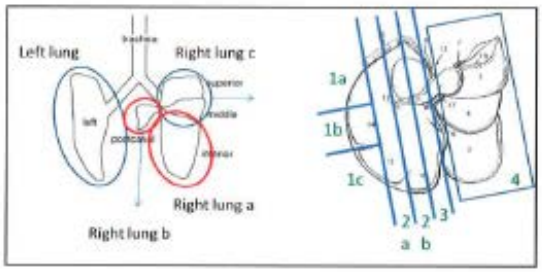


Figure 1. **Liver and lung necropsy for 'omics analysis.** Where described in the necropsy section, lungs may be partitioned into left, and 3 x right lung partitions (lung c: superior & middle; lung a: interior; lung b: postcaval) as depicted (left). The liver will be partitioned into multiple pieces, and described using the numbering system depicted above (right)

1.1.2 Lung inflammation

The parameters listed in Table 3 will be used to assess lung inflammation. These include the analysis of the cellular composition of the BALF (free lung cells), as well as the levels of specific proteins (multi-analyte profile (RodentMAP® v 3.0) – delegated phase 5) or their activities (MMP activity) from the cell-free BALF.

1.1.3 Collection of Bronchoalveolar fluid (BALF)

The collection of the BALF will be performed according to standard work instructions (PMI_RD_WKI_000319, 'Prepare lavage samples: Lavage of mouse lung – syringe method'). As there are different downstream processes, the BALF will be split as follows; Cycle 1 will be collected in PBS, and used for protein and enzyme analysis. Cycles 2 – 5 will be collected using PBS + BSA for collection of free lung cells, and processed using flow cytometry.

The BALF supernatant (1st lavage cycle) will be aliquoted into separate tubes for the following applications, in order of priority:

i) RodentMAP 3.0 (300 µl)

ii) MMP activity determination (optimal 150 µl, minimum 120 µl)

iii) Remaining supernatant

The fluid will be stored frozen at ≤ -70 °C until used for experimentation of shipment on dry ice to the test site (RBM). Any residual supernatant will be stored in a separate tube under the same conditions for potential future analysis.

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

Table 3 **Lung Inflammation**

Parameter	Biological Material	Method	WKI/SOP #
Collection of free lung cells (FLC)	Lungs of mice	Bronchoalveolar lavage of the lung	'Prepare lavage samples: Lavage of mouse lung – syringe method', PMI_RD_WKI_000319
Determination of cellular viability	BALF (cycles 2 to 5 plus FLC from cycle 1)	Flow cytometric analysis after staining with fluorescein diacetate and propidium iodide	'Perform analysis: Flow cytometric measurement of cell viability with simultaneous determination of cell count by means of BD TruCount beads', PMI_RD_WKI_000419
FLC count	BALF (cycles 2 to 5 plus FLC from cycle 1)	Flow cytometrical counting using Trucount® tubes	'Perform analysis: Flow cytometric measurement of cell viability with simultaneous determination of cell count by means of BD TruCount beads', PMI_RD_WKI_000419
FLC differentiation	BALF (cycles 2 to 5 plus FLC from cycle 1)	Flow cytometric analysis after staining with cell-type specific antibodies conjugated to fluorochromes	Perform analysis, Flow cytometric differential counting of mouse free lung cells by labeling with CD11c, Ly6G, CD3e, B220 and MHCII. See also Table 4 for revised antibody mixture, PMI_RD_WKI_000415 (draft version will be used).
FLC lymphocyte differentiation	BALF (cycles 2 to 5 plus FLC from cycle 1)	Flow cytometric analysis after staining with cell-type specific antibodies conjugated to fluorochromes	'Flow cytometric determination of lymphocyte differentiation in murine bronchoalveolar lavage fluid', PMI_WKI_RD_000647 (draft version will be used).
FLC macrophage activation	BALF (cycles 2 to 5 plus FLC from cycle 1)	Flow cytometric analysis after staining with cell-type specific antibodies conjugated to fluorochromes	'Flow cytometric quantification of activated marker expression in murine alveolar macrophages', PMI_RD_WKI_000648 (draft version will be used)
Inflammatory mediators (Multi-analyte profile)	BALF (cell-free supernatant of cycle 1)	Multiplexed bead array according to the RodentMAP® v 3.0 program	Rules-based medicine (Delegated phase 4)

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

MMP activity	BALF (cell-free supernatant of cycle 1)	Fluorescence method using fluorescein-conjugated gelatin	'Quantitative determination of matrix metalloproteinase (MMP) activity in rodent bronchoalveolar lavage fluid (BALF)' PMI_RD_WKI_000620.

The BALF cells (FLC, lavage cycles 1, and 2 through 5) will be processed as follows: Cells from cycle 1 will be combined with cycle 2 – 5 for viability and cell quantification. These will then be fixed, and partitioned for each different analytical endpoints (differential count, lymphocyte differentiation, and macrophage activation).

The antibody mixes used will be described in Table 4:

Table 4 **Free lung cell antibody mixes**

Assay	Name	Company	Cat#
Mouse DiffCount	CD11c-APC	Becton Dickinson	550261
	Ly6G (Gr1) - FITC	Becton Dickinson	551460
	CD3 - PE	Becton Dickinson	553064
	B220 - APC - Cy7	Becton Dickinson	552094
	MHCII - PerCP-eFluo710	e-Bioscience	46-4031-82
Lymphocyte differentiation	Ly6G-PE	BD biosciences	553141
	CD4-PerCP	BD biosciences	553052
	CD8a-PE-Cy7	BD biosciences	552877
	CD45R/B220-APC-Cy7	BD biosciences	552094
Macrophage activation	CD86-Pe-Cy7	BD Biosciences	560582
	CD54/ICAM-1-PE	BD Biosciences	553253
	CD11b-APC-Cy7	BD Biosciences	557657
	MHCII-PerCP-eFluor710	e-Biosciences	46-4031-82
	[1]CD11c-APC	Becton Dickinson	550261

[1]: CD11c included as a macrophage and dendritic cell marker in addition to the antibody mix described in the working instruction.

The MMP activity in the BALF supernatants will be determined using a commercially available assay kit which is based on the cleavage of fluorochrome-labeled gelatin by gelatinotytic metalloproteinases (MMP) in BALF (EnzChek® Gelatinase/Collagenase Assay Kit; Invitrogen).

1.1.4 Tissue processing for 'omics analysis (genomics, proteomics, lipidomics)

Tissues for 'Omics analysis (dissection block 'D') will be performed between 16 and 24 hours post exposure. The dissection will be as follows:

i) Anaesthesia – the mice will be anaesthetised using sodium pentobarbital

ii) Blood collection – through the retro-orbital plexus (whole blood for RNA, DNA, plasma for lipids,)

iii) Perfusion of lung tissue using 0.9% NaCl

iv) Tissue extraction and treatment.

To minimize RNA degradation during the removal process, all surfaces and instruments will be wiped with RNAse away between animals. Tissues will be frozen as quickly as possible, by immersion of the tube in liquid N2, or pouring liquid N2 into the containers.

