A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

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4. COMPLIANCE STATEMENT

This study was performed in compliance with the following Good Laboratory Practice (GLP) regulations:

- The Organisation for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice (ENV/MC/CHEM(98)17).

Exceptions from the above regulations are listed below.

- Stability data are currently being generated and no formal expiry date for the test or control items were provided (see Section 9.3).

This study was conducted in accordance with the procedures described herein. All deviations authorised/acknowledged by the Study Director are documented in the study records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

The Test Site, National Institute of Biological Standards and Controls, is not in the UK GLP compliance programme, however the work has been monitored by Charles River personnel and is considered to be in compliance with the principles of GLP.

Bruce Robertson, BSc
Study Director

Date 22 Feb 2012

The test and control items were used as supplied and their production and subsequent analysis are outside the scope of this compliance statement.
5. QUALITY ASSURANCE STATEMENT

The Charles River Quality Assurance Unit conducted a protocol review, protocol amendment reviews, study-based inspections and report audits on this study, as detailed below.

| Dates of QA Activity | Activity                                | Date of Report to Management and Study Director |
|----------------------|-----------------------------------------|-------------------------------------------------|
| 20 Jul 2011          | Facility Inspection                      | 01 Aug 2011                                     |
| 09 Aug 2011          | Protocol Review                          | 09 August 2011                                  |
| 11 Aug 2011          | Protocol Amendment 1 Review              | 11 August 2011                                  |
| 12 Aug 2011          | Protocol Amendment 2 Review              | 12 August 2011                                  |
| 16-17 Aug 2011       | Dose Dispensing                          | 22 August 2011                                  |
| 17 Aug 2011          | Dosing and Protocol Compliance           | 22 August 2011                                  |
| 05 Sep 2011          | Protocol Amendment 3 Review              | 05 Sep 2011                                     |
| 23 Sep 2011          | Protocol Amendment 4 Review              | 23 Sep 2011                                     |
| 21 Oct 2011          | Necropsy                                 | 21 Oct 2011                                     |
| 16 Nov 2011          | Antibody Bleeds                          | 17 Nov 2011                                     |
| 21 Nov 2011          | Protocol Amendment 5 Review              | 21 Nov 2011                                     |
| 22 Nov 2011          | Sample Analysis                          | 24 Nov 2011                                     |
| 28 Nov 2011          | Protocol Amendment 6 Review              | 28 Nov 2011                                     |
| 04-12 Jan 2012       | Draft Report Audit                       | 12 Jan 2012                                     |
| 09 Feb 2012          | Final Report Audit                       | 09 Feb 2012                                     |

Process-based inspections relevant to this study are scheduled once every quarter. The outcome of each inspection is reported to Management and, where relevant, the Study Director.

Facilities relevant to this study are included in Charles River’s annual facility inspection programme. The outcome of each inspection is reported to Management.

This report is considered to describe accurately and completely the procedures used in the study and the results obtained.

Caroline Garth BSc
Quality Assurance

Date: 22 February 2012
6. RESPONSIBLE PERSONNEL

6.1. Test Facility

Study Director: Bruce Robertson, BSc
(Study Initiation-01 Sep 2011, 23 Sep 2011- Study completion)
Elizabeth Donald, BSc
(02 Sep-22 Sep 2011)

Quality Assurance: Caroline Garth BSc
Stewart Fraser BSc

Report Peer Review: Adam Woolley, MSc, DABT, FRCPATH, ERT, CBiol, MSB
ForthTox Limited

6.2. Test Facility Individual Scientists (IS)

Pathology: Lise Bertrand, DVM, MSc, DESV, DiplECVP

Peer Review Pathologist: Petrina Rogerson BMVS, MRCVS

6.3. Sponsor-designated Responsible Scientists

Antibody analysis: Caroline Vipond, PhD
National Institute of Biological Standards and Control, Hertfordshire, UK

6.4. Sponsor

Sponsor Representative: Andrew J Pollard, FCRPCH, PhD
Professor of Paediatric Infection & Immunity, Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Room 02-46-07, Level 2, Children’s Hospital, Oxford, OX3 9DU
7. **SUMMARY**

This study evaluated the potential toxicity and reversibility of reactions to MenPF-1, a prophylactic vaccine for the prevention of infection from bacterial meningitis, when given by intramuscular injection for 4 occasions over a 9 week period to New Zealand White rabbits. In addition, immunogenicity was characterised.

