STUDY REPORT
90 DAY OECD INHALATION STUDY ON SPRAGUE-DAWLEY RATS TO CHARACTERIZE TOXICITY OF MAINSTREAM AEROSOL FROM ZURICH FPD 4.2 CIGARETTES AND COMPARISON TO 3R4F MAINSTREAM AEROSOL TOXICITY
STUDY NUMBER 15006

PART 7 – SUMMARY OF RESULTS FOR PLUS GROUP ANIMALS

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1 Abstract

The objective of the present study is to characterize the toxicity due to sub-chronic inhalation of mainstream (MS) aerosol from the next generation product (NGP) Zurich FPD 4.2 (ZRH) and to compare it to the toxicity inherent to the inhalation of mainstream smoke from the Reference Cigarette 3R4F (University_of_Kentucky, 2003) in a 90-day nose-only inhalation study on Sprague-Dawley rats. This is an exploratory phase of the main OECD study where in-life observations of exposed animals (hereby named as the PLUS group animals) as well as the dissection of animals were performed. The exposure phase of this study has been performed according to OECD Test Guideline 413 (OECD, 2009). Animals were exposed to aerosol from 3R4F reference cigarettes or ZRH sensorial media at 3 target nicotine concentrations 6 hours a day, 5 days per week for 90 days, followed by 42 days recovery period. Control group was exposed to fresh air (sham). Measurement of aerosol constituents indicated lowered content of formaldehyde, acrolein, acetaldehyde and carbon monoxide in the diluted aerosol from ZRH as compared to 3R4F. Measurement of nicotine, total particulate matter (TPM) and carbon monoxide (CO) in diluted aerosol indicated stable supply of aerosol to the experimental animals.

There was no difference in mortality between ZRH and 3R4F exposed groups. There was no remarkable exposure related in-life observations with the exception of a few incidences (no more than 2 animals per group per gender at each occurrence) of mild to moderate tremor occurring infrequently in rats exposed to high nicotine concentration. Aerosol exposure induced lower weight gain in 3R4F high dose male group compared to ZRH group of equal or higher nicotine concentration. Following 28 day exposure, 90 day exposure and 42 day post-recovery periods, animals were dissected for exploratory endpoints under non-GLP condition. Further processing and assessment of the harvested tissues will be reported in separate study report.

2 Introduction

See Part 1 of report.

3 Objectives

The objective of this part of the study was to expose PLUS group Sprague-Dawley rats for 90 days to aerosol from ZRH or MS from the University of Kentucky 3R4F cigarettes. The, PLUS group animals in each exposure dose were dissected in order to provide biological samples for further exploratory studies.

4 Experimental Design and Actual Study Conditions

4.1 Test and Reference Items

See Part 1 of the report

4.2 Treatment administration

The PLUS group animals were exposed in the same nose-only exposure chamber as OECD group animals (Part 1 of report). Exposure of PLUS group animals took place 1 week after the exposure start of OECD group animals. Hence the exposure phase of the PLUS animals fell on study days 8 to 103. The duration of the exposure was 13 weeks using a 5 days per week, 6 hours per day exposure regimen. A time adaptation phase was included during the first 7 days (1.5 hours for first and second days, 3 hours for third and fourth days and 4.5 hours for day 5 to 7) to acclimatize the rats to the target aerosol concentration. On study day 12 (adaptation week for PLUS group animals), 2 OECD animals were exposed for 4.5 hours (instead of 6 hours) while 2 PLUS animals were mistakenly exposed for 6 hours (instead of 4.5 hours) (Deviation 5). There was no impact on study outcome and animal welfare as these incidents happened during the adaptation week. Also, during the dissection week following 90 day exposure, the recovery animals were mistakenly exposed for 2 additional days (deviation 11). The impact on study when recovery animals were exposed for 2 additional days should be small as dissection animals were also additionally exposed for 2 days prior to dissection.

4.2.1 Test Atmosphere Generation and Characterization

Aerosol generation was described in Part 1 of report.

To obtain the target concentration levels, the aerosol was diluted with fresh filtered conditioned air immediately downstream from the pump. The aerosol was delivered via glass tubing from the smoking machines to the exposure chambers. The average inlet temperature and relative humidity (Sham only) of the test atmosphere for all exposure chambers are listed in Table 1 and Table 2. Air flow was monitored continuously and recorded in Citect during the exposure period. The flow through the exposure tubes was calculated for each of the groups and was at least 1.4 L/min per tube (Table 3).

Table 1: Average chamber inlet temperature

Short Name	Sham	3R4F-Low	3R4F-Medium	3R4F-High	ZRH-Low	ZRH-Medium	ZRH-High
Chamber No.	1	2	3	4	5	6	7
Mean (°C)	21.8	21.9	22.4	22.4	22.3	21.7	22.2
Standard Deviation	0.3	0.2	0.2	0.2	0.2	0.3	0.3
Number of measurement	74	74	74	74	74	74	74

Table 2: Average chamber inlet relative humidity

Short Name	Sham
Chamber No.	1
Mean (%)	55.1
Standard Deviation	1.1
Number of measurement	74

Table 3: Dilution and Air Flow

Group	First dilution flow (L/min)	2nd dilution flow (L/min)	pump flow (L/min)	waste flow (L/min)	Sampling flow(L/min)	Total flow to chamber (L/min)	No. of exposure tubes	Air flow to each tube (L/min)
1	91.8	NA	1.7	NA	2.7	90.8	60	1.5
2	191.4	68.0	1.6	164.0	5.5	91.5	56	1.6
3	186.6	44.6	1.6	141.0	5.7	86.2	56	1.5
4	138.4	36.6	1.6	83.9	4.6	88.1	56	1.6
5	218.8	NA	1.6	120.8	5.8	93.8	56	1.7
6	134.9	NA	1.6	40.4	4.5	91.7	56	1.6
7	114.9	NA	3.3*	26.9	4.5	86.7	56	1.5

Dilution air flows = pressure difference across a Venturi tube

*The pump flow for group 7 is obtained from the sum of pump flow of 2 smoking machines

Total flow to chamber = Dilution air flow (1st and 2nd) + pump flow - waste flow – sampling flow

Air flow per inhalation tube = total flow to chamber / number of exposure tubes

Characterization of the test atmosphere was performed through the analysis of several analytical parameters (see Part 1 of report). The puff volume was determined before and after daily exposure (Table 4). The values obtained were within specification of 55ml ± 1 ml before exposure and 55ml ± 2 ml after exposure.

Table 4: Puff volume generated by smoking machines

One smoking machine was used to supply aerosol to each of the exposure chamber with the exception of ZRH-High chamber where 2 smoking machines were used.

	Puff Volume (Before) (ml/puff)						
Short Name	3R4F-Low	3R4F-Medium	3R4F-High	ZRH-Low	ZRH-Medium	ZRH- High	
Chamber No.	2	3	4	5	6	7	
Mean	54.9	54.9	54.6	55.4	55.3	55.2	55.2
Standard Deviation	0.4	0.5	0.5	0.5	0.6	0.5	0.5
Number of daily measurement	74	74	74	74	74	74	74

	Puff Volume (After) (ml/puff)						
Short Name	3R4F-Low	3R4F-Medium	3R4F-High	ZRH-Low	ZRH-Medium	ZRH- High	
Chamber No.	2	3	4	5	6	7	
Mean	53.9	53.9	53.9	53.2	53.2	53.4	53.3
Standard Deviation	0.5	0.5	0.5	0.4	0.5	0.5	0.4
Number of daily measurement	74	74	74	74	74	74	74

Particle size distribution was determined using the PIXE impactor protocol at least once per week with the exception of some study days where data were excluded due to failed measurement (see deviation 12 and 17). The particle size distribution measurements indicated that particle sizes and distributions were very similar among the exposure groups (Table 5). The mass median aerodynamic diameter (MMAD) for ZRH aerosol ranged from 0.41 to 0.98 µm and geometric standard deviation (GSD) ranged from 1.01 to 1.64 while 3R4F aerosol's MMAD aerosol ranged from 0.56 to 0.86 µm and GSD ranged from 1.11 to 1.44. The particle size distribution measurements indicated that particle sizes are compatible with inhalation and equally respirable between all aerosol-exposed groups.

The 3R4F cigarettes were smoked to butt lengths (N=3) of 34 ± 1 mm, 35 ± 1 mm and 34 ± 1 mm respectively by smoking machines for 3R4F- Low, 3R4F- Medium and 3R4F- High groups. 3R4F cigarettes were smoked to puff counts (N=3) of 10 to 11 per cigarette (11 ± 0 for 3R4F Low, 10 ± 1 for 3R4F Medium, 10 ± 1 for 3R4F High). Due to the design of the smoking machine, the ZRH sensorial media were configured to smoke to 12 puffs per cigarette. Static burn rate of 3R4F cigarettes (N=3) were 433 ± 35 sec/ 40mm, 427 ± 37 sec/ 40mm and 423 ± 22 sec/ 40mm for smoking machines supplying aerosols to 3R4F- Low, 3R4F- Medium and 3R4F- High groups respectively.

Table 5: Particle size Distribution. Remarks: Collection time is 15 minutes.

Study Day	Group	MMAD(µm)	GSD
18	002	0.69	1.23
33	002	0.59	1.21
53	002	0.65	1.11
67	002	0.56	1.40
74	002	0.69	1.44
81	002	0.57	1.23
88	002	0.86	1.23
95	002	0.84	1.26
18	003	0.62	1.21
26	003	0.59	1.20
46	003	0.59	1.18
53	003	0.61	1.19
67	003	0.69	1.25
74	003	0.63	1.22
81	003	0.60	1.19
88	003	0.68	1.27
95	003	0.67	1.28
102	003	0.84	1.44
14	004	0.76	1.42
18	004	0.63	1.22
33	004	0.69	1.35
40	004	0.67	1.30
46	004	0.61	1.21
53	004	0.71	1.31
60	004	0.59	1.19
67	004	0.72	1.34
74	004	0.65	1.24
88	004	0.64	1.25
95	004	0.65	1.33
25	005	0.75	1.01
32	005	0.86	1.11
39	005	0.87	1.43
47	005	0.63	1.34
54	005	0.45	1.34
61	005	0.51	1.22
67	005	0.50	1.55
75	005	0.47	1.43

Study Day	Group	MMAD(µm)	GSD
82	005	0.47	1.34
96	005	0.45	1.49
103	005	0.52	1.64
25	006	0.51	1.26
39	006	0.98	1.57
54	006	0.41	1.24
61	006	0.40	1.43
67	006	0.46	1.32
75	006	0.52	1.31
82	006	0.51	1.39
89	006	0.49	1.63
96	006	0.47	1.32
103	006	0.57	1.46
13	007	0.61	1.20
25	007	0.57	1.25
32	007	0.83	1.39
39	007	0.67	1.30
47	007	0.58	1.25
54	007	0.51	1.37
61	007	0.52	1.28
67	007	0.52	1.26
75	007	0.58	1.40
82	007	0.48	1.38
89	007	0.51	1.58
96	007	0.44	1.30
103	007	0.51	1.36

4.2.2 Achieved aerosol concentrations

The study averages for nicotine concentration are shown in Table 6 and Figure 1, while the daily averages are shown in Figure 2.

Table 6: Average nicotine concentration in the exposure chamber

Short Name	Sham	3R4F-Low	3R4F-Medium	3R4F-High	ZRH-Low	ZRH-Medium	ZRH-High
Chamber No	1	2	3	4	5	6	7
Target concentration (µg/L)	0.00	8.00	15.00	23.00	15.00	23.00	50.00
Mean in exposure chamber (µg/L)	0.00	7.30	13.19	21.41	13.50	21.63	46.86
Standard Deviation	0.00	0.71	1.24	1.75	1.38	2.87	4.25
Number of daily measurement	18	31	30	30	74	74	74

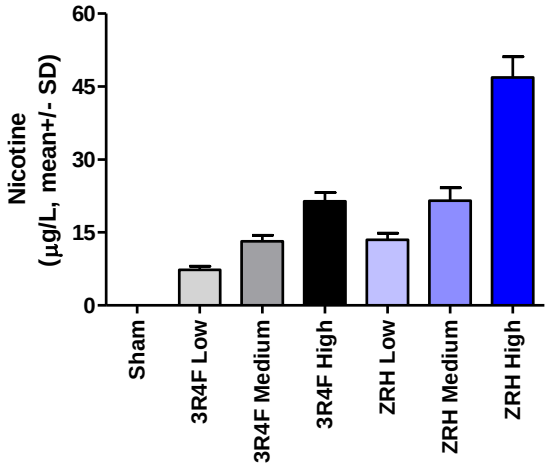


Figure 1: Average nicotine concentration in the exposure chamber

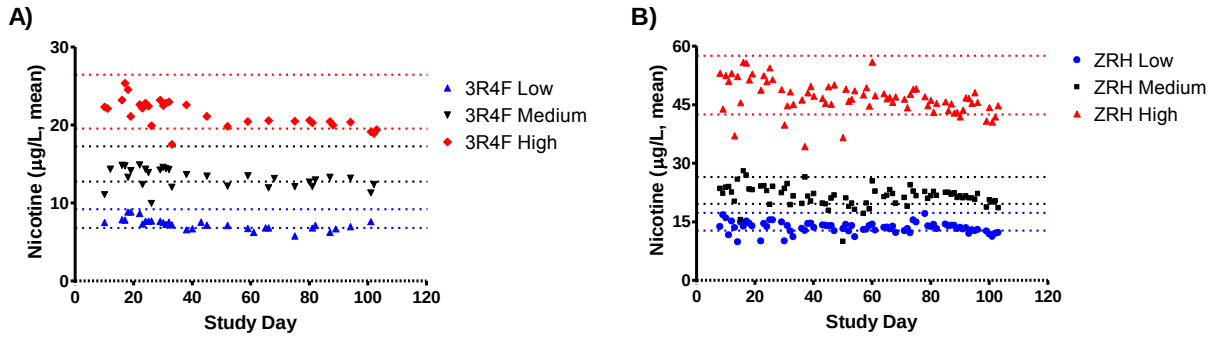


Figure 2: Daily average nicotine concentration

Daily average nicotine concentration in 3R4F chambers (A) and ZRH chambers (B). The upper and lower limits of nicotine concentration (target concentration ± 10%) for each exposure group are indicated as dotted lines in the graphs.