Note, tissues will be prioritised for time sensitivity. The target is to have the nasal epithelium removed within 15 minutes post-mortem (perfusion time). As all tissues may experience degradation, all tissues should be removed and processed/frozen by 20 minutes post-mortem.

For vascular tissues, the trimming for tissues destined for RNA will be performed in the presence of RNA later, the samples will incubate for 30 – 120 minutes in RNA later before snap freezing.

Left lung samples will be frozen using a 'slow' freeze procedure. The tissue will be set onto aluminium foil on a bed of dry ice, rather than snap-freezing. This is to minimize the risk of tissue fracture during the freezing process.

Samples will be stored at ≤-70°C until the completion of the study, after which the samples will be processed.

1.1.5 Blood collection

There are multiple downstream applications requiring blood collection at the necropsy phase, each requiring specific methods of collection and processing.

1.1.5.1 Whole blood for RNA

Blood samples will be collected from the retro-orbital plexus using an uncoated (i.e., no heparin) capillary or PE tube. The blood will be transferred straight to an RNA collection tube of appropriate size for the volume (Qiagen RNA protect animal blood tubes, 100 ul volume planned). The blood must be added up to the 'fill' line on the tubes as closely as possible (avoid overloading the tubes). The tube and blood

mixture will be mixed by inversion 10 times, followed by resting overnight (minimum 2 hours) at between 15°C and 25°C. The sample may then be placed at ≤-70°C for storage before further processing or shipment.

1.1.5.2 Whole blood for DNA collection

Blood samples will be collected from the retro-orbital plexus using an uncoated (i.e., no heparin) capillary or PE tube. The blood will be transferred straight into 4 ml EDTA coated tube. The blood will be mixed by inversion 8 times

1.1.5.3 Whole blood for haematology

Blood samples will be collected from the retro-orbital plexus using an uncoated (i.e. no heparin) PE tube. The blood will be collected into EDTA tubes, mixed well, and then placed on a tube rotator until analysis.

1.1.6 Serum for proteomics/inflammation

Blood will be collected from the retro-orbital plexus using non-heparinized capillary tubes. The blood will be transferred directly to a serum separator tube (SST) and processed according to our work instructions. Briefly, after centrifugation, the gel will form an interface between the serum and the supernatant. The serum is then transferred to two separate tubes, 70 µl for rules based medicine, analyte profiling, and 100 µl for ELISA . Importantly, there is no heat inactivation as this would denature or inactivate some of the heat labile components in the serum, thereby disrupting the proteomic or lipidomic analysis.

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

1.1.7 Lipidomics methods

1.1.7.1 Plasma for oxysterols

Plasma will be collected using standard methods. However, immediately after the isolation of the plasma, it will be stabilized with 200 uM BHT (butyl hydroxy-toluol). A 100 mM stock solution of BHT in ethanol will be prepared (i.e., 220.35 mg BHT in 10 ml ethanol), and stored in aliquots (low retention tubes) at -20°C for up to 2 weeks. The stock solution will be diluted to a 50x working solution - 10 mM (1/10 dilution) in ethanol, and added directly to the plasma (for example, 2 ul working BHT solution for 100 ul plasma). The samples will then be frozen on dry ice as quickly as possible.

1.1.7.2 Plasma for cholesterol and lipoprotein analysis

Plasma will be collected according to our standard procedures and in heparin tubes (not EDTA, which may interfere with the cholesterol analysis). The plasma may be stored at ≤ -70°C for up to 6 months prior to analysis. The analysis of cholesterol, HDL, LDL, glucose and triglycerides will be performed using the Unicel DxC 600 clinical analyser using methods to be described in a separate amendment or WKI.

1.1.7.3 Plasma for lipidomics (Delegated phase 3)

Plasma will be isolated according to the recommendations from Zora Biosciences. Blood will be collected into EDTA-plasma tubes, separated as soon as possible after collection, and snap-frozen at -80°C – note, samples to be frozen rapidly in order to ensure preservation of sphingosines..

1.1.7.4 Measurement of aortic plaque size

Digital images of aortic arches will be captured during dissection for study endpoints which require this measurement as an endpoint. Aortic arches will be stained with oil red o in accordance with PMIRL-S internal work instructions to identify atherogenic lesions. As a control for staining, each stained aortic arch will also have an unstained image captured before staining. Imaging will be performed using the LEICA camera mount on the dissection microscope. The images will be stored onto the shared drive.

Aortic arch images will be uploaded into Visiopharm, image analysis module. This software will be used to determine the area of the atherogenic lesion compared to the total area of the healthy aortic tissue. Note, plaque area size may also be measured using Image J software at the discretion of the study director.

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

1.1.7.5 Lung Volume Measurement

Immediately following lung instillation with EGAF solution, the volume of the lung will be estimated using volume displacement, which will be measured as the change in weight of the immersion solution (e.g EGAF solution) and jar before and after the lungs have been immersed. We will follow the following methodology:

i) Jar filled with fixative will be placed on a balance, the weighted clip, hanging from a retort stand, will be submersed.
ii) Balance will be zeroed
iii) The lungs will be submerged in the fixative via the clip. Ensure that both clip and lungs are fully submerged, and that there is no contact with the sides of the vessel.
iv) The weight differential (before and after the submerged lungs) corresponds to the volume of the organ in milliliters

1.1.8 Lung Function Measurement

All lung function manoeuvres will be performed using equipment (Scireq, Canada). This will be performed according to our standard working instructions, PMI_RD_WKI_000420, 'Perform terminal measurement: Lung function measurement'.

1.1.9 Histoprocessing and Staining of Respiratory Tract Organs

During dissection, all respiratory tracts organs, including nose and lungs will be fixed in EGAFS solution post instillation with EGAFS. Histological processing of these organs will be performed at PMIRL-S according to internal work instructions. In summary, three levels of the nasal cavity will be sectioned and will include critical landmarks most commonly affected by main stream cigarette smoke exposure. The entire left lung lobe will be serially sectioned at no more than 20 levels to provide an overall assessment of the lung including the mainstem bronchus. Each level will include a total of 12 consecutive sections of the lung. All three levels of the nose and the first 3 consecutive serial sections of the lung at each level will be stained with Hematoxylin and Eosion (H&E). The first level of the nose and the second -3 consecutive serial sections at each level of the lung with be stained with Alcian blue- Periodic acid Schiff (AB-PAS). The third- 3 consecutive serial sections of the lung at each level will be stained with Resorcin-Fuchsin (RF). The last 3 consecutive serial sections of the lung will be unstained reserve slides.

1.1.10 Histoprocessing and Staining of Non-Respiratory Tract Organs

At the time of necropsy, non-respiratory tract organs, including the liver and pancreas will be fixed in 4% paraformaldehyde (PFA). An additional section of liver will also be frozen using cryopreservation freezing media. 4-um thick sections of 4% PFA fixed tissue will be routinely processed and stained with H&E. In

addition to H&E, liver sections will also be stained with Periodic acid Schiff (PAS) and PAS pre-digested with diastase (PASD). 8-10 um thick cryosections of liver will be stained with Oil Red O. All histochemical staining is in accordance with internal PMIRL-S work instructions

1.1.11 Histopathological Evaluation of Respiratory and Non-Respiratory Tract Organs

Histopathological evaluation of left lung, nose, pancreas, and liver will be conducted internally in accordance with internal PMIRL-S work instructions. In summary, relevant histopathology parameters will be evaluated utilizing pre-defined severity scores as described in PMIRL-S work instructions. In addition any findings which are not pre-defined in the work instruction will be documented as an incident. Statistical analysis will only be performed on the parameters which are assigned a severity score.