Animals were treated into a hind limb muscle on Days 1, 22, 43 and 64 with necropsy on Days 66 and 92.

The study design was as follows:

| Group No. | Animal Numbers | Main Study | Recovery | Test Item     | Dosage (µg/dose) | Conc. (µg/mL) | Dose Volume (mL/dose) |
|-----------|----------------|------------|----------|---------------|------------------|---------------|-----------------------|
| 1         | 1-3            | 10-12      | 19-21    | 28-30         | MOX Control      | 0             | 0.5 mL                |
| 2         | 4-6            | 13-15      | 22-24    | 31-33         | MenPF-1          | 25            | 50 mL                 |
| 3         | 7-9            | 16-18      | 25-27    | 34-36         | MenPF-1          | 50            | 50 mL x 2 mL          |

The following parameters and end points were evaluated in this study: viability, clinical signs, injection site reactions, body weights, body weight changes, food consumption, ophthalmology, body temperatures, clinical pathology parameters (haematology, coagulation and clinical chemistry), antibody analysis, gross necropsy findings, organ weights, and histopathological examinations.

There were no unscheduled deaths during the observation period.

There were no systemic signs and no local irritation noted in any animal during the observation period. Body weight and food consumption profiles were unaffected by treatment and there were no eye changes that were considered to be treatment related. There were no differences in body temperatures recorded up to 48 h after injection with MenPF-1.

Other than higher neutrophil numbers, higher fibrinogen levels and minor disturbances in plasma proteins at Day 64 in animals that received MenPF-1, when compared with controls, there were no in-life findings that were considered to be related to treatment with the vaccine.

An increase in titre of specific IgG was observed with increasing dose and over time. Following completion of 4 week treatment-free period, titres on Day 92 were similar or higher in the majority of animals to those recorded on Day 64.

At Day 66 (2 days after the last injection), 50 µg/dose of MenPF-1 resulted in minor findings at the injection site with foreign material-laden macrophages and giant cells noted. Polymorphonuclear and mononuclear inflammation with myofibre necrosis and/or regeneration, interstitial fibrosis and/or mineralisation were also observed. Lumbar lymph node enlargement was observed at necropsy and this correlated with lymphoid hyperplasia. Accumulation of foreign material-laden macrophages and giant cells was also noted in the lumbar lymph nodes. After a 4 week recovery period, a number of findings persisted in treated injection sites, however were of a lesser severity and frequency.

There were no differences in organ weight that were considered to be related to MenPF-1.

In conclusion, administration of the vaccine, MenPF-1, when given by intramuscular injection for 4 occasions over a 9 week period, was well tolerated in rabbits up to 50 µg/dose.
There was only an expected, minor inflammatory response which was associated with vaccine administration, characterised by macrophages, giant cells and polymorphonuclear and mononuclear inflammation at the injection sites with on-going recovery noted. There was no evidence of systemic toxicity.
8. INTRODUCTION

The objective of this study was to determine the potential toxicity of MenPF-1, a prophylactic vaccine for the prevention of infection from bacterial meningitis, when given by intramuscular injection for 4 occasions over a 9 week period to rabbits, and to evaluate the potential reversibility of any findings. Data will support the use of MenPF-1 in humans. In addition, immunogenicity was characterised.

The design of this study was based on the study objectives, the overall product development strategy for the test item, including the following study design guidelines:

- CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/ICH/302/95), December 1997
- WHO guidelines on nonclinical evaluation of vaccines (WHO Technical report series No. 927, 2005)
- CPMP Note of Guidance on Nonclinical Local Tolerance Testing of Medicinal Products (CPMP/SWP/2145/00), March 2001

The Study Director signed the protocol on 03 Aug 2011, and dosing was initiated on 17 Aug 2011. The in-life phase of the study was completed on 16 Nov 2011. The experimental start date was 10 Aug 2011, and the experimental completion date was 16 Dec 2011. The study protocol, protocol amendments, and deviations are presented in Appendix 1.
9. MATERIALS AND METHODS