The average exposure nicotine concentrations, representing the entire exposure period are summarized as follows:

1. The average study nicotine concentration was within ± 10 % of target values for all exposure groups except the 3R4F-Medium group which was slightly below the lower limit of target concentration (see deviation 8). There were few incidences where daily average nicotine concentration was below the specification in the 3R4F-Medium group that resulted in the overall lowered study average. The impact of study average of 3R4F-Medium group falling below specification was low as the problems were quickly identified and corrective actions were implemented. Downtime of smoking machines was typically short (less than 30 minutes) and loss in exposure time could be compensated on the same day.
2. Very similar nicotine concentrations achieved by the ZRH and 3R4F groups with matched nicotine concentrations ie 3R4F-Medium *versus* ZRH-Low group, 3R4F-High *versus* ZRH-Medium group.
3. Reproducible and stable nicotine concentration throughout the 90 day exposure period with exception of few incidents where daily nicotine concentration was below daily target concentration (± 15 % of target concentration) and/or smoking machine downtime exceeding 30 minutes (see deviation 8). The frequency and duration of smoking machine downtime were more frequent and longer in the ZRH smoking machines compared to 3R4F smoking machines. The commonly faced problems with ZRH smoking machines include piston secure errors due to failure to load tobacco stick into the cigarette holder, failure to alternate between odd and even smoking ports, communication error between cigarette holders and smoking machine, and cigarette holders not fully charged in time for smoking. Corrective actions to upgrade the software in cigarette holders and change out parts in the smoking machines as well as installation of audible alarm to better react to machine faults were in place to overcome these problems. The maximum time the rats could stay in exposure tubes was also increased from 7 hours to 7.5 hours to allow more compensation time for machine downtime (see deviation 7 and amendment 7). The impact of technical faults with ZRH smoking machines was low as loss in exposure time could be compensated on the same day and study average of nicotine levels in most chambers were within specification (± 10 % of target values).

Beside nicotine, the TPM concentration and CO level were also determined daily in the breathing zone of the aerosols. The daily TPM and CO levels were highly reproducible throughout the entire exposure period (Table 7, Figure 3, Figure 4 and Figure 5). The aerosol generated from ZRH sensorial medium contained approximately 2-fold less TPM and 50- fold less CO compared to aerosol from 3R4F at the same nicotine concentration. At the start of the study, during the routine check of CO meter, the CO value obtained was not within the specification for chamber 6 and 7 (See deviation 2). There was no impact on the study as values were within specification when rechecked the next day.

Table 7: Average TPM and CO concentration in the exposure chamber

Short Name	Sham	3R4F-Low	3R4F-Medium	3R4F-High	ZRH-Low	ZRH-Medium	ZRH-High
Chamber No.	1	2	3	4	5	6	7
Mean TPM (µg/L)*	0.0	87.8	168.1	274.6	87.5	125.8	319.7
Standard Deviation TPM	0.0	9.9	8.8	14	8.4	25.5	29.8
Mean CO (ppm)**	< LOQ	110.8	196.3	335.0	4.4	6.4	12.9
Standard Deviation CO	NA	10.2	9.0	14.5	0.5	0.6	0.8
Number of daily measurement	74	74	74	74	74	74	74

*LOQ for TPM is defined as zero since the difference in filter weight before and after collection in Sham is zero. Records of the identity of the person who performed TPM measurement is not available, refer to deviation 18. Lower limit of quantification (LOQ) for CO is defined as 10 times of noise value (differences between max and min values from zero curve) which is 7.3 ppm.

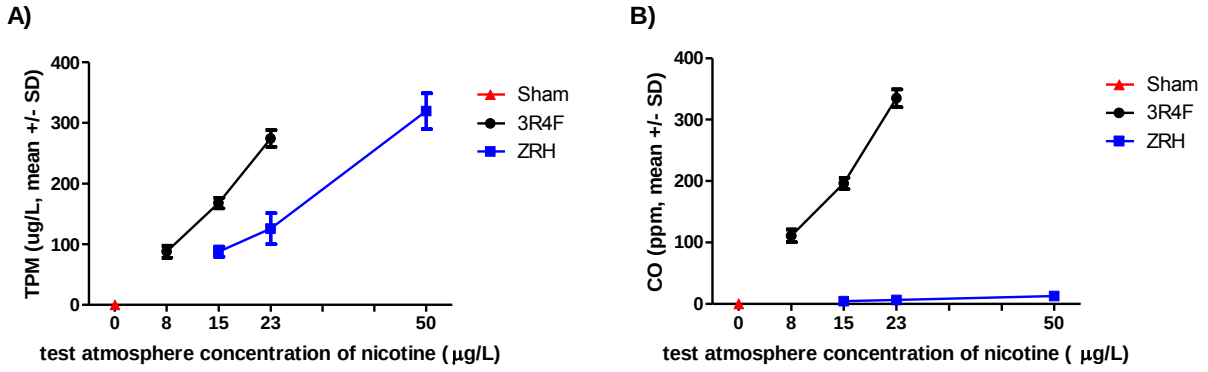


Figure 3: Average TPM and CO concentration in the exposure chamber

Average TPM (A) and CO (B) concentration in the exposure chamber

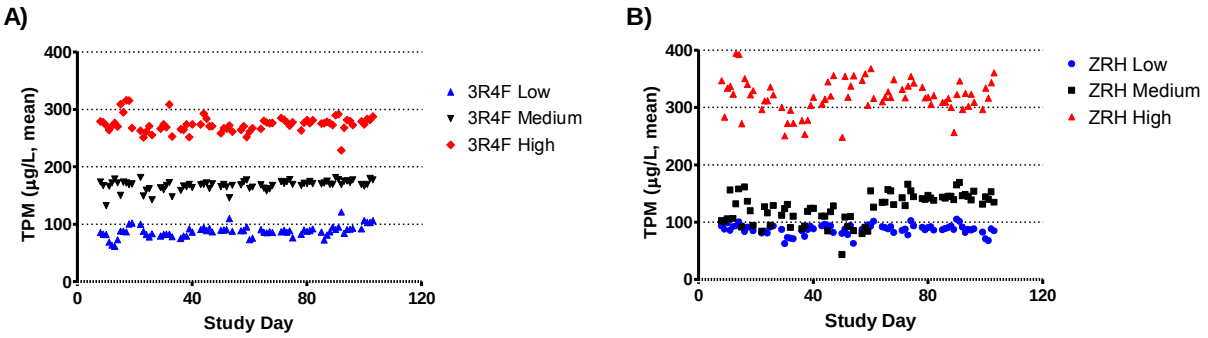


Figure 4: Daily average TPM concentration

Daily average TPM concentration in 3R4F chambers (A) and ZRH chambers (B).

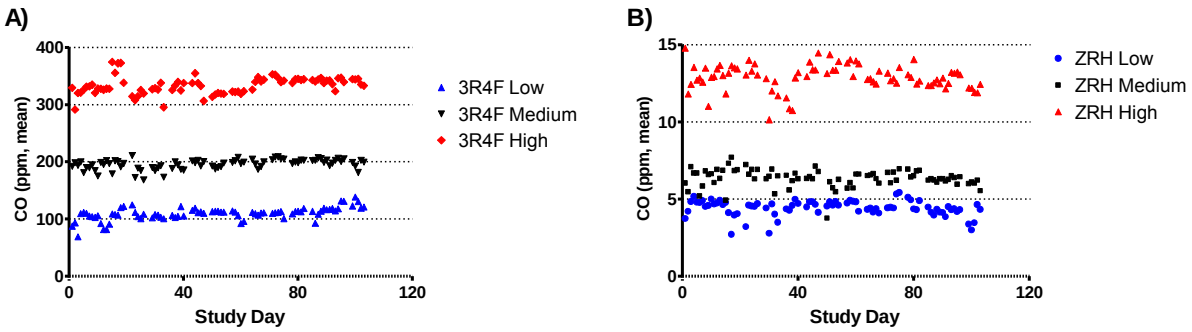


Figure 5: Daily average CO concentration

Daily average CO concentration in 3R4F chambers (A) and ZRH chambers (B).

The levels of aldehyde and acrolein in aerosol were determined weekly; see Table 8 and Figure 6 for study averages. At equal nicotine concentration, the amount of formaldehyde and acetaldehyde present in the aerosol generated from ZRH sensorial medium were approximately 5-fold less as compared to aerosol generated from 3R4F reference item. The amount of acrolein present in the aerosol generated from ZRH sensorial medium was approximately 12-fold less as compared to aerosol generated from 3R4F reference item.

Table 8: Average aldehydes and acrolein concentration in the exposure chamber

Short Name	3R4F-Low	3R4F-Medium	3R4F-High	ZRH- Low	ZRH-Medium	ZRH-High
Chamber No.	2	3	4	5	6	7
Mean formaldehyde (µg/l)	0.20	0.36	0.54	0.07	0.09	0.18
Standard Deviation formaldehyde	0.04	0.05	0.05	0.01	0.02	0.04
Mean acetaldehyde (µg/l)	5.87	10.29	16.84	2.19	3.35	6.59
Standard Deviation acetaldehyde	1.02	1.29	0.78	0.14	0.45	1.09
Mean acrolein (µg/l)	0.58	1.01	1.62	0.08	0.13	0.25
Standard Deviation acrolein	0.10	0.12	0.11	0.01	0.02	0.04
Number of daily measurement	15	15	14	14	15	15

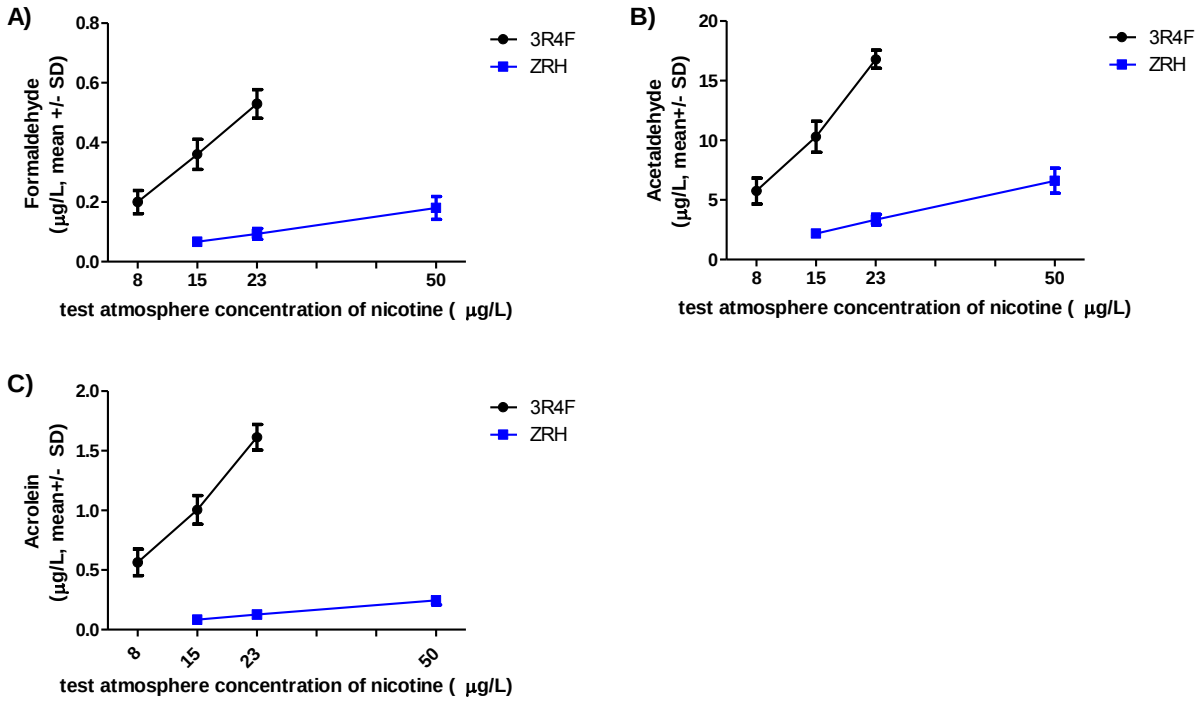


Figure 6: Tabulation of aldehyde levels present in the test atmosphere.