1.1.12 Endpoints for Pulmonary Emphysema

Consecutive serial step sections (4-um thick) stained with H&E as described in section 1.1.9 will be scanned and digitalized (Aperio digital imaging). These images will be used to evaluate pulmonary emphysema (newCAST, VisioPharm). Digitalized images will be uploaded into Visiopharm newCAST module for final quantification of emphysema. To determine the destruction of alveolar walls, total alveolar number and alveolar size will be quantified and normalized to the total lung volume (1.1.7.5). Alveolar surface area of the lungs will be determined using intersection counting and test lines followed by normalization to the total lung volume (1.1.7.5). Volume to surface ratios of acinar airspaces will be derived from volume and surface area measurements described above.

1.1.13 Apoptosis/Oxidative Stress in lungs and liver

The occurrence of oxidative stress in the sections of left lung and liver (if tissue available) will be assessed by measuring the level of the oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OH-dG) by immunohistochemistry. To identify apoptosis in lung cells, sections of left lung of fresh air or cigarette smoke-exposed mice will be subjected to terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay.

1.1.14 Vascular Inflammation

Flow cytometry-based method will be applied to analyze the immune cell composition in aortas of mice exposed to fresh air, 3R4F, ZRH aerosol. In brief, animal will be perfused (with PBS containing heparin) and the aortic arch and the thoracic aorta will be isolated and enzymatically digested according to

methods that will be described in a study plan amendment. After digestion, singe cell suspensions will be labeled with different set of antibodies to differentiate macrophages, neutrophils, dendritic cells, T and B lymphocytes by flow cytometry according to methods described in a study plan amendment.

1.1.15 Platelet aggregation assay

Flow cytometry-based assay will be applied to detect platelet aggregates using whole blood isolated from mice exposed to fresh air, 3R4F or ZRH aerosol (including cessation and switching groups). In brief, two portions of cells from the same donor mouse will be labeled with different fluorochromes of CD9 antibody and, after mixing and upon appropriate stimulation, analyzed for the formation of double-colored aggregates by flow cytometry. This will be performed according to methods described in a study plan amendment.

1.2 Insulin resistance (IR)– special methods

Longitudinal blood collection (fasting glucose) – blood/plasma will be collected for assessment of the endpoints described in Table 5. The collection of the blood will be via facial vein bleeding according to our standard working instructions (PMI_RD_WKI_000375) but with the following differences:

i) As inhaled anaesthetics interfere with metabolic parameters, the blood collection will be performed without anaesthetics.

ii) Analgaesics will be administered to the animals, in the form of orally dosed NSAIDS.

Study 15015 ApoE ZRH Switching Study Amendment 4, Attachment 1
Study director: Blaine Phillips, 16 Sept 2013.

Table 5 **Endpoints for longitudinal blood analysis (from 6 – 7 hour fasted animals)**

Primary Endpoint	**Method**	**Timing of the analysis**
Whole blood fasting glucose	Glucometer	Immediate in SG
Plasma fasting insulin	ELISA	Outsourced (Cambridge Biomedical)
Plasma fasting HbA1C	ELISA	Outsourced (Cambridge Biomedical)

Necropsy – special procedures for insulin resistance study

Procedures for organ extraction will be performed using standard working protocols. The following tissues will also be applied:

- Blood – samples will be collected from the retro-orbital plexus and processed as follows:

Table 6 **Blood samples for metabolic analysis**

Test	Sample	Preferred volume
Unicell DX-600 Lipid profile: HDL, LDL, VLDL, glucose, triglycerides	Serum	130 ul
Rat metabolic MAP, v2.0	Plasma	80 ul
Remainder	Plasma	remainder

- Liver (as described in section 1.1) – Liver will be weighed at necropsy, and process for histopathology (H&E, and cryosectioning), protein isolation for future validation work.
- Muscle – soleus + gastrocnemius – the entire muscle complex will be isolated and removed (**Figure 2**). Tissue will be snap-frozen for RNA extraction.

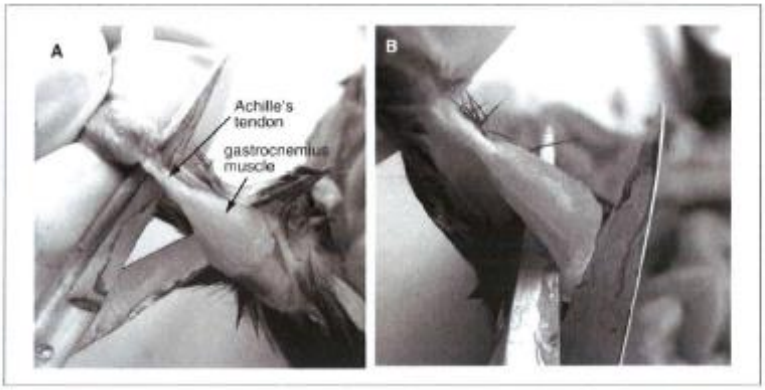


Figure 2. Dissection of gastrocnemius and soleus muscles. Picture taken from : Antal et al. 2007 Tissue collection for systematic phenotyping in the mouse. *Curr. Protoc. Mol. Biol 80:29A.4.1-29A.4.23.*

- Mesenteric fat – This is the fat deposition located within the folds of the intestines (Figure 3). The tissue will be removed and snap frozen in liquid N2 for RNA extraction.

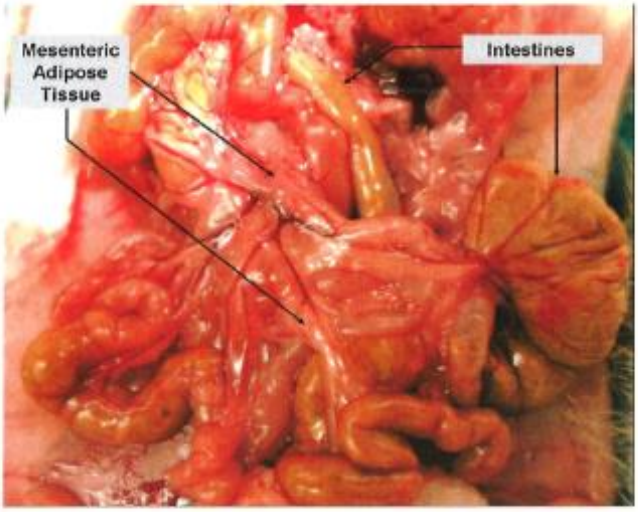


Figure 3. Dissection of mesenteric fat. Picture taken from: http://en.wikipedia.org/wiki/File:MES_Male_1.jpg.