9.1. Test Item

Identification: MenPF-1
Batch (Lot) No.: FMOX1102
Receipt Date: 07 July 2011
Expiration Date: Concomitant assessment, ongoing
Physical Description: Opaque, even milky suspension; easily redispersed
Purity: The active pharmaceutical ingredient (API), formulated as outer membrane vesicles, is a mixture of *Neisseria meningitidis* serogroup B outer membrane proteins that shows \(>93\%\) adsorption degree to aluminium hydroxide adjuvant. The API contains 8.0% 70kD FetA F3-3 variant protein, 21.7% Class 1 P1.7, 16 variant protein and 32.6% Class 3 P3.15 protein. The test item batch (i.e., vaccine product) contains 1.0 mg/mL aluminium. Dose calculations were not corrected for purity.
Concentration: 25 µg protein/dose of 0.5 mL
Storage Conditions: In a refrigerator set to maintain 4°C
Supplier: Norwegian Institute of Public Health, Oslo, Norway

9.2. Control Items

Identification: MOX Control
Batch (Lot) No.: FMOX1103
Expiration Date: Concomitant assessment, ongoing
Physical Description: Opaque, even milky suspension; easily redispersed
Purity: The product contains the adjuvant, Alhydrogel; specifically containing 1.1 mg/mL aluminium. Dose calculations were not corrected for purity.
Concentration: Nominal 0.333% w/v Alhydrogel in 3% sucrose solution
Storage Conditions: In a refrigerator set to maintain 4°C
Supplier: Norwegian Institute of Public Health, Oslo, Norway

Test and control items were monitored during transit to Charles River, Pre-Clinical Services, Edinburgh. Items were despatched refrigerated (2-8°C); an average temperature of 6.3°C was observed, with a high of 9.3°C which was maintained for 40 minutes. This deviation was considered to be transient and minor and not to have impacted on the integrity of the test and control items.

9.3. Test and Control Item Characterisation

The Sponsor provided to the Test Facility documentation of the identity, strength, purity, composition, and stability for the test and control items. A Certificate of Analysis for the vaccine, control item and the bulk vaccine from which the batch for this study was prepared, was provided to the Test Facility and these documents are presented in Appendix 2.
The vaccine, control item and the bulk vaccine from which the batch for this study was prepared were tested in accordance with Good Manufacturing Practice (GMP).

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test and control items, and this information is available to the appropriate regulatory agencies should it be requested.

No formal expiration dates were provided for the batches of MOX Control or MenPF-1 used on this study. The Sponsor indicated that batches of the control and test items were currently subject to stability testing. Data for the MenPF-1 batch including the latest available timepoint of 3 months were supplied, with further data being generated. The data indicated that at 3 months there was little difference between the results at this timepoint and the results at the initiation of testing. To provide stability data for the period of this preclinical study stability data of 4 months would be required, however, the current data suggested that there would be no reason to expect any degradation in MenPF-1 between 3 and 4 months.

For the MOX control item, current testing is underway, but no data were provided to the Test Facility. Given that this product has been subject to various testing before release, and as this is a control item, the lack of a definitive expiration date was considered not to have had any impact on the data generated from this study.

9.4. Reserve Samples

For each batch (lot) of test and control item, a reserve sample of one vial was retained under the appropriate storage conditions by the Test Facility.

9.5. Test and Control Item Inventory and Disposition

Records of the receipt, distribution, and storage of test and control items were maintained. With the exception of reserve samples, it is currently the intention to return all unused test and control items to the Sponsor after finalisation of the study report:

Andrew J Pollard, FRCPCH, PhD
Professor of Paediatric Infection & Immunity
Oxford Vaccine Group
Department of Paediatrics
University of Oxford
Room 02-46-07
Level 2, Children’s Hospital
Oxford, OX3 9DU
UK

9.6. Dose Formulation and Analysis

9.6.1. Dispensing of Control Item

The control item, MOX Control, was provided in single dose vials for administration to Group 1 control animals. No aliquoting of the control item was required. The vials were stored in a refrigerator set to maintain 4°C until use and on each day of injection an appropriate quantity was despatched to the animal unit for dosing. Details of the dispensing of the control item have been retained in the study records.
9.6.2. Dispensing of Test Item

The test item, MenPF-1, was also provided in single dose vials and no aliquoting of the test item was required. The vials were stored in a refrigerator set to maintain 4°C until use and on each day of injection an appropriate quantity was despatched to the animal unit for dosing. Details of the dispense of the test item have been retained in the study records.