4.3 Test System

See Part 1 of report for details.

The first body weight measurement (study day -12, 5 days after animal arrival) showed an average body weight of 285.8 g for the males (range 223.1 g to 323.1 g) and 212.5 g for the females (range 176.6 g – 241.4 g). The rats were acclimatized for 23 days prior to exposure to the test item.

4.4 Experimental Groups and Dosing

4.4.1 Test Groups

Male and female animals were distributed into exposure groups coded 011 to 017 (7 PLUS groups, Table 9). The PLUS group animals were exposed concurrently with OECD group animals.

4.4.2 Allocation of Animals to Treatment Groups

A total of 209 male and 179 nulliparous and non-pregnant female rats were randomly allocated to 10 OECD groups and 7 PLUS groups by a Provantis-generated randomization sequence, based on sex and body weight. The mean body weights of the groups on the day of allocation was 285.6 ± 10.9 g for the males, and 213.1 ± 10.0

g for the females, with a RSD of 3.8 % in the male groups, and 4.7 % in the female groups. After allocation, a maximum body weight RSD of 4.1 % in the male groups and 5.3 % in the female groups were obtained, both within the specification of 10 % RSD and 20% differences between groups (Table 10 and deviation 17). Space limitations in the exposure chamber restricted the OECD 'PLUS' component of the study (groups 011 – 017) to no more than 6 rats per gender per group.

Exposed animals that died due to technical reasons were replaced with aerosol-exposed reserve animals (See Section 6.1.3 on Mortality). Replacement of exposed animals with non-exposed spare animals took place within the first 7 exposure days.

Table 9: Aerosol Exposure PLUS Groups

Animals were allocated into 3 dissection time points- at the end of 28 day exposure period (28d), 90 day exposure period (90d) and 42 day post-exposure following 90 day exposure period (90+42d)

			28d	90d	90+42d
Group Code	Long Name	Target nicotine (μg/L)	M/F	M/F	M/F
011	Sham Plus	0	6/0	6/6	5/5
012	3R4F- Low Plus	8	6/0	6/6	6/6
013	3R4F- Medium Plus	15	6/0	6/6	6/6
014	3R4F- High Plus	23	0/0	6/6	6/6
015	ZRH- Low Plus	15	6/0	6/6	6/6
016	ZRH- Medium Plus	23	6/0	6/6	6/6
017	ZRH- High Plus	50	0/0	6/6	0/0
	Total		30/0	42/42	35/35

Table 10: Body weight variation for the PLUS exposure groups

Group Name	Sham Plus		3R4F- Low Plus		3R4F- Medium Plus		3R4F- High Plus	
Group Code	011		012		013		014	
Sex of animals	Male	Female	Male	Female	Male	Female	Male	Female
mean body weight (g)	285.5	213.0	285.7	213.3	286.1	213.2	285.1	212.9
SD	11.7	10.3	11.3	11.2	11.2	10.2	11.0	10.2
RSD	4.1	4.8	4.0	5.3	3.9	4.8	3.9	4.8

Group Name	ZRH- Low Plus		ZRH- Medium Plus		ZRH- High Plus	
Group Code	015		016		017	
Sex of animals	Male	Female	Male	Female	Male	Female
mean body weight (g)	285.3	212.7	285.2	213.4	285.7	212.6
SD	11.1	10.8	11.3	10.8	11.4	10.3
RSD	3.9	5.1	4.0	5.1	4.0	4.8

4.5 Procedures

Biological parameters were selected parameters specified in the OECD Test Guideline 413 (OECD, 2009). General in-life condition and health of the rats were monitored, as described in Section 4.5.1. Necropsies of PLUS groups (Groups 011 to 017) were performed at the end of the 28 day inhalation period, 90 day inhalation period and after 42 day recovery following 90 day inhalation period, see section 4.5.2.

4.5.1 Parameters to Monitor General Condition and Health

Parameters to monitor general condition and health (Table 11) were:

- in-life observation
- mortality/morbidity
- body weight

Table 11: General Condition and Health

Parameter	Method	Frequency	Number of Rats	Remarks
Body weight	Gravimetry	≥2 times*/week during exposure	All rats	Individually
In-life Observations	Individual observations according to checklist in Provantis	Daily	Each rat systematically observed ≥4 times within the 13 weeks exposure period	Immediately after removal from exposure tube. Input data into Provantis
	Group observations according to checklist in Provantis	Daily	All rats	Immediately after removal from exposure tube. Input data into Provantis
Mortality	Observation	Daily	All rats	Immediately after removal from exposure tube. Input data into Provantis

* Body weight is performed once a week before exposure starts

4.5.2 Dissection and tissue collection for PLUS group animals

Dissection and tissue collection for PLUS groups 011 to 017 took place 16 – 24 hours post exposure under non-GLP conditions. Tissues were collected for purpose of histopathological evaluation, proteomics, transcriptomics, genomics and lipidomics. See Table 12 for tissue collection after 28 day inhalation period. See Table 13 for tissue collection list for dissection at 90 day and 90+42 day. Further processing and analysis of the collected tissues will be described in separate study reports.

Efforts to minimize RNA degradation were included by careful following of the relevant work instructions, using sterile collection tubes and petri plates that were RNase free, using surfaces and surgical tools cleaned with RNase AWAY® reagent, keeping the tissues chilled, and rapidly freezing the tissues for storage and all samples were frozen in dry ice or liquid nitrogen within 15 minutes after animal exsanguination (animal death) and stored at -80°C until shipment.

Table 12 Summary of tissues collection 28 day after inhalation

See text for details. RT: Room temperature; LCM: laser capture microscopy

Blood/ tissues	Collection/ Processing	Collection	Storage	Future analysis
Whole blood	RNAprotect animal blood tube.	RT	-80°C	RNA
Whole blood	EDTA tubes	RT	-80°C	DNA
Serum	microfuge tube	see text	-80°C	Protein
Liver	See section on liver below	Liq N_2	-80°C	Lipidomic and RNA
Larynx And Trachea	Remove and trim	Liq N_2	-80°C	RNA
Left lung	fix in EGAFS	RT	RT	Histology
Right lung	Perfuse with saline, slow freeze individual lobes in dry ice	Dry Ice	-80°C	LCM
Nose	fix in EGAFS	RT	RT	Histology

Table 13: Summary of tissues collection at 90 day after inhalation and at 90+42d.

See text for details. RT: Room temperature; RNE: Respiratory nasal epithelium.

Blood/ tissues	Collection/ Processing	Collection	Storage	Future analysis
Whole blood	RNAprotect animal blood tube	RT	-80°C	RNA
Whole blood	EDTA tubes	RT	-80°C	DNA
Serum	microfuge tube	see text	-80°C	Protein
Liver	See section on liver below	Liq N_2	-80°C	Lipidomic and RNA
Larynx and Trachea	Remove and trim	Liq N_2	-80°C	RNA
Left lung	Perfuse with saline, slow freeze in dry ice	Dry Ice	-80°C	LCM
Right lung	Perfuse with saline, slow freeze individual lobes in dry ice	Dry Ice	-80°C	LCM
Left RNE	No perfusion	Liq N_2	-80°C	RNA/ DNA
Right RNE	No perfusion	Liq N_2	-80°C	Protein

Detailed protocols:

1. **Collection of whole blood and serum**

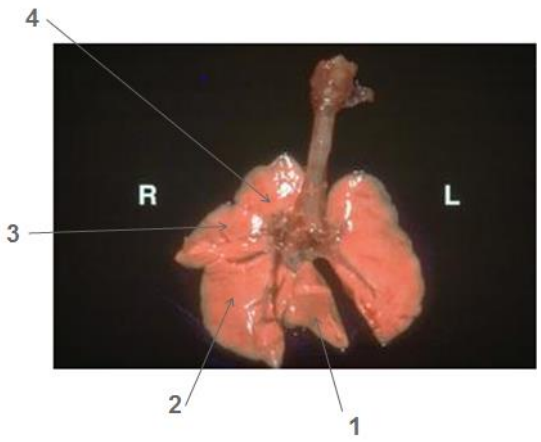
   Blood from rat were collected via retro-orbital puncture (WKI Exsanguinate: Retro-orbital Blood Collection). 500 µl of whole blood collected each for RNA and DNA isolation. For RNA, blood was collected in RNAprotect tube, inverted 10 times and stored at 15-25°C for 2h before freezing**.** For DNA, blood was collected in EDTA tube, inverted 10 times and frozen immediately**.** For collection of serum, approximately 4 ml blood was collected in SST tube and allowed to clot at room temperature for 30 minutes. The collection tube was centrifuged according to WKI Exsanguinate: Serum and plasma preparation. To avoid modification of protein content, heat inactivation of serum was not performed. The serum was aliquoted (250 µl aliquots) and stored at -80°C until shipment. The blood samples were stored at -80°C until shipment.

2. **Lung Perfusion**

   Prior to the removal of the organs, the animals' lung was perfused with cold saline to remove contaminating blood cells according to WKI Performing dissection: Lung Perfusion. For 28 day dissection, to perfuse only the right lung, the left bronchus/pulmonary artery were ligated before perfusion.

3a. Removal and freezing of right lung for Omics (28d dissection)

Each of the 4 lobes was dissected and labeled separately (figure below). Each lobe was trimmed and placed on aluminum foil and frozen slowly in crushed dry ice. See link below for further details.



3b. Left Lung for histopathology (28 day dissection)

The left lung was excised at the ligature. The left lung was instilled and fixed in EGAFS according to WKI Fixate tissues: Instillation of lungs for 48 hours before placement in 70% ethanol.

Paraffin-embedded sections were stained with haematoxylin-eosin (HE) and alcian blue periodic acid-Schiff (AB-PAS) (Table 14). Images of stained slides were captured using Aperio Digital Pathology system. Histopathology assessment will be described in separate study report.

Table 14: Histological processing of lung and nose at 28 day time point

Parameter	Method	Remarks
histopathology	light microscopic evaluation of	Nose: 4 transverse sections:
	histological slides after HE stain	1 immediately posterior to the upper incisor teeth,
		1 posterior to incisive papilla,
	nose at level 1 and	1 at second palatal ridge, and
	left lung at level 1 were	1 between first and second
	additionally stained with Alcian	
	blue/periodic acid Schiff's stain	Lungs: 3 longitudinal sections of left lobe:
		1 section at main bronchus
		2 sections at 1000 μm apart

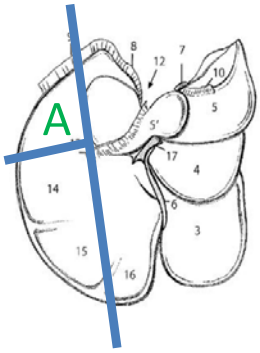
3c. Left Lung for Omics (90d and 90+42d dissection)

The left lungs were trimmed and placed on aluminum foil and frozen in dry ice as described in section 3a.

The rat's body was next halved into the upper and lower body from which the larynx, trachea, nose and liver were collected.

4a. Liver

A tissue segment indicated with "A" in the figure below was divided into 2 pieces for lipodomics and RNA isolation. For RNA isolation, a tissue size approximately 3x3x3mm was excised from segment "A". Both pieces of tissues were snapped frozen in Liq N_2.



4b. Larynx with trachea

Larynx with trachea was trimmed free from esophagus, thyroid/ parathyroid and associated muscle tissue and snapped freezing in Liq N_2.

4c Nose for histopathology (28d dissection)

The head was excised and the lower jaw, fur, calvaria, brain and the eyes were removed from the skull. The nasal tract was flushed with EGAFS and the entire nose was fixed in EGAFS together with the left lung for 48 hours. See Table 14 for histoprocessing.

4d. Nasal Epithelium (90d and 90+42d)

The head was excised and the lower jaw, fur, calvaria and the brain were removed from the skull. An incision sagittal to the nose was made; the nose was prised into left and right halves before removing the nasal epithelium from the exposed grooves. The left and right nasal epithelium was snap freeze in liquid nitrogen.

5 Statistical Methods

5.1 Statistical Design

Descriptive statistics (N, mean, standard deviation or standard error on mean) of the main aerosol characteristics (TPM, carbon monoxide, nicotine, formaldehyde, acetaldehyde, acrolein), inhalation chamber condition (temperature, relative humidity (sham group only), airflow) as well as smoking parameters (butt length & puff count (3R4F groups only), puff volume, burning rate) were computed for each exposure chamber. Reported statistics are mean ± standard deviation unless otherwise stated. The MMAD and GSD for particle size distribution were listed for each sample collected and no summary statistics was computed.

5.2 In-life observation

Findings in In-life observations and mortality were listed based on groups or individuals, no summary statistics was computed. For body weights data: descriptive statistics (number of valid measurements, mean, standard deviation (unless otherwise stated), median, minimum and maximum) were computed for each exposure group and gender, and when relevant, for various time points. Additionally and separately for each gender, comparative statistics between groups as described in Table 15 was estimated.