Aortic arch - Atherosclerotic plaque measurements will be conducted in the aortic arch of ApoE mice. The aortic arch will be dissected out, sectioned and

cleaned from connective and perivascular fat. All procedures will be conducted under a dissection microscope. The aortic arch will be opened by sectioning the midline of the lesser aortic curvature, from the proximal to the distal end. A short cut from the proximal end of the larger curvature to the bifurcation of the left subclavian artery will be made and the tissue pinned down for microscope image recording. Tissue will then be stained with oil-red O dye (ORO) and another image taken. Plaque area will be determined by planimetry using the ORO-stained images. Procedures for dissection, cutting, image acquisition, ORO staining and planimetric measurements have all been described in detail in WKI documents.

Heart and thoracic aorta - We will also conduct gene expression, transcriptomic analysis of cardiac and aortic tissues. Heart and thoracic aorta will be collected. After the thoracic aorta is excised, a 0.5 cm segment from the proximal end will be sectioned and fixed in paraformaldehyde for paraffin embedding. The rest of the thoracis aorta will be processed for RNA extraction and transcriptomics after the, perivascular adipose and connective tissues are mechanically removed and discarded. This procedure will ensure that only the vascular wall is used for RNA analysis. The apex segment of the heart (left ventricle), approximately 0.5 cm, will be sectioned transversally and collected in RNAse-free tubes. Both tissues will then be snap-frozen in liquid nitrogen for mRNA extraction and high-throughput hybridization experiments (Affymetrix chips).

Consequences of change and measures to be taken

None

FORM
STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name: PMI_RD_FOR_000175

P -/	Short Title	Amendment 5
15015	APOE-ZRH switching - IR dissection update	

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	11 Nov 13

Subject

The scheduled IR arm dissection updated.

Reason(s) for Change

This amendment updates the dissection plan to include:
1) details of timing for glucose fasting collection and pre-dissection exposure.
2) one additional tissue endpoint.

Change

1. The final fasting glucose in the barriers will be Saturday 16 Nov 2013. There will be no exposure for the IR animals on 16 Nov, 2013, but the mice will be exposed Sunday, 17 Nov. 2013 as scheduled.
2. Mesenteric fat will be collected for:
   1) RNA - maxylysen tube
   2) lipids - eppendorf tube.

Consequences of Change and Measures to be taken

The animals can not be exposed on same day as blood collection, and will therefore have only 1 day weekend exposure for the Monday dissection group. This is unlikely to have any significant effect on results.

APPROVED

Controlled Copy

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Remarks

NA

Author:	11 Nov 13 Date/Signature
Study Director:	11 Nov 13 Date/Signature
TFM:	12 NOV 2013 Date/Signature
Sponsor:	Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [2]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site
[2] If applicable

Controlled Copy

APPROVED

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 20

Cdus name : PMI_RD_FOR_000175

P -/	Short Title	Amendment 6
15015	ApoE-ZRH Switching - Individual urine collection pilot	

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	14 Nov 2013

Subject

Individual housed metabolic cases will be assessed for small sample (3 pergroup) of ApoE mice.

Reason(s) for Change

Current 4 mice per case set-up has significant loss of urine in the chamber surfaces.

Change

3 mice per group will be housed overnight in individual metabolic cases.

Consequences of Change and Measures to be taken

No consequence.

APPROVED **Controlled Copy** Page 1 of 2

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Remarks

NA

Author:	14 Nov 13 Date/Signature
Study Director:	14 Nov 13 Date/Signature
TFM:	14 NOV 2013 Date/Signature
Sponsor:	Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [2]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site

[2] If applicable

Controlled Copy

Page 2 of 2

APPROVED

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdus name: PHLRD_FOR_000175

P -/	Short Title
15015	Apo E-ZRH Switching – Additional dissection end points IR Arm Amendment-7

Study Director/Substitute Study Director	Author	Date Submitted
Blaine Phillips/Oviedo Albrecht	Danial Sharma	18 Nov 13

Subject

Tissues will be collected for additional end points with weighing them as an additional activity for the insulin resistance arm dissection.

Reason(s) for Change

Lipidomic profiling of these tissue may add information significant for the interpretation of the insulin resistance part of the study.

Change

please see attached slides.

Consequences of Change and Measures to be taken

Dissection team and concerned people performing this activity has updated the work flow for dissection accordingly – No consequence

APPROVED

Controlled Copy

FORM
STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Remarks

NA → None -

Author: 18 Nov 13 — Date/Signature

Study Director: 18 Nov 13 — Date/Signature

TFM: 18 NOV 2013 — Date/Signature

Sponsor: — Date/Signature

Test Site [1]: —

PI [2]: — Date/Signature

TSM [2]: — Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site

[2] If applicable

Controlled Copy

APPROVED

FORM

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2014

Cdus name: PMI_RD_FOR_000175

P -/ Short Title Amendment 8

15015 ApoE - ZRH switching - Dissection grouping VI arm

Study Director: Blaine Phillips

Author: Blaine Phillips

Date Submitted: 24/9/14

Subject

Update for housing of VI animal groups and distribution during dissection

Reason(s) for Change

See attached

Change

See attached

Consequences of Change and Measures to be taken

See attached

APPROVED Controlled Copy Page 1 of 2

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 20

Remarks

NA

Author:	24 Apr 14 Date/Signature
Study Director:	24/Apr/14 Date/Signature
TFM:	24 Apr 2014 Date/Signature
Sponsor:	NA Date/Signature
Test Site [1]:	NA
PI [2]:	NA Date/Signature
TSM [2]:	NA Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments to the study plan should be justified and approved by dated signature of the principal investigator and/or study director and maintained with the study plan. They should be distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site

[2] If applicable

Controlled Copy

Study 15015 ApoE ZRH Switching Study Amendment 8, Attachment 1
Study director: Blaine Phillips, 19 Dec 2013.

Reason for change

A total of 106 animals were initially allocated to the vascular inflammation (VI) dissection arm, with scheduled dissection on month 1, 3, and 6. However, a redistribution of the groupings is necessary in order to perform method development steps.

Change

1. **Redistibution of VI-arm animals to the dissection groups**

The assays for the vascular inflammation dissection arm (platelet aggregation assay, and vascular inflammation assay) are in development. The development/optimization steps require a re-distribution of the animals. The numbers of animals per groups are updated as follows:

Study plan groupings

Group	Month 1	Month 3	Month 6	Total
Sham	10	8	8	26
3R4F	8	8	8	24
ZRH	8	8	8	24
Cessation		8	8	16
Switch		8	8	16
			Total	106

Updated groupings

Group	Month 1	Month 3	Month 6	Numira (month 3)	Total
Sham	8	6	9	3	26
3R4F	8	6	7	3	24
ZRH	8	6	7	3	24
Cessation	0	8	8		16
Switch	0	8	8		16
				Total	106

This also includes the inclusion of a pilot study for imaging (Numira) which uses 9 animals.

2. **Housing of animals**

The above redistribution will result in 6 animals from month 3 (2 from each of the following groups: sham, 3R4F, ZRH) to be dissected instead in month 6. These animals will therefore be housed with the month 6 animals (of the corresponding exposure group).

For group observations – the animals will all be scored with the month 6 mice (group 106, 206, 306).