9.6.3. Sample Collection and Analysis

The test and control items were used as received from the Sponsor, therefore, dose formulation analysis was not conducted at the Test Facility.

9.7. Test System

9.7.1. Species and Receipt

Eighteen male and 18 female New Zealand White rabbits were received from Harlan UK Ltd, Bicester, Oxon, UK on 02 August 2011.

On despatch, the animals were approximately 11-12 weeks old and weighed approximately 2.5 kg. At the start of treatment all animals were approximately 13-14 weeks old and weighed in the range of 2.6-2.8 kg for males while females weighed in the range of 2.7-3.1 kg (see protocol deviations in Appendix 1).

9.7.2. Justification for Test System and Number of Animals

The intramuscular route of administration was selected for this study as this route has been defined by the Sponsor as the route of clinical application/human exposure. The rabbit was selected by the Study Director in consultation with the Sponsor as the test model:

i. to satisfy regulatory requirements for toxicity testing

ii. because of the availability of background data in this species and proven suitability in toxicology studies

iii. because at this time, studies in laboratory animals provide the best available basis for extrapolation to humans and acceptable models which do not use live animals currently do not exist.

iv. because immunogenicity can be investigated in this species.

The numbers of animals chosen for this study was the smallest number considered necessary to provide sufficient data.

9.7.3. Animal Identification

Each animal received a unique ear tag which identified it individually within the study and which corresponded to that animal’s on-study number.

9.7.4. Environmental Acclimation

The animals were allowed to acclimate to the Charles River, Edinburgh rabbit toxicology accommodation for a period of 15 days before the first administration (see protocol deviations in Appendix 1).
9.7.5. Selection, Assignment, and Replacement of Animals

Animals were removed in a random order from their transport boxes and allocated to dose groups on arrival by placing them in separate cages. Cages were housed on racks according to treatment and labelled with the study, animal and group number. Control animals were housed on a separate rack.

A cage plan is presented in Figure 1.

During the week before commencement of dosing, the animals were approved for entry into the experiment on the basis of satisfactory clinical observation records and body weight profiles. There was no replacement of animals.

9.7.6. Disposition

All animals remained on-study until completion of in-life phases at which point designated animals were humanely euthanised by an intravenous overdose of a barbiturate. Details are retained in the study records.

9.7.7. Husbandry

9.7.7.1. Housing

Animals were housed individually in stainless steel cages (approximate dimensions 77 x 70 x 48 cm) with a ‘Noryl’ dual level interior, perforated floor, a mesh top, and a metal food hopper. Beneath each cage was a suspended tray containing absorbent paper. Paper was changed once a week.

Cages, cage racks, hoppers and bottles were changed weekly throughout the course of the study.

Animal room floors and work surfaces were washed daily with disinfectant solution. The ceiling, walls and all other surfaces within the animal room were washed weekly. Cage racks remained in the room throughout washing procedures.

9.7.7.2. Environmental Conditions

The environmental conditions are continually monitored and recorded every 15 min. Target ranges for temperature and humidity were 16-20°C and 40-85%, respectively, with a room air flow intended to give a minimum of 15 air changes per hour. From animal arrival, until study completion, the average daily ranges for temperature was 15-21°C and for humidity was 33-64% (see protocol deviations in Appendix 1).

Lighting was controlled to provide a 12 h light/dark cycle, normally being 0700-1900 hours.

9.7.7.3. Food

Harlan Irradiated Certified Global Rabbit Diet, supplied by Harlan, UK, was available ad libitum throughout the study. Each animal was also offered a supplement of hay at least 3 times per week.

Each batch of diet is routinely analysed by the supplier for various nutritional components and chemical and microbiological contaminants.

The results of the diet analysis did not provide evidence of contamination, and so did not prejudice the outcome of the study. Certificates of analysis for each batch used are retained at the Test Facility. The hay is not analysed.
9.7.7.4. Water

Water taken from the public supply (Scottish Water, Edinburgh, Midlothian, UK) was available ad libitum throughout the study.

The quality of water supply is stipulated by Water Quality (Scotland) Regulations 2001 and certificates of analysis for dissolved materials, heavy metals, pesticide residues, pH, nitrates, nitrites and selected bacteria are periodically provided. These analyses are based on water samples taken from these laboratories.