Table 15: List of Pairwise Comparisons

N°	Group 1	Vs.	Group 2
1	Sham PLUS	Vs.	3R4F- Low PLUS
2	Sham PLUS	Vs.	3R4F- Medium PLUS
3	Sham PLUS	Vs.	3R4F- High PLUS
4	Sham PLUS	Vs.	ZRH- Low PLUS
5	Sham PLUS	Vs.	ZRH- Medium PLUS
6	Sham PLUS	Vs.	ZRH- High PLUS
7	ZRH- Low PLUS	Vs.	3R4F- Medium PLUS

8	ZRH- Medium PLUS	Vs.	3R4F- High PLUS
9	ZRH- High PLUS	Vs.	3R4F- High PLUS

5.3 General considerations for data analysis

5.3.1 Missing Values

No imputation for missing values is performed.

5.3.2 Values under the Limit of Quantification

For aerosol data, values below limit of quantification (LOQ) was handled in the following manner:

- analyte not detectable or with no value was replaced by limit of detection/ 2 (LOD/2)
- negative value was replaced by zero
- measured values that were below LOQ was not replaced
- Averages falling below the LOQ was reported as “< LOQ”.

5.3.3 Minimization of variation

The major source of variation comes from:

- the individual response difference of rats to smoking exposure. To control this variation:
  - same species and strain of rats was ordered from one single supplier,
  - the rats were allocated into groups using a Provantis- generated randomization sequence so that the mean body weights for all groups at the start of the study will be similar.

5.4 Sample Size Justification / Power Considerations

The sample size was determined as 6 male and/or 6 female rats in each group

6 Results of Biological Endpoints

6.1 General Condition and Health of the Animals- Observations

6.1.1 Group observations in PLUS groups

Typical signs of stress related to nose-only exposure and restraint including Harderian gland secretion, wet fur and reduced corneal and pinna reflexes were observed in the PLUS groups at comparable frequencies with the OECD groups. There were sporadic incidences of tremor during adaptation phase in the female 3R4F Low group (group 012) and female ZRH Low group (group 015). There was a higher incidence of tremor in the ZRH High nicotine exposure group (Group 017), clustered around the first 6 days of exposure and after day 50 of exposure (Table 16). The tremor that occurred during the adaptation phase of the study was mild and may be linked to animals being cold and shivering following the exposure period. 2 female animals belonging to ZRH High group were noted to show repeated tremor in 2 or more and sometimes consecutive exposure days towards the later phase of the 90 day exposure period. The tremor in these animals was more intense compared to shivers

observed during adaptation phase. The lower body weights in female rats, in combination with stress may account for higher incidence of tremor in the female high nicotine concentration group. The animals with tremor typically recovered within 30 minutes upon removal from exposure, which is typical of nicotine toxicity and short half-life of nicotine *in vivo*. The incidences of tremor in rats exposed to 50 µg/L nicotine were not unexpected as tremor was also noted in the female rats during ZRH dose range finding study (study 15008).

Table 16: Incidences of tremor in PLUS groups. NR: Not recorded

Group	Day of exposure	No. of animal (s) with tremor	Animal code
017	6	2 male	NR
017	64	1 male	NR
017	78	1 male	NR
017	5	2 female	NR
017	6	2 female	NR
017	50	1 female	NR
017	61	1 female	NR
017	71	1 female	NR
017	65-67, 73, 78, 79, 85, 86, 89	1 female	0172001
017	68, 81	1 female	0172002
015	6	2 male	NR
015	5	3 female	NR
015	6	1 female	NR
012	5	2 female	NR
012	6	1 female	NR

6.1.2 Individual observations in PLUS groups

Similar to the OECD groups, there were sporadic occurrences of abnormal individual observations in the PLUS groups, summarized as follows:

1. Spontaneous activity: One occurrence of reduced activity in ZRH Medium and one occurrence of increased activity in ZRH High group noted in week 3 and week 10, respectively.
2. Increased irritability (vocalizations): Seven occurrences in different exposure groups (3R4F Low, ZRH Low, ZRH Medium, ZRH High) and occurrences were distributed over the exposure period.
3. Reduced turning reflex: One incident in ZRH High group in week 1 of exposure.
4. Reduced gripping ability: Nine incidents distributed in 3R4F and ZRH aerosol exposed groups and occurrences were distributed over the exposure period.

Aerosol exposed animals particularly in the 3R4F High groups (15 cases) were also noted with higher incidences of wounds (on paws/ legs, tail base, ear, cheeks), sneezing and/ or closed/ swollen eyes, probably arising due to animal struggling in exposure tube and irritation from aerosol exposure.

6.1.3 Mortality in PLUS groups

A total of 8 animals from PLUS groups (group 011 to 017) were found dead or moribund during the study (Table 17 and deviation 3). 3 of 8 PLUS animals that died were replaced. There were 7 technical deaths in the PLUS groups. Animal 9991002 and 0141002 were found dead during exposure but with no obvious trauma and no findings during post-mortem necropsy. Animal 8821001, 0141012, 0142001, 0142008 and 9991009 died due to asphyxiation from turning in the tube and blocking the air supply. Animal 0142006 was found dead in cage and had wounds on the front paws. The cause of paw wounds was technical, due to animal struggling during exposure and abrasion with the exposure tube. Upon necropsy and histopathological evaluation, animal 0142006 was found with pyo-granulomatous necrotising inflammation of kidney. The prolonged non-healing wounds and severe kidney inflammation were probably linked, and possibly aggravated by stress from tube restrain and exposure. The severe kidney inflammation was concluded as the likely cause of death for 0142006. In conclusion, as most premature deaths were of technical causes, the incidents of exposure related mortality was negligible and did not differ between ZRH and 3R4F exposed groups.

Table 17: Animal mortality in PLUS groups during the experimental phase

Animals with organs sent for histopathological evaluation are in bold. Animal 9991002 and 9991009 were not sent for histopathological evaluation, see deviation 3 for details. CAN: coded animal number; UAN: uncoded animal number. No bone marrow or organ weight data was collected for these animals.

Group	Dead Animal CAN	Dead Animal UAN	Replacement animal (CAN)	Replacement animal (UAN)	Date of replacement
012	910172	0121015	910024	9991002	14-Feb-13
017	910153	0171004	910069	9991009	14-Feb-13
014	**920012**	**0142008**	None	None	NA
012	910200	0121014	**910086**	**8821001**	14-Mar-13
014	**910032**	**0141002**	None	None	NA
014	**910116**	**0141012**	None	None	NA
014	**920193**	**0142001**	None	None	NA

6.2 Body Weight

An overview of the mean body weights and comparative statistics of both male and female rats can be found in Figure 7, Table 18 to Table 21. The body weights trends before exposure, during first week of adaptation and progression during 90 day exposure and post-exposure periods were very similar to that of OECD groups. The mean body weights between groups showed the following trends:

1. Male rats at 90 day exposure period:

- Lower mean body weights in 3R4F and ZRH exposed animals which were significant ($P<0.05$) when compared to Sham group. Statistically significant weight differences between Sham group and 3R4F High was observed starting from study days 25 to 81, lower in all 3R4F groups from study days 53 to 81, lower in ZRH Low group from study days 39 to 92 and lower in ZRH Medium group on study days 46 to 92.
- Mean body weight in 3R4F High was lower than ZRH High groups ($P<0.05$) during study days 53- 88

2. Male rats at 42 day recovery period:

- Weight gain in all animals with mean body weights approaching that of Sham group

3. Female rats at 90 day exposure period:

- Weight loss in Sham group was greater than the aerosol exposed groups during first week of dose adaptation. As a result, the Sham group had the lowest mean body weight throughout the exposure period. The weight loss is likely due to stress from tube restrain during nose only exposure.
- Higher mean body weight in ZRH Medium group compared to Sham group (P<0.05) from study day 18 to 92. We considered this largely technical due to lower body weight in Sham animals.
- At equivalent nicotine dose, mean body weight in 3R4F High was lower than ZRH Medium group (P<0.05) during study days 25 to 92.

4. Female rats at 42 day recovery period:

- Weight gain in all animals with mean body weights of aerosol exposed animals approaching that of Sham groups
- Mean body weight of 3R4F High group was lower than ZRH Medium group (P<0.05)

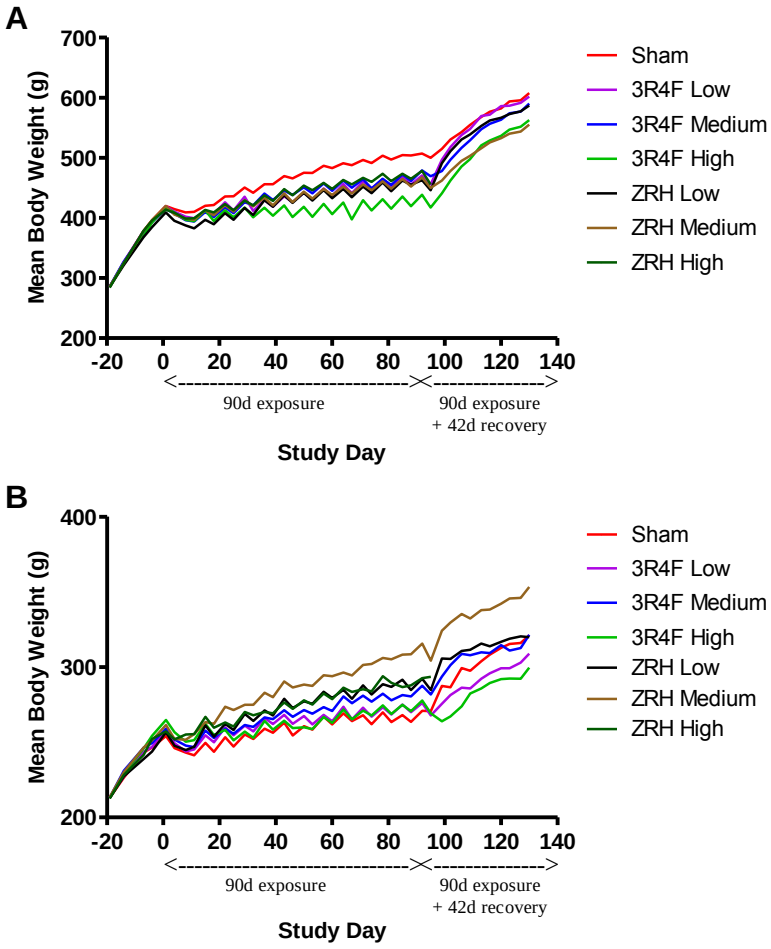


Figure 7: Mean body weight
Mean Body weight, male rats (A) and female rats (B) from PLUS groups

6.3 Dissection and tissue collection for PLUS group animals

Organs from all PLUS group animals with the exception of moribund and unscheduled death animals were successfully harvested and shipped out for further analysis.

7 Discussion

The objective of this 90 day inhalation study was to determine whether subchronic (90 day) inhalation of ZRH aerosol induces toxicity in Sprague-Dawley rats and to compare it to the toxicity induced by subchronic inhalation of MS from the University of Kentucky 3R4F cigarettes. In-life observations during the exposure phase were conducted in accordance to GLP guidelines while the dissection of PLUS group animals was conducted under non-GLP conditions. Male and female Sprague-Dawley rats were exposed for 90 days, 5 days per week, 6 hours per day. The recovery animals were exposed for 90 days before 42 days of post-exposure period. The target aerosol concentration of ZRH test item was 15 µg/L, 23 µg/L and 50 µg/L nicotine. The target aerosol concentration of 3R4F reference item was 8 µg/L, 15 µg/L and 23 µg/L nicotine. Frequent monitoring of nicotine concentrations in the breathing zone of the exposure chambers demonstrated that the average nicotine concentrations were within specified range (± 10% of the target concentration) in all chambers except 3R4F Medium (Deviation 8). The impact of lower nicotine concentration in 3R4F Medium group was minimal because the concentration of ZRH group at equivalent nicotine dose was very similar (13.17 µg/L for 3R4F Medium group versus 13.46 µg/L for ZRH Low group). Even though there were some daily fluctuations in the aerosol concentrations (Deviation 8) mostly attributed to smoking machines downtime, the impact on the study was negligible as the loss in exposure time was mostly compensated. The overall study average nicotine concentration between groups, along with reproducible particle size distributions of the aerosols indicate that the aerosol was delivered in a consistent manner to the exposure chambers with levels approaching the target concentrations. Measurement of other aerosol components also indicated significantly lower levels of harmful constituents such as formaldehyde, acrolein, acetaldehyde and CO in the aerosol from ZRH compared to conventional cigarette.