Individual observations are performed by cage – this new housing assignment will therefore not impact the individual observations.

Consequence of change

This will not impact the study. The use of these animals for assay development was anticipated.

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPME

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2

Cdus name : PHLRD_FOR_000175

P -/	Short Title	Amendment 9
15015	ApoE-ZRH Switching - Plasma for nicotine analysis	

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	11 Jun 14

Subject

Plasma collection for biomarkers of exposure (nicotine and cotinine) analysis

Reason(s) for Change

see attachment

Change

see attachment

Consequences of Change and Measures to be taken

see attachment

APPROVED **Controlled Copy**

STUDY PLAN AMENDMENT | PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001591 Version N°: 1.0 Project No: Effective Date : 8 Oct 2

Remarks

NA.

Author:	11 June 14 Date/Signature
Study Director:	11 June 14 Date/Signature
TFM:	11 JUNE 2014 Date/Signature
Sponsor:	NA Date/Signature
Test Site [1]:	NA
PI [2]:	NA Date/Signature
TSM [2]:	NA Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and, if applicable, PI and TSM

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, amendments t the study plan should be justified and approved by dated signature of the principa investigator and/or study director and maintained with the study plan. They should b distributed to all recipients of the study plan.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If applicable, specify which test site
[2] If applicable

Controlled Copy

Page 2 of 2

APPROVED

Study 15015 ApoE ZRH Switching Study Amendment 9, Attachment 1
Study director: Blaine Phillips, 24 Apr 2014.

Reason for change

The determination of plasma nicotine and cotinine levels post-exposure has been requested by the sponsor.

Change

An additional blood collection will be performed on 40 animals (8 mice per exposure group). The blood will be collected from the facial vein under inhaled anaesthetic (the same procedure will be followed as per the HbCO blood collection, as documented in our working instruction, PMI_RD_WKI_000375, collect blood facial vein). The blood will be collected in EDTA-coated tubes. Following plasma separation, the plasma will be stored at -80°C until analysis or shipment.

The following animals will be selected for blood collection:

	24-Apr	25-Apr	24-Apr	25-Apr	24-Apr
	sham	3R4F	ZRH	Cess	Switch
1	1062043	2062043	3992005	4992001	5062044
2	1082034	2992002	3032046	4992005	5062045
3	1082035	2992003	3082029	4992008	5992001
4	1082036	2992007	3082030	4062039	5992002
5	1082037	2082017	3082031	4062045	5992003
6	1082038	2082018	3082032	4082019	5992004
7	1082019	2082019	3082019	4082020	5992006
8	1082020	2082020	3082020	4082021	5992007

Consequence of change

This analysis will support the HbCO and urinary nicotine metabolites analysis, and strengthen the overall demonstration of aerosol uptake.

FORM
DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001590 Version N°: 1.0 Project No: Effective Date : 8 Oct 20

Deviation #1

P -/	Short Title
15015	Apo E-ZRH Switching – Food consumption in high fat diet group

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	11 Sept 13

Description of Deviation

Food consumption in high fat diet group will be stopped.

Explanation

The high fat diet crumbles, resulting in inaccurate weight for food-out. Much diet is lost in the bedding.

Assessing the Impact of the Deviation and Corrective Action to be taken

If food consumption is required in future studies, we must test the diet for 'crumbling' before use.
We will only have data for chow group consumption.

Controlled Copy

APPROVED

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001590 Version N°: 1.0 Project No: Effective Date : 8 Oct 20

Remarks

Author:	11 Sept 2013 Date/Signature
Study Director:	11 Sept 2013 Date/Signature
TFM:	13 Sep 2013 Date/Signature
Test Site [1]:	
PI [2]:	Date/Signature
TSM [3]:	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and Sponsor[4]

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, deviations from the study plan should be described, explained, acknowledged, and dated in a timely manner by the principal investigator and/or study director and maintained with the study raw data. The principal investigator and/or study director should assess and document the impact of any deviations from the study plan on the quality and integrity of the study, and take appropriate corrective action if necessary.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If the deviation is related to the delegated phase, specify which test site

[2] If the deviation is related to the delegated phase, the PI is responsible for

[3] If required by the SOPs of the test site

[4] To be determined by SD, in concertation with TFM

APPROVED Controlled Copy

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001580 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name: PMI_RD_FOR_000174

Deviation 2

P -/	Short Title
15015	ApoE-ZEH Switching - Exposure, nicotine out of limits

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	11 June 14

Description of Deviation

see attachment

Explanation

see attachment

Assessing the Impact of the Deviation and Corrective Action to be taken

see attachment

APPROVED

Controlled Copy

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001590 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name : PMLRD_FOR.000179

Remarks

NA.

Author:	11 Jun 14 Date/Signature
Study Director:	11 Jun 14 Date/Signature
TFM:	11 JUN 2014 Date/Signature
Test Site [1]:	NA
PI [2]:	NA Date/Signature
TSM [3]:	NA Date/Signature

Distribution: Original SD (PMF)

Copies: DC, QS and Sponsor[4]

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, deviations from the study plan should be described, explained, acknowledged, and dated in a timely manner by the principal investigator and/or study director and maintained with the study raw data. The principal investigator and/or study director should assess and document the impact of any deviations from the study plan on the quality and integrity of the study, and take appropriate corrective action if necessary.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If the deviation is related to the delegated phase, specify which test site

[2] If the deviation is related to the delegated phase, the PI is responsible for

[3] If required by the SOPs of the test site

[4] To be determined by SD, in concertation with TFM

Page 2 of 2

APPROVED

Controlled Copy

Study 15015 ApoE ZRH Switching Study Deviation 2, Attachment 1
Study director: Blaine Phillips, 11 June 2014.

Description of deviation

During exposure, the target nicotine concentrations for the 3R4F and the THS 2.2 (ZRH) chambers was set to 29.9 ug/l, with an acceptable daily range tolerance of +/- 20%.

In the course of the study, the daily average chamber concentrations fell outside of this range on 32 occasions with the following distribution by chamber:
3R4F 1: 2 times
3R4F 2: 4 times
3R4F 3: 1 time
ZRH 1 (THS 2.2): 15 times
ZRH 2 (THS 2.2): 10 times

These deviations from the daily limits are also depicted in table 1, in which the out of specified limits values are in red font.

Explanation

Daily variance in chamber concentrations may be the consequence of the following:
i) There is an absence of real-time monitoring of nicotine concentrations, meaning that the daily concentrations could only be assessed after the exposure was completed. This makes 'fine tuning' of the exposure levels difficult.

ii) Exposure concentrations are sensitive to the number of animals in the chambers. There were frequent dissection activities, meaning that the air flow levels needed frequent fine tuning to compensate for the reduced animal numbers.

Assessing the impact of the deviation and the corrective actions to be taken

There is no impact to the study, as despite the occasional daily out-of-specification nicotine measurements, the overall nicotine concentrations were well within the of +/- 10% study target.

Study 15015 ApoE ZRH Switching Study Deviation 2, Attachment 1
Study director: Blaine Phillips, 11 June 2014.