Results of water analysis did not provide evidence of contamination, and so did not prejudice the outcome of the study. Certificates of analysis relevant to the study are retained at the Test Facility.

9.7.7.5. Animal Enrichment

For environmental enrichment wooden chewsticks, produced by Datesand, Manchester, UK were placed in each cage and treats ‘Bunny blocks’ as supplied by William Lillico & Son Ltd, UK were also provided.

Analyses of these were considered to indicate that there were no additional substances in sufficient concentration to have any influence on the outcome of the study. Certificates of analysis for these items are retained at Charles River, Edinburgh.

9.8. Veterinary Care

All animals were under the care of Charles River’s clinical veterinary surgeons, who were available at all times to provide advice and assistance.

On veterinary advice, a lesion to the right hind limb of Animal 27 (Group 3, Recovery Male), which resulted from clipping the injection site, was bathed twice a day for 4 consecutive days from Days 22-25 with aqueous chlorhexidine (an anti-septic). No further advice was required.

9.9. Experimental Design

Animals were treated on Days 1, 22, 43 and 64 with necropsy on Days 66 and 92. The Study design was as follows:

| Group No. | Main Study | Recovery | Test Item | Dosage (µg/dose) | Conc. (µg/mL) | Dose Volume (mL/dose) |
|-----------|------------|----------|-----------|-----------------|---------------|-----------------------|
| 1         | 1-3        | 10-12    | 19-21     | 28-30           | MOX Control   | 0                     | 0.5 mL                |
| 2         | 4-6        | 13-15    | 22-24     | 31-33           | MenPF-1       | 25                    | 50                    | 0.5 mL                |
| 3         | 7-9        | 16-18    | 25-27     | 34-36           | MenPF-1       | 50                    | 50                    | 2 x 0.5 mL            |

9.9.1. Administration of Test and Control Items

The test and control items were administered to the appropriate rabbits by intramuscular injection on Days 1, 22, 43 and 64.

The injection sites (left hind limb – Injection site 1) were clipped free from hair. The aliquots of test and control item were removed from the refrigerator and allowed to warm to room temperature for at least 30 minutes before dosing. To ensure homogeneity, the vials were inverted before dosing and the dose volume required to meet the dosage was administered;
0.5 mL for controls and animals receiving 25 µg/dose or 2 x 0.5 mL µg/dose. The injection sites were delineated.

Animal 27 (Group 3; 50 µg/dose) also received injection in the right hind limb (Injection Site 2) on Day 22. This was due to a small injury caused by clipping of the injection site.

The injection sites were clipped free from hair and delineated before necropsy.

9.9.2. Justification of Route and Dosage Levels

The intramuscular route of administration was selected for this study as this route has been defined by the Sponsor as the route of clinical application/human exposure.

The dose levels were agreed with the Sponsor and took into account the maximum tolerated dose in the test model and other factors such as anticipated therapeutic dose. The test item was produced with similar methodology as for the vaccine product MenBvac (Norwegian Institute of Public Health), based on deoxycholate extracted outer membrane vesicles from Neisseria meningitidis. MenBvac is known to be moderately reactogenic but safe in humans (Nøkleby et al. Vaccine 2007:25:3080-3084).

Clinical injections are planned every 6 to 8 weeks, with three doses intended. In this study, injections were given to rabbits over a shorter period and one more injection (n + 1) was also given. The intended clinical dose may include a dosage of up to 50 µg/dose. This amount was tested in this preclinical study and based on body weight ratio of rabbit 3 kg: human 60 kg and the administration of an additional injection, this was considered to provide adequate safety data.

9.10. Definition of Day

The first day of treatment (Day 1) ran from midnight before the first administration until 24 h later, subsequent day numbers (Day 2 etc) also followed this pattern. Body weights and food consumption recorded immediately before dosing on the day of treatment started (Day 1 of the study) were classified as Day 0, relating to the number of days of treatment completed. Subsequent recordings also followed this pattern (that is, body weights, food consumption and clinical signs recorded at the end of the 1st day of treatment, Day 2 of the study, were documented as Day 1). Any body weights or food consumption recorded before Day 0 were classified as Day -1, etc. Recording of laboratory investigation bleeds and terminal kills were carried out according to study days, that is, Day 1 being the day treatment started.