In-life observations, mortality and body weight development of PLUS group animals were similar and consistent with that of OECD group animals. There were no differences in mortality rate between the 3R4F and ZRH groups. Low incidences of tremor due to high nicotine dose (50 µg/L) from ZRH test item were observed. This was not unexpected as mild to moderate tremor was observed in female rats exposed to 50 µg/L nicotine in past study (Wong, 2013). Smoke/aerosol exposure-related reduction in body weight gain is considered as a general systemic effect of toxicity. Reduced weight gain was observed in male 3R4F groups and ZRH groups. Exposure to chemicals such as nicotine (Chowdhury, 1990) and acrolein (Feron et al., 1978) leads to reduce weight gain in experimental animals. The body weight effect in the male rats exposed to 3R4F aerosol is probably due to harmful smoke constituents such as acrolein. Due to the very low levels of acrolein in the ZRH groups, the body weight effects in the male rats exposed to ZRH aerosol are probably driven by nicotine. For both male and female rats, the body weight gain in 3R4F High group was lower than ZRH Medium and High groups likely due to lower toxicity from ZRH aerosol. Reduced body weight gain was also observed in female Sham rats and was considered as a consequence of restrain-related stress. Effectively, no additional pathological changes were observed in the routine health checks, systemic analysis and histopathological assessment of the fixed tissues from the Sham animals.

8 Conclusion

This study evaluated the biological effect of inhaled aerosol from ZRH in comparison with the 3R4F reference cigarette. The 90-day inhalation study was performed according to the OECD Test Guideline 413 (OECD, 2009). Aerosol generation was reproducible as indicated from aerosol monitoring parameters. In-life observations, mortality and body weight development were consistent with OECD group animals and lower toxicity induced by ZRH test item as compared to conventional cigarettes was evident from higher body weight gained over time.

9 References

Chowdhury, P. (1990). Endocrine and metabolic regulation of body mass by nicotine: role of growth hormone. Annals of clinical and laboratory science *20*, 415-419.

Feron, V.J., Kruysse, A., Til, H.P., and Immel, H.R. (1978). Repeated exposure to acrolein vapour: subacute studies in hamsters, rats and rabbits. Toxicology *9*, 47-57.

OECD (2009). OECD Test Guideline 413: Subchronic Inhalation Toxicity: 90-Day Study, revised Toxicity Guideline

University_of_Kentucky (2003). University of Kentucky Tobacco Research and Development Center: The reference cigarette.

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10 Abbreviations and Definition of Terms

Abbreviation/Term	
AP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BALF	bronchioalveolar lavage fluid
CO	carbon monoxide
EDTA	ethylenediamine tetraacetic acid
EGAFS	ethanol glycerol acetic acid formaldehyde solution
FLC	free lung cell
FTC	Federal Trade Commission (USA)
GLP	Good Laboratory Practice
GSD	geometric standard deviation
HbCO	carboxyhaemoglobin
HB	haemoglobin
HCT	haematocrit
HEPA	high efficiency particulate air
ISO	International Organization for Standardization
KMIS	Knowledge Management Information Services
LIMS	Laboratory Information Management System
IU	international units
LLOQ	lower limit of quantification
LPT	Laboratory of Pharmacology and Toxicology
MMAD	mass mean aerodynamic diameter
MRS	mean relative to sham
MS	mainstream smoke

Abbreviation/Term	
N	number of individual values (or determinations etc.)
NA	not applicable
NACLAR	National Advisory Committee for Laboratory Animal Research
NADH	nicotinamide adenine dinucleotide
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
p	probability of receiving the value of the test statistic or a more extreme value of the test statistic, if the null hypothesis holds
PLT	platelet(s)
PMIRL	Philip Morris International Research Laboratories
PMRL	Philip Morris Research Laboratories
QL	quantification limit
RSD	relative standard deviation
SD	standard deviation
SE(M)	standard error (of the mean)
SOP	standard operating procedure
SPF	specific pathogen-free
TPM	total particulate matter

11 Tables and Figures

11.1 Body Weight

Table 18: Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure
Table 19: Descriptive and comparative statistics for body weights of male PLUS groups during recovery period
Table 20: Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure
Table 21: Descriptive and comparative statistics for body weights of female PLUS groups during recovery period

Note:

Significance level

- *** when p-values are lower than 0.001
- ** when p-values are lower than 0.01
- * when p-values are lower than 0.05

Table 18: Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	-19	-14	-7	-4	1	4
Sham PLUS (011)	Mean	285.5	325.2	378.2	395.9	419.7	414.8
	Standard Deviation	11.7	14.1	21.4	24.3	27.6	29.8
	Maximum	306.9	346.7	417.1	435.5	466.4	471.4
	Minimum	265.0	299.8	344.0	352.9	370.3	363.6
	Standard Error of Mean	2.8	3.4	5.2	5.9	6.7	7.2
	Number of samples	17	17	17	17	17	17
3R4F Low PLUS (012)	Mean	284.7	326.6	377.9	392.9	419.6	410.6
	Standard Deviation	12.7	18.4	24.1	26.1	33.9	32.5
	Maximum	306.4	353.8	416.1	437.6	475.3	466.7
	Minimum	261.2	300.3	343.4	355.9	369.2	359.1
	Standard Error of Mean	3.0	4	6	6	7.98	7.66
	Number of samples	18	18	18	18	18	18
3R4F Medium PLUS (013)	Mean	286.1	326.9	374.6	393.1	414.9	407.0
	Standard Deviation	11.2	13.3	20.9	25.9	31.3	31.1
	Maximum	306.0	355.3	410.1	444.0	484.5	472.1
	Minimum	268.2	304.1	332.1	351.3	362.3	356.4
	Standard Error of Mean	2.6	3.1	4.9	6.1	7.4	7.3
	Number of samples	18	18	18	18	18	18

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	-19	-14	-7	-4	1	4
3R4F High PLUS (014)	Mean	285.1	324.7	369.5	392.3	417.7	408.7
	Standard Deviation	11.0	17.6	20.6	25.8	28.9	29.7
	Maximum	303.4	354.2	398.7	430.9	458.9	455.8
	Minimum	269.5	296.0	337.8	351.2	369.9	361.2
	Standard Error of Mean	3.2	5.1	6.0	7.5	8.4	8.6
	Number of samples	12	12	12	12	12	12
ZRH Low PLUS (015)	Mean	285.3	320.8	367.6	384.2	408.9	395.2
	Standard Deviation	11.1	17.0	24.8	27.4	31.4	26.1
	Maximum	306.0	351.2	408.4	428.1	454.5	432.2
	Minimum	267.4	287.4	316.8	325.6	340.8	341.7
	Standard Error of Mean	2.6	4.0	5.8	6.5	7.4	6.2
	Number of samples	18	18	18	18	18	18
ZRH Medium PLUS (016)	Mean	285.2	324.5	377.8	396.0	419.6	405.5
	Standard Deviation	11.3	12.2	19.8	23.8	27.2	26.7
	Maximum	303.4	348.4	419.0	447.0	477.3	472.3
	Minimum	265.8	300.0	338.4	356.7	373.5	363.6
	Standard Error of Mean	2.7	2.9	4.7	5.6	6.4	6.3
	Number of samples	18	18	18	18	18	18

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	-19	-14	-7	-4	1	4
ZRH High PLUS (017)	Mean	283.9	323.9	378.3	395.7	413.4	408.3
	Standard Deviation	14.4	19.8	27.7	30.9	33.6	28.2
	Maximum	303.1	341.1	399.6	424.7	447.7	437.9
	Minimum	261.8	288.7	330.0	340.4	353.7	361.7
	Standard Error of Mean	5.9	8.1	11.3	12.6	13.7	11.5
	Number of samples	6	6	6	6	6	6

Continue …

		study day					
Group Name	Statistics	8	11	15	18	22	25
Sham PLUS (011)	Mean	408.8*(015)	410.3**(015)	420.1*(015)	421.3*(014),**(015)	435.5*(015)	435.8*(012),*(013),**(014),**(015),*(016)
	Standard Deviation	29.8	29.4	34.8	31.0	33.6	31.6
	Maximum	469.9	473.4	495.6	482.3	498.1	490.4
	Minimum	360.3	364.0	370.7	363.2	369.9	376.4
	Standard Error of Mean	7.2	7.1	8.5	7.5	8.1	7.7
	Number of samples	17	17	17	17	17	17

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	8	11	15	18	22	25
3R4F Low PLUS (012)	Mean	401.8	399.3	411.0	406.5	425.6	412.5
	Standard Deviation	31.1	34.6	34.2	34.8	37.7	42.2
	Maximum	460.1	466.5	484.7	476.3	504.7	492.8
	Minimum	350.7	347.0	361.2	354.0	369.0	353.0
	Standard Error of Mean	7.3	8	8	8	8.88	9.94
	Number of samples	18	18	18	18	18	18
3R4F Medium PLUS (013)	Mean	395.8	393.7	409.7	401.3	417.4	407.9
	Standard Deviation	31.6	28.4	30.6	28.8	31.7	31.9
	Maximum	463.4	455.9	477.9	470.2	490.6	482.6
	Minimum	342.1	344.0	362.5	362.6	368.8	360.8
	Standard Error of Mean	7.5	6.7	7.2	6.8	7.5	7.5
	Number of samples	18	18	18	18	18	18
3R4F High PLUS (014)	Mean	396.7	394.5	412.6	394.6	412.9	400.7
	Standard Deviation	29.1	28.9	31.3	28.3	31.2	30.1
	Maximum	440.4	436.2	451.9	434.4	452.4	440.3
	Minimum	347.7	347.8	359.9	353.8	371.8	354.7
	Standard Error of Mean	8.4	8.3	9.0	8.2	9.0	8.7
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	8	11	15	18	22	25
ZRH Low PLUS (015)	Mean	387.3	382.4	396.7	389.4	407.7	396.8
	Standard Deviation	26.2	25.2	30.6	27.2	27.8	27.2
	Maximum	425.5	418.0	437.7	426.8	445.8	443.3
	Minimum	339.0	339.5	341.2	339.6	357.5	345.9
	Standard Error of Mean	6.2	6.0	7.2	6.4	6.5	6.4
	Number of samples	18	18	18	18	18	18
ZRH Medium PLUS (016)	Mean	398.6	396.8	412.9	404.1	420.9	410.4
	Standard Deviation	25.7	25.5	27.0	27.4	29.0	29.6
	Maximum	460.9	456.9	473.1	458.4	478.5	461.3
	Minimum	355.8	352.8	362.5	353.3	366.6	351.6
	Standard Error of Mean	6.1	6.0	6.4	6.5	6.9	7.0
	Number of samples	18	18	18	18	18	18
ZRH High PLUS (017)	Mean	398.6	399.5	413.1	408.8	422.6	412.7
	Standard Deviation	37.9	36.9	37.1	38.8	43.4	46.5
	Maximum	441.1	443.7	454.1	450.9	471.2	466.9
	Minimum	330.8	334.6	346.7	337.9	343.5	327.1
	Standard Error of Mean	15.5	15.1	15.1	15.8	17.7	19.0
	Number of samples	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day						
Group Name	Statistics	29	32	36	39	43	46	50
Sham PLUS (011)	Mean	450.5*(013),*(014),**(015)	441.9*(012),**(014),**(015)	455.9*(014)	456.1*(012),**(014),*(015),*(016)	469.3*(012),**(014),*(015)	465.5*(012),**(014),**(015),*(016)	474.9*(012),**(014),*(015),*(016)
	Standard Deviation	32.9	30.3	30.6	30.6	32.0	27.4	28.8
	Maximum	514.0	485.7	500.2	498.0	511.6	501.0	509.1
	Minimum	387.8	384.8	400.6	397.2	404.3	402.3	409.7
	Standard Error of Mean	8.0	8.1	9.2	9.2	9.7	8.3	8.7
	Number of samples	17	14	11	11	11	11	11
3R4F Low PLUS (012)	Mean	435.0	411.5	429.6	418.7	436.0	426.4	441.6
	Standard Deviation	39.5	37.5	40.0	41.0	42.9	43.3	43.9
	Maximum	511.9	490.0	520.7	509.0	535.9	522.5	539.6
	Minimum	375.3	368.5	384.0	373.4	389.5	378.0	399.6
	Standard Error of Mean	9.3	10.8	12	12	12	12.48	12.68
	Number of samples	18	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	426.1	420.5	440.2	429.7	445.1	438.1	450.6
	Standard Deviation	34.5	34.0	35.8	37.2	40.3	37.6	39.1
	Maximum	499.6	487.4	507.9	503.0	522.1	515.1	530.9
	Minimum	378.4	379.3	395.8	387.3	394.0	396.6	409.2
	Standard Error of Mean	8.1	9.8	10.3	10.7	11.6	10.9	11.3
	Number of samples	18	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day						
Group Name	Statistics	29	32	36	39	43	46	50
3R4F High PLUS (014)	Mean	417.1	400.9	416.9	403.4	420.5	401.1*(017)	418.5
	Standard Deviation	32.2	30.9	32.8	30.8	30.0	29.5	29.1
	Maximum	463.2	439.5	461.7	441.2	459.2	431.1	460.4
	Minimum	366.4	353.2	370.7	355.3	375.6	341.9	371.8
	Standard Error of Mean	9.3	8.9	9.5	8.9	8.7	8.5	8.4
	Number of samples	12	12	12	12	12	12	12
ZRH Low PLUS (015)	Mean	416.4	404.9	429.5	418.2	437.5	425.0	441.7
	Standard Deviation	29.4	29.9	31.6	31.3	32.2	30.5	30.8
	Maximum	460.7	454.4	463.9	452.8	473.8	460.7	485.8
	Minimum	363.9	349.5	366.0	352.4	378.1	361.4	379.2
	Standard Error of Mean	6.9	7.0	9.1	9.0	9.3	8.8	8.9
	Number of samples	18	18	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	429.6	418.8	433.8	420.5	440.6	426.1	443.7
	Standard Deviation	32.0	31.3	34.8	36.4	38.7	37.5	38.7
	Maximum	486.3	477.8	493.8	482.1	506.2	490.4	513.3
	Minimum	364.2	360.8	376.2	367.8	380.9	374.8	385.1
	Standard Error of Mean	7.5	7.4	10.0	10.5	11.2	10.8	11.2
	Number of samples	18	18	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day						
Group Name	Statistics	29	32	36	39	43	46	50
ZRH High PLUS (017)	Mean	428.9	420.5	437.4	427.9	447.9	437.5	453.7
	Standard Deviation	43.4	46.8	46.0	48.8	49.3	47.2	49.5
	Maximum	476.5	474.8	492.8	482.8	509.2	490.1	512.5
	Minimum	348.9	334.8	355.0	339.2	360.8	349.9	363.5
	Standard Error of Mean	17.7	19.1	18.8	19.9	20.1	19.3	20.2
	Number of samples	6	6	6	6	6	6	6