Table 1. Daily average nicotine values by chamber

Nicotine (ug/L)		1D	2D	3D	4D	5D	6D	7D
Date	Sday	Blank 1	SHAM 2	SHAF 1	SHAF 3	SHAF 5	ZRH 1	ZRH 2
2-Sep	1			6.443	5.526	6.084	7.178	4.804
3-Sep	2			5.662	8.547	8.820	5.362	4.832
4-Sep	3	0.000	0.000	15.484	14.584	15.229	10.322	13.003
5-Sep	4			25.030	15.308	15.648	23.225	17.534
6-Sep	5			24.504	22.511	23.521	28.046	28.537
7-Sep	6			23.812	22.908	18.735	23.750	16.292
8-Sep	7			23.348	22.475	23.571	33.132	18.930
9-Sep	8			29.858	30.363	30.839	28.931	23.048
10-Sep	9			30.162	31.819	31.489	26.068	31.690
11-Sep	10	0.000	0.000	33.211	28.807	34.594	37.323	27.608
12-Sep	11			30.984	30.612	30.154	29.256	28.195
13-Sep	12			27.896	26.815	26.181	26.219	26.922
14-Sep	13			26.391	27.893	24.295	24.868	25.877
15-Sep	14			28.728	30.122	29.780	35.677	24.247
16-Sep	15	0.000	0.000	26.674	28.876	29.573	32.443	31.364
17-Sep	16			29.580	26.579	30.735	31.899	28.585
18-Sep	17			28.694	28.302	28.124	33.744	25.838
19-Sep	18			27.708	27.884	27.584	28.412	23.289
20-Sep	19			30.364	30.527	28.269	30.198	26.112
21-Sep	20	0.000	0.000	29.947	25.908	30.379	27.474	27.066
22-Sep	21			30.538	30.570	31.895	28.568	25.538
23-Sep	22			27.398	25.894	30.536	27.354	23.318
24-Sep	23			28.263	28.306	27.502	24.986	22.415
25-Sep	24			29.555	31.585	29.689	30.397	30.660
26-Sep	25			28.954	31.971	26.479	28.438	29.861
27-Sep	26			29.806	33.865	27.889	28.714	27.269
28-Sep	27			26.953	28.160	25.134	27.405	27.600
29-Sep	28			26.052	24.990	24.056	26.208	25.475
30-Sep	29			29.506	28.125	29.581	28.095	30.034
1-Oct	30	0.000	0.000	28.144	26.201	28.388	26.583	28.549
2-Oct	31			33.014	31.525	31.080	30.530	33.406
3-Oct	32			30.536	31.068	30.277	29.226	26.513
4-Oct	33			30.558	29.444	29.304	29.280	30.395
5-Oct	34			31.958	32.391	31.629	27.368	27.548
6-Oct	35	0.000	0.000	31.728	32.149	33.485	28.932	32.818
7-Oct	36			33.052	33.712	33.759	31.542	35.224
8-Oct	37		0.000	30.622	29.778	29.185	28.225	29.963
9-Oct	38			27.861	27.997	27.788	26.913	23.808
10-Oct	39			30.827	29.283	30.609	28.758	25.301
11-Oct	40	0.000	0.000	30.942	30.680	28.549	29.063	25.688
12-Oct	41			30.480	28.628	29.430	29.730	33.256
13-Oct	42			28.766	28.156	26.070	27.791	23.878
14-Oct	43			26.830	25.356	25.183	28.790	28.853
15-Oct	44			30.211	26.690	27.343	28.694	27.991
16-Oct	45	0.000	0.000	30.776	26.703	27.181	30.260	31.794
17-Oct	46			35.749	34.802	29.509	31.557	36.157
18-Oct	47	0.000		34.482	28.205	28.351	30.182	28.604
19-Oct	48				25.128		27.653	28.063
20-Oct	49				28.438		27.538	28.403
21-Oct	50	0.000	0.000		30.523		27.189	25.158
22-Oct	51				30.950		28.287	32.698
23-Oct	52				28.704		24.607	28.398
24-Oct	53				29.758		26.180	26.798
25-Oct	54				31.967		27.563	28.901
26-Oct	55	0.000	0.000		32.125		28.461	28.254
27-Oct	56				33.153		29.242	30.806
28-Oct	57		0.000		31.082		27.064	24.673
29-Oct	58				35.881		32.875	31.780
30-Oct	59				28.377		27.282	28.951
31-Oct	60				30.748		27.282	30.806
1-Nov	61	0.000	0.000		31.794		25.520	28.918
2-Nov	62				34.942		24.822	28.340
3-Nov	63		0.000		29.758		27.801	29.217
4-Nov	64				29.485		26.080	31.067
5-Nov	65				29.705		26.856	28.798
6-Nov	66	0.000	0.000		30.137		30.185	28.619
7-Nov	67				31.034		33.817	35.519
8-Nov	68		0.000		29.164		26.989	32.963
9-Nov	69				30.091			33.858
10-Nov	70				30.639			29.238
11-Nov	71				29.763		29.565	28.345
12-Nov	72				26.697		26.528	28.866
13-Nov	73	0.000	0.000		28.288		24.056	26.468
14-Nov	74				30.513		27.269	27.218
15-Nov	75				27.986		30.303	27.989
16-Nov	76				27.193		23.178	24.216
17-Nov	77				31.038		28.039	32.189
18-Nov	78	0.000	0.000		29.432		26.645	30.320
19-Nov	79				28.281		31.637	31.879
20-Nov	80				27.398		29.625	31.806
21-Nov	81				26.782		24.609	28.968
22-Nov	82				29.026		27.916	29.196
23-Nov	83	0.000	0.000		27.026		24.383	25.481
24-Nov	84				30.124		28.603	32.050
25-Nov	85				29.785		27.298	
26-Nov	86				26.387		26.126	
27-Nov	87				26.961		24.496	
28-Nov	88	0.000			26.962		32.080	
29-Nov	89				28.143		30.591	
30-Nov	90				26.926		27.323	
1-Dec	91				27.492		27.642	
2-Dec	92				31.346		32.774	