9.11. In-life Procedures, Observations, and Measurements

The in-life procedures, observations, and measurements listed below were performed for all animals.

9.11.1. Mortality/Moribundity Checks

Animals were checked early morning and as late as possible each day for viability.

9.11.2. Clinical Observations

9.11.2.1. Detailed Clinical Observations

Once each week, starting during the pretrial period, animals received a detailed clinical examination including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.
9.11.2.2. Postdose Observations

Animals were examined regularly throughout the day on each dosing day, and once on each non-dosing day. Particular attention was paid to the animals during and for the first hour after dosing. The onset, intensity and duration of any signs were recorded.

9.11.3. Dermal Scoring

Dermal scoring was conducted 0 h (immediately before dosing), 24 h and 48 h after each injection. On 2 separate occasions, scoring was recorded up to 120 h following injections due to erythema observed. Skin was assessed for erythema and eschar formation, oedema formation, skin thickening, desquamation and any other reaction to treatment. The scoring system below was used for assessing erythema, eschar and oedema formation.

| Erythema and Eschar Formation | Grade |
|------------------------------|-------|
| No erythema                  | 0     |
| Very slight erythema (barely perceptible) | 1     |
| Well defined erythema         | 2     |
| Moderate to severe erythema   | 3     |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4     |

| Oedema Formation | Grade |
|------------------|-------|
| No oedema        | 0     |
| Very slight oedema (barely perceptible) | 1     |
| Slight oedema (edges of area well defined by definite raising) | 2     |
| Moderate oedema (edges raised approximately 1 mm) | 3     |
| Severe oedema (raised by more than 1 mm and extending beyond the area of exposure) | 4     |

9.11.4. Body Weights

Body weights were recorded once during the pretrial period then twice weekly during the dosing and recovery periods.

9.11.5. Food Consumption

The quantity of food consumed by each animal was measured and recorded twice weekly from the beginning of the pretrial period until the end of the study.

9.11.6. Water Consumption

Water consumption was not monitored.

9.11.7. Ophthalmic Examinations

Ophthalmic examinations were carried out on all animals during pretrial and after the completion of dosing. Examinations were conducted by a veterinary surgeon.

The eyes were examined using an indirect ophthalmoscope after the application of a mydriatic agent (1% Tropicamide, Mydriacyl®). The anterior, lenticular and fundic areas were examined.
9.11.8. Body Temperature

The body temperature of each animal was measured by digital thermometer inserted into the ear and recorded once during the pretrial period, then 0 h (immediately before dosing), 1 h, 3 h, 24 h and 48 h after each injection.

9.12. Laboratory Evaluations

9.12.1. Clinical Pathology

9.12.1.1. Sample Collection

Blood was collected from an auricular artery. Animals were not fasted prior to sampling. After collection, samples were transferred to the clinical pathology laboratory at Charles River, Edinburgh for processing.

Samples were collected from all animals according to Text Table 3.

| Group Nos. | Time Point | Haematology | Coagulation | Clinical Chemistry |
|------------|------------|-------------|-------------|--------------------|
| 1-3        | Pretrial   | X           | X           | X                  |
| 1-3        | Day 66     | X           | X           | X                  |
| 1-3        | Day 92     | X           | X           | X                  |

X = Sample to be collected;

On occasion, repeat samples were collected (where possible) due to clotting of samples. Details are retained in the study records. Values obtained from repeat samples have been reported and used for statistical analysis.

9.12.1.2. Haematology

Blood samples (0.5 mL) were collected into tubes containing EDTA and analysed for the parameters specified in Text Table 4.

| Red blood cell count | Haemoglobin | Haematocrit | Mean cell volume | Mean cell haemoglobin concentration | Mean cell haemoglobin | Reticulocytes (percentage) | Reticulocyte count (absolute) | Red blood cell distribution width | Platelet count | Blood Smear | White blood cell count | Neutrophils | Lymphocytes | Monocytes | Eosinophils | Basophils | Large unstained cells | Other cells (as appropriate) |
|----------------------|-------------|-------------|------------------|------------------------------------|-----------------------|---------------------------|-------------------------------|-------------------------------|----------------|------------|------------------------|-------------|-------------|-----------|-------------|---------|------------------------|------------------------|
|                      |             |             |                  |                                    |                       |                           |                               |                               |                |            |                       |             |             |           |             |         |                       |            |