Continue …

		study day					
Group Name	Statistics	53	57	60	64	67	71
Sham PLUS (011)	Mean	474.8**(012),**(013),**(014),**(015),**(016)	486.5*(012),**(014),*(015),*(016)	482.7**(012),**(013),**(014),**(015),**(016)	490.6*(012),**(014),*(015),*(016)	487.7**(012),**(013),**(014),**(015),**(016)	496.3*(012),**(014),**(015),*(016)
	Standard Deviation	28.5	29.9	27.7	30.8	30.2	30.7
	Maximum	512.8	525.5	524.3	538.7	529.0	533.4
	Minimum	414.2	415.9	422.3	423.9	421.3	430.3
	Standard Error of Mean	8.6	9.0	8.4	9.3	9.1	9.3
	Number of samples	11	11	11	11	11	11

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	53	57	60	64	67	71
3R4F Low PLUS (012)	Mean	430.2	448.9	436.6	456.4	441.1	460.6
	Standard Deviation	45.8	44.0	45.6	49.0	47.7	48.3
	Maximum	532.1	545.3	538.0	569.4	546.2	564.7
	Minimum	384.3	405.7	389.0	407.0	394.6	407.5
	Standard Error of Mean	13.2	13	13	14	13.75	13.94
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	440.6	457.1	444.7	462.0	449.9	464.1
	Standard Deviation	38.4	36.4	36.5	40.7	40.5	41.5
	Maximum	517.7	517.8	508.6	535.3	517.5	538.2
	Minimum	398.2	415.2	399.4	414.6	402.8	413.9
	Standard Error of Mean	11.1	10.5	10.6	11.8	11.7	12.0
	Number of samples	12	12	12	12	12	12
3R4F High PLUS (014)	Mean	401.6*(016),*(017)	423.3	405.9*(017)	425.5	397.5*(016),**(017)	429.5
	Standard Deviation	28.7	29.1	29.5	30.4	42.3	31.9
	Maximum	430.8	462.8	443.2	465.5	448.0	464.0
	Minimum	341.4	372.7	357.1	378.6	312.1	377.1
	Standard Error of Mean	8.3	8.4	8.9	9.2	12.8	10.1
	Number of samples	12	12	11	11	11	10

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	53	57	60	64	67	71
ZRH Low PLUS (015)	Mean	428.7	446.4	432.5	448.2	434.5	451.8
	Standard Deviation	31.4	32.8	33.8	34.1	34.1	34.3
	Maximum	472.0	492.1	480.6	500.5	482.3	507.6
	Minimum	364.5	380.2	364.6	384.9	371.7	395.7
	Standard Error of Mean	9.1	9.5	9.8	9.9	9.9	9.9
	Number of samples	12	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	432.0	448.3	437.5	451.8	440.2	455.5
	Standard Deviation	40.1	40.9	42.4	42.8	42.4	42.0
	Maximum	509.1	519.8	521.5	532.1	523.5	533.9
	Minimum	376.5	383.4	381.2	391.8	382.9	397.4
	Standard Error of Mean	11.6	11.8	12.2	12.4	12.3	12.1
	Number of samples	12	12	12	12	12	12
ZRH High PLUS (017)	Mean	445.7	458.2	448.1	463.1	455.7	466.6
	Standard Deviation	49.2	50.8	53.8	53.1	55.9	53.0
	Maximum	501.7	518.4	510.6	526.2	522.6	525.9
	Minimum	355.4	365.2	350.9	367.6	356.1	370.0
	Standard Error of Mean	20.1	20.7	22.0	21.7	22.8	21.7
	Number of samples	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	74	78	81	85	88	92
Sham PLUS (011)	Mean	491.0*(012),*(013),**(014),**(015),**(016)	503.4*(012),*(013),**(014),*(015),*(016)	497.0*(012),*(013),**(014),**(015),**(016)	504.5*(012),**(014),*(015),*(016)	503.6**(012),**(013),**(014),*(015),**(016)	507.0*(012),**(014),*(015),*(016)
	Standard Deviation	30.3	31.2	30.6	29.3	33.6	33.5
	Maximum	533.6	547.6	537.7	545.0	544.4	550.9
	Minimum	426.1	438.8	428.9	437.5	428.9	441.4
	Standard Error of Mean	9.1	9.4	9.2	8.8	10.1	10.1
	Number of samples	11	11	11	11	11	11
3R4F Low PLUS (012)	Mean	448.7	464.0	452.5	468.1	455.3	470.6
	Standard Deviation	46.2	47.4	48.9	48.0	48.7	50.1
	Maximum	543.8	570.5	555.6	571.8	555.6	573.2
	Minimum	398.9	412.6	395.2	415.9	400.4	409.1
	Standard Error of Mean	13.3	14	14	14	14.07	14.45
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	449.0	464.9	454.6	471.9	461.2	478.8
	Standard Deviation	42.9	40.2	40.6	42.4	41.3	42.5
	Maximum	524.9	538.5	526.8	550.3	535.6	555.4
	Minimum	409.6	428.6	411.3	428.7	418.6	433.4
	Standard Error of Mean	12.4	11.6	11.7	12.2	11.9	12.3
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	74	78	81	85	88	92
3R4F High PLUS (014)	Mean	412.2*(017)	430.9*(017)	415.2*(017)	435.3	419.6*(017)	438.4
	Standard Deviation	31.3	32.8	32.1	31.8	30.3	29.6
	Maximum	444.4	469.2	448.2	470.3	449.1	473.2
	Minimum	356.3	374.4	359.2	381.8	367.8	386.2
	Standard Error of Mean	9.9	10.4	10.2	10.1	9.6	9.4
	Number of samples	10	10	10	10	10	10
ZRH Low PLUS (015)	Mean	441.2	458.8	444.5	461.8	454.8	462.6
	Standard Deviation	32.4	33.4	31.6	33.2	29.6	33.1
	Maximum	492.3	517.2	492.1	516.5	491.7	515.1
	Minimum	377.0	394.8	383.8	401.9	415.9	399.3
	Standard Error of Mean	9.3	9.7	9.1	9.6	10.5	9.5
	Number of samples	12	12	12	12	8	12
ZRH Medium PLUS (016)	Mean	445.8	460.7	448.3	464.4	452.0	467.3
	Standard Deviation	44.4	43.0	42.9	43.0	43.6	43.3
	Maximum	536.7	544.2	533.2	545.8	540.8	552.5
	Minimum	388.8	402.7	395.0	406.8	399.8	407.0
	Standard Error of Mean	12.8	12.4	12.4	12.4	12.6	12.5
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during 90d exposure

		study day					
Group Name	Statistics	74	78	81	85	88	92
ZRH High PLUS (017)	Mean	459.5	473.4	461.2	473.4	464.9	478.4
	Standard Deviation	56.9	57.4	58.0	57.7	61.4	59.7
	Maximum	523.0	544.0	531.5	541.8	534.1	544.9
	Minimum	356.7	374.5	361.2	372.5	356.5	372.4
	Standard Error of Mean	23.2	23.5	23.7	23.6	25.1	24.4
	Number of samples	6	6	6	6	6	6

Table 19: Descriptive and comparative statistics for body weights of male PLUS groups during recovery period

		study day						
Group Name	Statistics	95	99	102	106	109	113	116
Sham PLUS (011)	Mean	500.0*(012),**(014),**(014),**(015),*(016)	514.8**(014),*(016)	530.3*(014)	542.6	554.3	568.0	576.4
	Standard Deviation	29.9	23.2	25.1	26.7	28.5	32.7	34.1
	Maximum	537.1	553.3	570.0	582.6	599.0	617.4	629.2
	Minimum	436.2	493.1	505.3	513.1	528.4	534.9	545.2
	Standard Error of Mean	10	10.4	11.2	12.0	12.8	14.6	15.2
	Number of samples	9	5	5	5	5	5	5
3R4F Low PLUS (012)	Mean	455.4	496.8	517.6	537.5	547.7	569.2	571.7
	Standard Deviation	53.4	63.0	67.0	66.6	67.7	68.8	71.6
	Maximum	552.0	584.5	610.6	631.6	641.1	662.4	680.7
	Minimum	396.3	418.1	428.1	445.2	452.2	466.5	466.2
	Standard Error of Mean	16.9	25.7	27	27	28	28.09	29.24
	Number of samples	10	6	6	6	6	6	6
3R4F Medium PLUS (013)	Mean	468.9	478.0	496.5	516.5	529.0	547.5	556.2
	Standard Deviation	43.4	47.8	54.5	56.4	59.8	63.4	65.8
	Maximum	542.5	574.2	604.5	626.0	644.6	671.6	684.5
	Minimum	420.9	452.5	462.2	476.1	486.9	500.7	506.6
	Standard Error of Mean	13.7	19.5	22.2	23.0	24.4	25.9	26.8
	Number of samples	10	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during recovery period

			study day					
Group Name	Statistics	95	99	102	106	109	113	116
3R4F High PLUS (014)	Mean	417.1	440.5	461.9	485.3	497.8	520.6	528.6
	Standard Deviation	31.1	33.0	33.7	37.3	40.0	43.0	41.4
	Maximum	457.7	476.1	500.7	531.2	544.8	566.8	575.0
	Minimum	368.3	403.9	423.3	445.3	451.9	478.0	482.5
	Standard Error of Mean	10.4	14.8	15.1	16.7	17.9	19.2	18.5
	Number of samples	9	5	5	5	5	5	5
ZRH Low PLUS (015)	Mean	446	490.8	510.7	530.3	538.7	553.0	561.9
	Standard Deviation	34.4	37.5	39.3	38.9	36.2	36.4	37.2
	Maximum	500.8	534.7	557.2	575.8	580.1	595.2	604.1
	Minimum	386.1	436.8	451.3	468.5	481.3	494.8	502.3
	Standard Error of Mean	9.9	15.3	16.1	15.9	14.8	14.9	15.2
	Number of samples	12	6	6	6	6	6	6
ZRH Medium PLUS (016)	Mean	449.6	461.8	477.9	494.8	502.9	515.5	525.3
	Standard Deviation	33.4	29.0	30.0	28.9	28.7	28.4	27.4
	Maximum	516.3	503.1	521.1	534.7	544.0	555.7	563.8
	Minimum	403.0	421.3	437.1	453.5	461.0	474.9	483.2
	Standard Error of Mean	10.6	11.8	12.3	11.8	11.7	11.6	11.2
	Number of samples	10	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during recovery period

		study day						
Group Name	Statistics	95	99	102	106	109	113	116
ZRH High PLUS (017)	Mean	455.6	NA	NA	NA	NA	NA	NA
	Standard Deviation	74.3	NA	NA	NA	NA	NA	NA
	Maximum	537.7	NA	NA	NA	NA	NA	NA
	Minimum	358.6	NA	NA	NA	NA	NA	NA
	Standard Error of Mean	37.2	NA	NA	NA	NA	NA	NA
	Number of samples	4	0	0	0	0	0	0