Nicotine (ug/L)		1D	2D	3D	4D	5D	6D	7D
Date	Sday	Sham 1	SHAM 2	SHAF 1	SHAF 3	SHAF 5	ZRH 1	ZRH 2
1-Jan	122	0.000			32.205		31.342	
2-Jan	123				35.186		31.814	
3-Jan	124				30.352		30.815	
4-Jan	125				26.597		28.062	
5-Jan	126				27.276		25.985	
6-Jan	127				28.140		28.439	
7-Jan	128	0.000			30.125		31.192	
8-Jan	129				29.698		27.658	
9-Jan	130				27.875		28.826	
10-Jan	131				30.197		31.079	
11-Jan	132				30.784		29.695	
12-Jan	133	0.000			32.834		33.610	
13-Jan	134				28.867		24.902	
14-Jan	135				25.227		26.211	
15-Jan	136				30.530		29.012	
16-Jan	137				31.035		30.678	
17-Jan	138				31.256		31.101	
18-Jan	139				30.650		25.537	
19-Jan	140				28.136		26.886	
20-Jan	141				31.140		26.267	
21-Jan	142	0.000			30.758		31.166	
22-Jan	143				29.568		31.907	
23-Jan	144				26.480		23.178	
24-Jan	145				26.597		26.358	
25-Jan	146				29.552		28.507	
26-Jan	147	0.000			30.342		27.087	
27-Jan	148				30.713		28.110	
28-Jan	149				30.265		25.966	
29-Jan	150				27.642		25.598	
30-Jan	151				28.781		29.495	
31-Jan	152				27.872		28.305	
1-Feb	153	0.000			29.135		31.353	
2-Feb	154				29.045		31.435	
3-Feb	155				26.892		26.568	
4-Feb	156				27.436		27.043	
5-Feb	157	0.000			29.892		31.081	
6-Feb	158				26.967		28.171	
7-Feb	159				27.376		31.367	
8-Feb	160	0.000			26.722		30.498	
9-Feb	161				27.669		30.948	
10-Feb	162	0.000			27.788		28.732	
11-Feb	163				27.813		32.375	
12-Feb	164				26.385		27.770	
13-Feb	165				33.124		28.920	
14-Feb	166				30.231		26.958	
15-Feb	167	0.000			30.937		29.085	
16-Mar	182				31.937		30.968	
17-Mar	183				30.693		31.246	
18-Mar	184				30.340		28.824	
19-Mar	185				30.765		33.456	
20-Mar	186				29.091		28.513	
21-Mar	187				30.003		33.357	
22-Mar	188	0.000			28.084		24.852	
23-Mar	189				30.106		31.709	
24-Mar	190				27.244		32.270	
25-Mar	191				29.230		27.485	
26-Mar	192				28.649		27.736	
27-Mar	193	0.000			27.089		27.830	
28-Mar	194				27.276		28.466	
29-Mar	195				26.096		25.627	
30-Mar	196				24.299		25.128	
31-Mar	197				26.834		28.508	
1-Apr	198	0.000			26.807		26.076	
2-Apr	199				27.671		30.991	
3-Apr	200				23.966		28.545	
4-Apr	201				27.118		26.645	
5-Apr	202				27.583		28.170	
6-Apr	203	0.000			26.812		27.308	
7-Apr	204				26.993		30.065	
8-Apr	205				26.462		31.285	
9-Apr	206				27.255		28.290	
10-Apr	207	0.000			25.042		33.124	
11-Apr	208				26.348		28.428	
12-Apr	209				30.849		28.404	
13-Apr	210				27.346		26.589	
14-Apr	211				29.879		28.963	
15-Apr	212				29.825		29.857	
16-Apr	213	0.000			31.256		32.080	
17-Apr	214				31.895		30.338	
18-Apr	215				27.343		28.233	
19-Apr	216				28.435		27.576	
20-Apr	217				27.881		31.209	
21-Apr	218	0.000			31.531		30.243	
22-Apr	219				32.813		31.857	
23-Apr	220	0.000			31.632		29.771	
24-Apr	221				31.558		33.903	
25-Apr	222				28.734		30.909	
26-Apr	223				29.756		27.973	
27-Apr	224				32.606		31.867	
28-Apr	225	0.000			28.867		32.950	
29-Apr	226				28.260		34.718	

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001590 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cclucs name: PMI_RD_FOR_000174

P -/	Short Title	Deviation 3
15015	ApoE-ZRH Switching – scale bar correction for plaque area images	

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	17 June 14

Description of Deviation

See attached

Explanation

See attached

Assessing the Impact of the Deviation and Corrective Action to be taken

see attached.

APPROVED

Controlled Copy

Page 1 of 2

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001590 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name: PMLRD_FOR_000174

Remarks

NA

Author: 20 Jun 14
Date/Signature

Study Director: 20 Jun 14
Date/Signature

TFM: 20 Jun 2014
Date/Signature

Test Site [1]:

PI [2]:
Date/Signature

TSM [3]:
Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and Sponsor[4]

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, deviations from the study plan should be described, explained, acknowledged, and dated in a timely manner by the principal investigator and/or study director and maintained with the study raw data. The principal investigator and/or study director should assess and document the impact of any deviations from the study plan on the quality and integrity of the study, and take appropriate corrective action if necessary.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If the deviation is related to the delegated phase, specify which test site
[2] If the deviation is related to the delegated phase, the PI is responsible for
[3] If required by the SOPs of the test site
[4] To be determined by SD, in concertation with TFM

APPROVED

Controlled Copy

Study 15015 ApoE ZRH Switching Study Deviation 3, Attachment 1
Study director: Blaine Phillips, 20 June 2014.

Description of deviation

During the analysis of the aortic arch images from months 1 through month 3, it was noted that the scale bar was labelled with an implausible value (10 um or 20 um, when the vessel is really approximately 2 mm in circumference). The cause was due to incorrect calibration of the scale bar during the camera set-up.

The following table shows the corrected measurements corresponding to the scale bar:

Month	Folder	Calibration date	Software magnification setting	Scale bar value	Scale bar unit	Actual value	Actual value unit	Pixel/mm conversion for scale bar
1	21013	02-10-13-1	1.60	10	µm	1.19	mm	356
	31013	03-10-13-1	1.25	20	µm	2.30	mm	553
2	301013	30-10-13-1	1.25	20	µm	2.30	mm	553
	311013	31-10-13-1	1.25	20	µm	2.38	mm	561
3	21213	02-12-13-1	1.00	20	µm	2.10	mm	408
	31213	03-12-13-1	1.00	20	µm	2.10	mm	404
	41213	04-12-13-1	1.00	20	µm	2.00	mm	384
	51213	05-12-13-1	1.00	20	µm	2.00	mm	404
6	30314	03-03-14-1	1.25	2	mm	2.00	mm	493.5
	40314	04-03-14-1	1.25	2	mm	2.00	mm	493
	50314	05-03-14-1	1.25	2	mm	2.00	mm	487.5
	60314	06-03-14-1	1.00	2	mm	2.00	mm	402
8	10514	01-05-14-1	1.00	2	mm	2.10	mm	403
	280414	28-04-14-1	1.00	2	mm	2.00	mm	399.5
	290414	29-04-14-1	1.00	2	mm	2.00	mm	389
	300414	30-04-14-1	1.00	2	mm	2.00	mm	404

Note:
- **Scale bar value** is the value displayed on each image for the indicated month
- **Actual value** is the real distance corresponding to the scale bar
- **Pixel/mm conversion for scale bar** is the number of pixels for the scale bar (actual value) for images taken on the indicated day.

Study 15015 ApoE ZRH Switching Study Deviation 3, Attachment 1
Study director: Blaine Phillips, 20 June 2014.

Explanation

An error in the calibration process was only identified during the aorta area analysis, performed after month 3 samples were imaged.

Assessing the impact of the deviation and the corrective actions to be taken

The calibration parameters are recorded in the camera software, which enables the study director to correct the values mathematically. This deviation will therefore have no impact on the values determined for plaque area measurements.

The images, however, have an annotated scale bar with the incorrect scale bar distance marker. It is not possible to change this value.