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during recovery period

		study day				
Group Name	Statistics	120	123	127	130	134
Sham PLUS (011)	Mean	582.0	593.4	595.6	607.2	618.3
	Standard Deviation	35.9	38.3	39.7	41.5	46.1
	Maximum	636.9	646.8	654.6	664.0	682.3
	Minimum	549.8	556.2	559.2	567.4	574.5
	Standard Error of Mean	16.1	17.1	17.8	18.6	20.6
	Number of samples	5	5	5	5	5
3R4F Low PLUS (012)	Mean	585.8	586.7	591.4	601.8	611.1
	Standard Deviation	74.0	73.5	77.2	74.3	78.2
	Maximum	699.4	708.0	708.0	713.1	727.6
	Minimum	477.7	482.2	481.2	493.1	494.6
	Standard Error of Mean	30.2	30	32	30	31.91
	Number of samples	6	6	6	6	6
3R4F Medium PLUS (013)	Mean	563.5	573.7	576.9	590.4	594.8
	Standard Deviation	67.3	72.7	78.3	83.4	86.4
	Maximum	693.8	716.6	730.8	753.1	763.0
	Minimum	509.6	517.9	517.2	520.8	526.7
	Standard Error of Mean	27.5	29.7	32.0	34.0	35.3
	Number of samples	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of male PLUS groups during recovery period

		study day				
Group Name	Statistics	120	123	127	130	134
3R4F High PLUS (014)	Mean	536.3	546.4	551.2	562.5	570.3
	Standard Deviation	44.9	49.3	48.4	53.8	51.1
	Maximum	591.9	603.6	607.0	625.1	626.7
	Minimum	494.6	495.4	499.0	506.3	514.6
	Standard Error of Mean	20.1	22.1	21.7	24.1	22.9
	Number of samples	5	5	5	5	5
ZRH Low PLUS (015)	Mean	566.4	572.8	577.2	586.8	592.2
	Standard Deviation	38.8	37.5	37.8	37.2	37.8
	Maximum	610.7	615.5	617.0	629.3	634.1
	Minimum	507.0	515.7	518.1	529.2	532.5
	Standard Error of Mean	15.8	15.3	15.4	15.2	15.4
	Number of samples	6	6	6	6	6
ZRH Medium PLUS (016)	Mean	532.4	539.7	543.5	555.1	558.7
	Standard Deviation	28.8	31.5	32.0	32.8	30.6
	Maximum	570.3	578.6	581.3	594.2	598.2
	Minimum	483.8	486.4	487.3	500.9	511.1
	Standard Error of Mean	11.8	12.9	13.1	13.4	12.5
	Number of samples	6	6	6	6	6

Continue …

Table 20: Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	-19	-14	-7	-4	1	4
Sham PLUS (011)	Mean	213.0	226.4	243.2	246.5	254.1	246.1
	Standard Deviation	10.3	16.5	18.8	18.0	19.9	20.4
	Maximum	227.5	253.3	278.1	282.9	290.3	281.6
	Minimum	196.5	203.0	215.3	219.5	227.6	210.1
	Standard Error of Mean	3.1	5.0	5.7	5.4	6.0	6.1
	Number of samples	11	11	11	11	11	11
3R4F Low PLUS (012)	Mean	213.3	227.2	243.7	246.9	258.9	249.3
	Standard Deviation	11.2	14.2	18.1	20.1	23.3	22.9
	Maximum	231.3	248.2	278.1	289.1	310.3	301.1
	Minimum	192.0	199.6	212.2	212.9	221.0	212.7
	Standard Error of Mean	3.2	4	5	6	6.74	6.60
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	213.2	231.2	246.3	249.5	258.9	251.9
	Standard Deviation	10.2	13.0	10.3	10.5	12.8	14.0
	Maximum	228.8	249.8	262.5	265.8	278.2	275.9
	Minimum	197.2	214.4	232.6	235.1	243.0	236.3
	Standard Error of Mean	2.9	3.8	3.0	3.0	3.7	4.0
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	-19	-14	-7	-4	1	4
3R4F High PLUS (014)	Mean	212.9	229.2	245.9	254.3	264.9	256.8
	Standard Deviation	10.2	11.3	15.7	18.6	19.1	15.0
	Maximum	229.4	250.3	269.3	283.8	296.0	284.7
	Minimum	198.1	211.3	222.8	225.4	234.0	235.6
	Standard Error of Mean	3.0	3.3	4.5	5.4	5.5	4.3
	Number of samples	12	12	12	12	12	12
ZRH Low PLUS (015)	Mean	212.7	227.4	238.8	243.8	256.8	247.7
	Standard Deviation	10.8	14.4	17.1	23.2	23.1	19.7
	Maximum	228.5	251.7	269.5	291.2	305.5	288.5
	Minimum	193.5	206.8	215.2	213.1	223.9	221.4
	Standard Error of Mean	3.1	4.2	4.9	6.7	6.7	5.7
	Number of samples	12	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	213.4	230.1	246.4	251.2	261.5	252.1
	Standard Deviation	10.8	13.1	16.8	19.4	23.8	19.9
	Maximum	231.9	258.2	273.2	284.5	297.6	281.2
	Minimum	196.2	208.7	224.0	226.7	219.9	227.2
	Standard Error of Mean	3.1	3.8	4.8	5.6	6.9	5.8
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	-19	-14	-7	-4	1	4
ZRH High PLUS (017)	Mean	212.6	228.3	241.6	252.2	257.2	252.0
	Standard Deviation	10.3	8.2	14.6	13.8	14.0	11.4
	Maximum	227.5	239.3	254.0	266.6	272.9	263.9
	Minimum	199.2	215.6	216.1	232.3	234.7	236.2
	Standard Error of Mean	4.2	3.4	5.9	5.6	5.7	4.7
	Number of samples	6	6	6	6	6	6

Continue …

		study day					
Group Name	Statistics	8	11	15	18	22	25
Sham PLUS (011)	Mean	243.2	241.3	249.6	243.6*(016)	253.3*(016)	247.3**(016)
	Standard Deviation	20.5	21.7	23.8	18.7	21.0	19.8
	Maximum	273.9	273.6	279.3	264.6	282.5	274.8
	Minimum	209.5	197.3	201.6	207.4	214.6	214.0
	Standard Error of Mean	6.2	6.6	7.2	5.6	6.3	6.0
	Number of samples	11	11	11	11	11	11

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	8	11	15	18	22	25
3R4F Low PLUS (012)	Mean	243.9	245.2	254.6	250.1	259.9	254.6
	Standard Deviation	21.4	21.3	23.6	23.7	24.6	24.0
	Maximum	290.4	285.4	297.0	290.4	305.7	300.3
	Minimum	212.7	209.4	212.3	207.8	219.2	215.1
	Standard Error of Mean	6.2	6	7	7	7.09	6.92
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	247.8	246.7	257.8	252.8	259.0	255.8
	Standard Deviation	14.1	15.3	16.3	15.6	12.3	15.1
	Maximum	275.1	281.4	291.3	284.1	285.6	289.3
	Minimum	235.0	228.6	237.4	236.7	237.9	231.5
	Standard Error of Mean	4.1	4.4	4.7	4.5	3.6	4.4
	Number of samples	12	12	12	12	12	12
3R4F High PLUS (014)	Mean	250.3	251.3	262.3	254.9	258.2	251.3*(016)
	Standard Deviation	14.7	16.2	17.9	17.2	16.5	16.9
	Maximum	276.2	282.2	299.9	289.0	296.0	289.9
	Minimum	225.9	232.5	241.9	232.5	232.5	229.1
	Standard Error of Mean	4.3	4.7	5.2	5.0	5.0	5.1
	Number of samples	12	12	12	12	11	11

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	8	11	15	18	22	25
ZRH Low PLUS (015)	Mean	244.9	247.1	261.2	253.8	262.3	258.0
	Standard Deviation	18.9	18.1	19.9	17.9	19.5	18.8
	Maximum	287.2	289.3	302.6	292.0	306.2	300.3
	Minimum	218.0	222.4	232.7	228.5	238.1	237.7
	Standard Error of Mean	5.5	5.2	5.8	5.2	5.6	5.4
	Number of samples	12	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	251.7	255.5	263.5	262.3	273.7	271.5
	Standard Deviation	20.4	20.0	20.8	19.7	23.9	21.7
	Maximum	284.0	292.8	302.5	302.8	310.8	312.0
	Minimum	224.0	227.5	234.9	235.1	245.7	245.6
	Standard Error of Mean	5.9	5.8	6.0	5.7	6.9	6.3
	Number of samples	12	12	12	12	12	12
ZRH High PLUS (017)	Mean	255.1	255.4	266.8	259.7	263.2	260.5
	Standard Deviation	15.6	16.0	17.2	13.7	15.5	15.9
	Maximum	272.0	269.5	281.9	274.4	276.5	277.5
	Minimum	233.0	227.8	237.6	237.7	235.5	234.5
	Standard Error of Mean	6.4	6.5	7.0	5.6	6.3	6.5
	Number of samples	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	29	32	36	39	43	46
Sham PLUS (011)	Mean	255.2*(016)	252.1**(016)	258.9**(016)	256.3**(016)	263.1**(016)	254.5*(015), **(016)
	Standard Deviation	20.4	22.5	22.9	23.6	25.5	23.6
	Maximum	282.5	280.3	289.0	279.6	296.5	285.0
	Minimum	218.7	209.7	217.4	210.2	213.2	213.5
	Standard Error of Mean	6.1	6.8	6.9	7.1	7.7	7.1
	Number of samples	11	11	11	11	11	11
3R4F Low PLUS (012)	Mean	261.1	257.3	266.2	262.2	268.3	262.3
	Standard Deviation	23.1	21.7	21.5	22.3	23.1	22.6
	Maximum	304.6	296.7	307.3	305.4	313.6	307.2
	Minimum	217.0	218.0	230.1	223.6	230.3	225.0
	Standard Error of Mean	6.7	6	6	6	6.66	6.53
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	261.6	260.2	266.6	265.3	271.3	266.9
	Standard Deviation	16.6	17.2	15.1	18.1	15.6	17.6
	Maximum	298.7	294.5	297.1	303.7	308.4	305.9
	Minimum	240.4	233.7	243.4	235.9	249.8	243.2
	Standard Error of Mean	4.8	5.0	4.4	5.2	4.5	5.1
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	29	32	36	39	43	46
3R4F High PLUS (014)	Mean	257.1*(016)	253.1*(016)	264.2*(016)	258.4*(016)	264.3**(016)	259.5**(016)
	Standard Deviation	17.1	17.4	21.9	19.6	20.8	19.4
	Maximum	299.7	291.8	309.9	298.0	312.0	301.8
	Minimum	235.4	225.9	231.2	226.7	230.6	229.0
	Standard Error of Mean	5.2	5.2	6.6	5.9	6.3	5.9
	Number of samples	11	11	11	11	11	11
ZRH Low PLUS (015)	Mean	268.8	264.3	271.2	267.8	279.0	272.7
	Standard Deviation	16.9	18.3	22.1	19.3	17.7	14.4
	Maximum	301.7	303.7	327.6	316.7	324.2	307.7
	Minimum	241.4	230.4	241.8	239.9	251.8	251.8
	Standard Error of Mean	4.9	5.3	6.4	5.6	5.1	4.2
	Number of samples	12	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	274.9	274.9	283.0	280.0	290.5	286.4
	Standard Deviation	26.7	24.8	24.2	24.4	27.6	26.8
	Maximum	331.3	332.7	331.8	330.4	354.8	350.4
	Minimum	236.2	246.5	251.3	248.9	262.9	257.8
	Standard Error of Mean	7.7	7.2	7.0	7.1	8.0	7.7
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	29	32	36	39	43	46
ZRH High PLUS (017)	Mean	270.2	267.9	270.2	269.3	276.4	272.3
	Standard Deviation	19.0	20.2	17.1	16.4	19.5	15.8
	Maximum	290.8	292.2	287.9	289.0	294.5	293.1
	Minimum	237.4	235.0	242.3	241.8	241.1	248.5
	Standard Error of Mean	7.8	8.2	7.0	6.7	7.9	6.4
	Number of samples	6	6	6	6	6	6

Continue …

		study day					
Group Name	Statistics	50	53	57	60	64	67
Sham PLUS (011)	Mean	260.8**(016)	258.4**(016)	267.0**(016)	261.8**(016)	269.3**(016)	264.1**(016)
	Standard Deviation	22.5	24.7	25.8	23.2	25.7	25.4
	Maximum	290.4	291.4	301.2	292.1	301.2	298.6
	Minimum	220.0	213.5	221.8	216.3	220.2	219.2
	Standard Error of Mean	6.8	7.5	7.8	7.0	7.7	7.7
	Number of samples	11	11	11	11	11	11

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	50	53	57	60	64	67
3R4F Low PLUS (012)	Mean	267.5	261.9	268.3	263.8	273.6	265.3
	Standard Deviation	21.3	21.8	22.9	21.8	21.7	21.4
	Maximum	307.3	301.6	309.6	301.3	315.8	302.7
	Minimum	231.8	222.8	225.6	228.3	236.3	227.2
	Standard Error of Mean	6.2	6	7	6	6.25	6.19
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	271.5	269.0	273.5	270.8	280.4	276.0
	Standard Deviation	16.0	19.3	19.5	20.0	19.0	20.3
	Maximum	303.4	308.3	316.7	316.1	312.5	314.1
	Minimum	249.7	240.9	246.8	240.3	253.3	245.5
	Standard Error of Mean	4.6	5.6	5.6	5.8	5.5	5.9
	Number of samples	12	12	12	12	12	12
3R4F High PLUS (014)	Mean	259.8**(016)	258.9**(016)	266.8**(016)	262.9**(016)	271.8*(016)	265.3**(016)
	Standard Deviation	16.8	17.2	20.9	20.8	21.5	21.1
	Maximum	292.8	296.7	313.0	308.2	310.7	308.8
	Minimum	232.9	232.4	238.2	232.1	238.5	233.3
	Standard Error of Mean	5.1	5.2	6.3	6.3	6.5	6.4
	Number of samples	11	11	11	11	11	11

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	50	53	57	60	64	67
ZRH Low PLUS (015)	Mean	277.4	275.5	283.6	279.5	285.0	279.0
	Standard Deviation	19.1	20.0	21.7	20.0	20.3	17.0
	Maximum	320.6	321.4	340.9	330.4	336.4	320.3
	Minimum	249.5	240.6	250.6	247.8	257.0	248.9
	Standard Error of Mean	5.5	5.8	6.3	5.8	5.9	4.9
	Number of samples	12	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	288.4	287.5	294.6	293.9	296.4	294.5
	Standard Deviation	27.5	25.7	30.8	29.1	32.5	30.8
	Maximum	351.5	345.9	361.8	360.6	375.6	371.1
	Minimum	256.0	254.5	259.1	254.1	253.0	257.3
	Standard Error of Mean	7.9	7.4	8.9	8.4	9.4	8.9
	Number of samples	12	12	12	12	12	12
ZRH High PLUS (017)	Mean	278.0	275.1	282.3	278.7	286.3	283.5
	Standard Deviation	17.0	17.2	19.5	20.1	16.1	17.0
	Maximum	296.6	298.3	311.2	308.1	309.7	307.2
	Minimum	252.2	249.2	256.0	253.4	267.7	267.9
	Standard Error of Mean	6.9	7.0	8.0	8.2	6.6	6.9
	Number of samples	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	71	74	78	81	85	88
Sham PLUS (011)	Mean	268.0*(015),**(016)	262.0**(016)	269.8**(016)	263.4*(015),**(016),*(017)	268.0*(015),**(016)	263.7**(016)
	Standard Deviation	24.9	23.2	23.3	26.9	26.7	27.7
	Maximum	302.8	295.2	302.7	300.7	305.8	305.6
	Minimum	224.7	221.2	227.4	213.6	221.6	210.1
	Standard Error of Mean	7.5	7.0	7.0	8.1	8.0	8.3
	Number of samples	11	11	11	11	11	11
3R4F Low PLUS (012)	Mean	272.9	267.0	273.4	268.5	275.1	270.0
	Standard Deviation	22.7	20.6	21.3	23.4	23.3	21.4
	Maximum	313.5	304.8	317.8	311.7	318.9	306.6
	Minimum	231.0	230.7	234.6	224.2	233.7	236.5
	Standard Error of Mean	6.6	6	6	7	6.73	6.17
	Number of samples	12	12	12	12	12	12
3R4F Medium PLUS (013)	Mean	281.1	277.0	282.2	277.9	281.3	280.5
	Standard Deviation	20.9	22.4	22.0	21.6	17.2	20.5
	Maximum	320.1	319.6	333.2	323.4	320.0	324.8
	Minimum	253.7	245.8	255.6	245.5	255.8	250.1
	Standard Error of Mean	6.0	6.5	6.3	6.2	5.0	5.9
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	71	74	78	81	85	88
3R4F High PLUS (014)	Mean	271.0**(016)	267.8**(016)	274.5**(016)	268.9**(016)	274.9**(016)	270.7**(016)
	Standard Deviation	21.0	22.6	27.7	26.0	24.0	22.5
	Maximum	319.2	315.8	332.7	321.9	323.9	314.7
	Minimum	240.1	234.2	240.7	234.6	240.1	236.1
	Standard Error of Mean	6.3	6.8	8.8	8.2	7.6	7.1
	Number of samples	11	11	10	10	10	10
ZRH Low PLUS (015)	Mean	288.0	281.8*(016)	288.7	286.9	291.6	284.7*(016)
	Standard Deviation	19.3	20.2	18.9	20.0	19.3	19.7
	Maximum	335.9	331.4	332.1	335.1	344.6	334.0
	Minimum	257.6	247.9	262.7	262.1	264.3	252.7
	Standard Error of Mean	5.6	5.8	5.5	5.8	5.6	5.7
	Number of samples	12	12	12	12	12	12
ZRH Medium PLUS (016)	Mean	301.5	302.1	306.1	305.2	308.3	308.4
	Standard Deviation	32.8	33.7	37.4	35.0	37.0	38.5
	Maximum	383.1	385.0	405.5	396.8	408.7	416.5
	Minimum	258.6	254.0	262.4	262.1	277.2	271.4
	Standard Error of Mean	9.5	9.7	10.8	10.1	10.7	11.1
	Number of samples	12	12	12	12	12	12

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		study day					
Group Name	Statistics	71	74	78	81	85	88
ZRH High PLUS (017)	Mean	285.3	284.0	293.9	289.6	286.8	288.0
	Standard Deviation	18.7	18.6	18.9	21.4	19.0	17.6
	Maximum	317.2	316.8	318.6	322.6	312.6	318.2
	Minimum	261.3	262.3	267.9	261.1	260.2	267.8
	Standard Error of Mean	7.6	7.6	7.7	8.7	7.7	7.2
	Number of samples	6	6	6	6	6	6

Continue …

		Study day
Group Name	Statistics	92
Sham PLUS (011)	Mean	270.9**(016)
	Standard Deviation	27.6
	Maximum	312.2
	Minimum	219.4
	Standard Error of Mean	8.3
	Number of samples	11

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		Study day
Group Name	Statistics	92
3R4F Low PLUS (012)	Mean	276.2
	Standard Deviation	22.2
	Maximum	317.0
	Minimum	240.2
	Standard Error of Mean	6.4
	Number of samples	12
3R4F Medium PLUS (013)	Mean	287.6
	Standard Deviation	20.3
	Maximum	328.8
	Minimum	265.2
	Standard Error of Mean	5.9
	Number of samples	12
3R4F High PLUS (014)	Mean	277.8**(016)
	Standard Deviation	24.7
	Maximum	330.5
	Minimum	247.8
	Standard Error of Mean	8.2
	Number of samples	9

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during 90d exposure

		Study day
Group Name	Statistics	92
ZRH Low PLUS (015)	Mean	292.2*(016)
	Standard Deviation	21.0
	Maximum	346.4
	Minimum	260.9
	Standard Error of Mean	6.1
	Number of samples	12
ZRH Medium PLUS (016)	Mean	315.7
	Standard Deviation	41.4
	Maximum	425.2
	Minimum	266.5
	Standard Error of Mean	11.9
	Number of samples	12
ZRH High PLUS (017)	Mean	292.7
	Standard Deviation	17.0
	Maximum	317.6
	Minimum	266.5
	Standard Error of Mean	6.9
	Number of samples	6

Continue …

Table 21: Descriptive and comparative statistics for body weights of female PLUS groups during recovery period

		study day						
Group Name	Statistics	95	99	102	106	109	113	116
Sham PLUS (011)	Mean	270.6**(016)	287.5*(016)	286.5*(016)	299.4	297.5	303.9	308.3
	Standard Deviation	27.5	34.8	34.4	36.1	39.1	40.6	43.9
	Maximum	314.6	320.6	325.2	335.8	340.1	348.6	355.7
	Minimum	216.1	228.4	231.3	240.3	236.5	241.9	240.0
	Standard Error of Mean	8.7	15.6	15.4	16.1	17.5	18.1	19.6
	Number of samples	10	5	5	5	5	5	5
3R4F Low PLUS (012)	Mean	267.8	275.5	281.4	286.3	285.9	292.3	295.8
	Standard Deviation	23.3	30.5	31.1	31.4	32.9	35.2	39.6
	Maximum	307.7	330.1	336.1	339.9	342.1	351.3	362.8
	Minimum	230.7	238.3	244.9	247.3	246.0	248.2	246.2
	Standard Error of Mean	7.4	12.5	13	13	13	14.36	16.18
	Number of samples	10	6	6	6	6	6	6
3R4F Medium PLUS (013)	Mean	281.8	293.7	301.4	308.8	308.0	309.8	309.4
	Standard Deviation	21.7	28.1	32.0	28.6	30.2	23.4	23.5
	Maximum	323.2	348.7	363.2	364.2	363.8	349.9	353.6
	Minimum	252.5	268.5	273.3	282.8	280.0	285.0	290.8
	Standard Error of Mean	6.9	11.5	13.1	11.7	12.3	9.5	9.6
	Number of samples	10	6	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during recovery period

		study day						
Group Name	Statistics	95	99	102	106	109	113	116
3R4F High PLUS (014)	Mean	269.3**(016)	264.0**(016)	267.3**(016)	273.8**(016)	282.4*(016)	285.8**(016)	289.5*(016)
	Standard Deviation	26.7	14.5	12.0	16.2	16.1	18.5	18.2
	Maximum	325.6	273.5	278.8	292.0	297.8	304.5	307.7
	Minimum	234.5	239.5	249.0	249.3	258.4	262.6	261.4
	Standard Error of Mean	9.4	6.5	5.4	7.2	7.2	8.3	8.1
	Number of samples	8	5	5	5	5	5	5
ZRH Low PLUS (015)	Mean	284.8	305.6	305.5	310.8	311.6	315.7	313.9
	Standard Deviation	25.5	30.8	30.3	33.6	32.9	35.5	34.9
	Maximum	342.7	358.8	354.3	369.2	372.6	381.4	375.8
	Minimum	252.6	265.0	262.6	266.1	274.2	276.2	278.0
	Standard Error of Mean	8.1	12.6	12.4	13.7	13.4	14.5	14.2
	Number of samples	10	6	6	6	6	6	6
ZRH Medium PLUS (016)	Mean	304.3	324.2	329.7	335.4	332.4	337.9	338.3
	Standard Deviation	25.0	21.9	22.1	25.6	25.9	26.6	26.9
	Maximum	343.3	347.5	347.7	355.3	356.4	366.1	368.6
	Minimum	260.9	284.2	287.7	290.0	287.7	293.6	299.5
	Standard Error of Mean	7.9	8.9	9.0	10.5	10.6	10.9	11.0
	Number of samples	10	6	6	6	6	6	6

Continue …

		study day						
Group Name	Statistics	95	99	102	106	109	113	116
ZRH High PLUS (017)	Mean	293.6	NA	NA	NA	NA	NA	NA
	Standard Deviation	23.7	NA	NA	NA	NA	NA	NA
	Maximum	320.0	NA	NA	NA	NA	NA	NA
	Minimum	263.0	NA	NA	NA	NA	NA	NA
	Standard Error of Mean	11.9	NA	NA	NA	NA	NA	NA
	Number of samples	4	0	0	0	0	0	0

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during recovery period

		study day				
Group Name	Statistics	120	123	127	130	134
Sham PLUS (011)	Mean	312.9	315.4	316.1	321.2	322.9
	Standard Deviation	45.2	47.4	49.5	52.1	58.3
	Maximum	363.5	369.3	373.4	381.5	392.2
	Minimum	244.8	244.1	242.8	249.2	240.5
	Standard Error of Mean	20.2	21.2	22.1	23.3	26.1
	Number of samples	5	5	5	5	5
3R4F Low PLUS (012)	Mean	299.3	299.2	303.0	309.0	311.7
	Standard Deviation	38.2	39.4	40.4	40.8	44.5
	Maximum	362.0	366.1	374.1	377.1	385.0
	Minimum	250.2	254.6	259.1	256.7	253.1
	Standard Error of Mean	15.6	16	16	17	18.15
	Number of samples	6	6	6	6	6
3R4F Medium PLUS (013)	Mean	314.7	311.0	312.7	321.5	321.9
	Standard Deviation	25.5	21.7	20.7	23.9	22.9
	Maximum	363.6	350.4	351.3	365.9	364.1
	Minimum	294.4	290.3	294.1	297.4	298.5
	Standard Error of Mean	10.4	8.9	8.5	9.8	9.3
	Number of samples	6	6	6	6	6

Continue …

Descriptive and comparative statistics for body weights of female PLUS groups during recovery period

		study day				
Group Name	Statistics	120	123	127	130	134
3R4F High PLUS (014)	Mean	291.9*(016)	292.4*(016)	292.2*(016)	299.7*(016)	298.1*(016)
	Standard Deviation	21.2	23.1	23.8	23.5	25.0
	Maximum	312.1	314.9	316.3	329.7	329.3
	Minimum	263.9	262.5	260.3	269.2	268.7
	Standard Error of Mean	9.5	10.3	10.6	10.5	11.2
	Number of samples	5	5	5	5	5
ZRH Low PLUS (015)	Mean	316.8	319.0	320.5	320.0	322.3
	Standard Deviation	37.2	41.1	42.1	40.6	43.2
	Maximum	384.9	395.7	398.3	395.2	402.8
	Minimum	277.5	275.8	277.6	277.1	277.3
	Standard Error of Mean	15.2	16.8	17.2	16.6	17.6
	Number of samples	6	6	6	6	6
ZRH Medium PLUS (016)	Mean	342.3	345.6	346.1	353.3	350.0
	Standard Deviation	28.2	30.9	30.1	30.9	36.8
	Maximum	371.0	374.8	380.8	381.0	383.9
	Minimum	300.0	300.4	304.3	306.6	304.8
	Standard Error of Mean	11.5	12.6	12.3	12.6	15.0
	Number of samples	6	6	6	6	6

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