The corrective actions performed were:
i) the study director showed the technicians the correct way to perform the calibration, explained the importance of having correct scale bar for downstream analysis, and insisted that the procedure was performed exactly according to the WKI.

ii) The study director requested that an image of the microscopic ruler was taken and saved for every new calibration, so the values may be confirmed during the analysis to confirm accuracy.

The scale bar is correct for months 6 and 8.

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001590 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name: PMI_RD_FOR_000174

Deviation 4

P -/	Short Title
15015	ApoE-ZRH Switching - Vascular Inflammation group

Study Director	Author	Date Submitted
Blaine Phillips	Blaine Phillips	22 July 14

Description of Deviation

see attachment

Explanation

See attachment

Assessing the Impact of the Deviation and Corrective Action to be taken

See attachment

APPROVED

Controlled Copy

Page 1 of 2

FORM

DEVIATION FROM STUDY PLAN, SOP OR WKI

PMI RESEARCH & DEVELOPMENT

Document Number : FOR_QM001580 Version N°: 1.0 Project No: Effective Date : 8 Oct 2010

Cdocs name : PMLRD_FOR_000179

Remarks

NA

Author:	22 July 2014	Date/Signature
Study Director:	22 July 2014	Date/Signature
TFM:	22 JUL 2014	Date/Signature
Test Site [1]:	NA	
PI [2]:	NA	Date/Signature
TSM [3]:	NA	Date/Signature

Distribution: Original SD (PMF)
Copies: DC, QS and Sponsor[4]

Remarks: According to the Revised OECD Principles of Good Laboratory Practice, deviations from the study plan should be described, explained, acknowledged, and dated in a timely manner by the principal investigator and/or study director and maintained with the study raw data. The principal investigator and/or study director should assess and document the impact of any deviations from the study plan on the quality and integrity of the study, and take appropriate corrective action if necessary.

Name	Function	Justification	Date	Signature
Phillips, Blaine	Staff Scientist	Signed as owner	8 Oct 2010	

[1] If the deviation is related to the delegated phase, specify which test site

[2] If the deviation is related to the delegated phase, the PI is responsible for

[3] If required by the SOPs of the test site

[4] To be determined by SD, in concertation with TFM

APPROVED Controlled Copy

Study 15015 ApoE ZRH Switching Study Deviation 4, Attachment 1
Study director: Blaine Phillips, 22 July 2014.

Description of deviation

A total of 106 animals were originally planned for analysis using the exploratory flow cytometry endpoint, 'vascular inflammation'. However, analysis of the data after month 1 and month 3 indicated that:
i) The method is technically quite complicated, and additional development work would be necessary to finalize the methods for this endpoint.
ii) The preliminary results did not show any obvious exposure (3R4F) effects.

Therefore, it was decided by the study director to re-allocate the remaining animals from the vascular inflammation dissection group to send for µCT imaging with Numira Biosciences (Delegated phase 6)

Explanation

Originally, 106 animals were distributed across all groups for vascular inflammation development and assay work (Table 1). However, as described in amendment 8, 9 animals (3 each from sham, 3R4F, and THS 2.2/ZRH) were re-allocated to a pilot imaging study with Numira Biosciences (Table 2).

Finally, and largely based on the great success of the imaging pilot study, it was decided to instead distribute the remaining animals for micro CT imaging rather than continue with the flow cytometry-based vascular inflammation assay (Table 3).

Table 1. Original animal distribution in the vascular inflammation group (study plan)

Group	Month 1	Month 3	Month 6	Total
Sham	10	8	8	26
3R4F	8	8	8	24
ZRH	8	8	8	24
Cessation	0	8	8	16
Switch	0	8	8	16
			Total	106

Table 2. Re-distribution of vascular inflammation group detailed in Amendment 8

Group	Month 1	Month 3	Month 6	Numira (month 3)	Total
Sham	8	6	9	3	26
3R4F	8	6	7	3	24
ZRH	8	6	7	3	24
Cessation	0	8	8		16
Switch	0	8	8		16
				Total	106

Study 15015 ApoE ZRH Switching Study Deviation 4, Attachment 1
Study director: Blaine Phillips, 22 July 2014.

Table 3. Final distribution of vascular inflammation animals

Group	Month 1	Month 3	Month 6	Numira (month 3)	Numira Month 6	Reserve	Total
Sham	8	6	0	3	6	3	26
3R4F	8	6	0	3	6	1	24
ZRH	8	6	0	3	6	1	24
Cessation	0	8	0	0	6	2	16
Switch	0	8	0	0	6	2	16
						Total	106

Assessing the impact of the deviation and the corrective actions to be taken

This deviation (along with amendment 8) describes the redistribution of animals from the exploratory vascular inflammation endpoint into groups for imaging. Therefore we did not obtain a final and comprehensive data set for the vascular inflammation endpoint. This, however, is offset by the addition of an imaging endpoint, which very interesting results and overall strengthening the study data set.

FORM

DEVIATION FROM STUDY PLAN

PMI RESEARCH & DEVELOPMENT

Document Number : PMI_RD_FOR_000174 Version N°: 2.0 APPROVED «Status»

Global Quality Management Quality Implementation 15 OCT 2014 Effective Date:

15015 14

Study # 15015 **Short Title** ApoE-THS2.2 switch - Individual housed where metabolic cages

Deviation # 5

Study Director Blaine Phillips **Author** Blaine Phillips **Date Submitted** 13 Nov 14

Description of Deviation

see attachment 1

Explanation

see attachment 1

Assessing the Impact of the Deviation and Corrective Action to be taken

see attachment 1

Controlled Copy

Related Document(s) : PMI_RD_WKI_000252 - Deviation from Study Plan

Page 1 of 2

FORM

DEVIATION FROM STUDY PLAN

PMI RESEARCH & DEVELOPMENT

Document Number : PMI_RD_FOR_000174 Version N°: 2.0

Global Quality Management Quality Implementation

APPROVED «Status»

15 OCT 2014 Effective Date:

Remarks and incident number

NA

Author:	13 Nov 14 Date/Signature
Study Director:	13 Nov 14 Date/Signature
Test Facility Management:	27 NOV 2014 Date/Signature
Sponsor	NA Date/Signature
Test Site Name [1]:	NA
Principal Investigator [1]:	NA Date/Signature
Test Site Management [1]:	NA Date/Signature

[1] If applicable

Controlled Copy

Related Document(s) : PMI_RD_WKI_000252 - Deviation from Study Plan

Page 2 of 2

Study 15015 ApoE ZRH Switching Study Deviation 5, Attachment 1
Study director: Blaine Phillips, 13 Nov 2014.

Description of deviation

From month 3 and onwards, urine samples were collected using individually-housed urine metabolic cages.

Explanation

Urine recovery in the first collections resulted in low urine recoveries, which was determined to be due to significant loss of urine in the group-housed metabolic cages. Following a successful trial of individual housed urine collection (amendment 6), the study director decided to continue using individual urine collections for all remaining sampling points.

Assessing the impact of the deviation and the corrective actions to be taken

This technical deviation improved the subsequent urine samples for the collections taken at month 3, 6, and 8. This resulted in generally improved urine yields, and therefore higher metabolite recoveries in these months. This technical change must therefore be taken into account when analyzing the data.

End of Document