A blood smear was prepared from each haematology specimen. Blood smears were labelled, stored and archived. Blood smears were not evaluated as there were no abnormal haematological findings and it was considered that examination would not yield any further information.
9.12.1.3. Coagulation

Blood samples (0.9 mL) were collected into tubes containing 3.8% (w/v) trisodium citrate, processed for plasma, and the plasma analysed for the parameters listed in Text Table 5.

| Activated partial thromboplastin time | Prothrombin time |
|--------------------------------------|------------------|
| Fibrinogen                           |                  |

9.12.1.4. Clinical Chemistry

Blood samples (1.5 mL) were collected into tubes containing lithium heparin, processed for serum, and the serum analysed for the parameters specified in Text Table 6.

| Urea                  |
|-----------------------|
| Glucose               |
| Aspartate aminotransferase |
| Alanine aminotransferase |
| Alkaline phosphatase  |
| Creatine phosphokinase |
| Lactate dehydrogenase |
| Sodium                |
| Potassium             |
| Chloride              |
|-----------------------|
| Total protein         |
| Albumin               |
| Globulin              |
| Albumin/globulin ratio|
| Cholesterol           |
| Creatinine            |
| Total bilirubin       |
| Calcium               |
| Inorganic Phosphate   |

9.12.1.5. Antibody Sample Collection, Processing and Analysis

Blood samples (2 mL) were collected from an auricular artery once during prettrial, and before dosing on Days 22, 64 and 92. Blood samples were allowed to stand at room temperature for a minimum period of 30 min and processed to serum by centrifugation (at least 1500g at 2-8°C for 10 min).

The serum samples were stored in a freezer set to maintain -80°C and then shipped to the Responsible Scientist on dry ice:

Caroline Vipond, Department of Bacteriology, National Institute of Biological Standards and Control (NIBSC), Blance Lane, Potters Bar, South Mimms, Hertfordshire, EN6 3QG, UK.

The samples were to remain frozen throughout transit. Although the temperature was not recorded during transportation, there is evidence that the samples were despatched frozen and were received frozen and in good condition at NIBSC. The immunology laboratory was notified before shipment of the samples, and upon receipt, were stored at ≤-20°C (see protocol deviations in Appendix 1).

The samples were analysed for antibodies against MenPF-1 using a validated ELISA analytical method. No validation was performed for the plate reader software, however, the calibration performed by the service engineer confirmed Operational Qualification (OQ) and standards, and QC samples run with each batch of samples confirmed Performance Qualification (PQ) of the reader.

Any residual anti-therapeutic antibody samples may be retained for research purposes. The results of any subsequent analysis of these samples are not covered in this study.
9.13. Terminal Procedures

Terminal procedures are summarised in Text Table 7.

Text Table 7
Terminal Procedures

| Group No. | No. of Animals | Scheduled Euthanasia Day | Necropsy Procedures | Histology | Histopathology |
|-----------|----------------|--------------------------|--------------------|-----------|----------------|
|           | M  F           |                          |                    |           |                |
| 1         | 3 3            | 66                       | X                  | Full Tissue | Full Tissue |
| 2         | 3 3            |                          | X                  | None      | None          |
| 3         | 3 3            |                          | X                  | Full Tissue | Full Tissue |
| 1         | 3 3            | 92                       | X                  | Select Tissues | Select Tissues |
| 2         | 3 3            |                          | X                  | None      | None          |
| 3         | 3 3            |                          | X                  | Select Tissues | Select Tissues |

X = Procedure to be conducted

a See Tissue Collection and Preservation table for listing of tissues.
b Injection site and lumbar and inguinal lymph node.

9.13.1. Unscheduled Deaths

There were no unscheduled deaths during the study.

9.13.2. Scheduled Euthanasia

Main and recovery study animals were euthanised by an intravenous overdose of a barbiturate, weighed and major blood vessels severed to exsanguinate. The animals were euthanised rotating across dose groups such that similar numbers of animals from each group, including controls were necropsied at similar times throughout the day. Animals were not fasted before their scheduled necropsy.

9.13.3. Necropsy

All main and recovery study animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Scheduled necropsy examinations were conducted by a trained technician and consisted of an external and internal examination and recording of observations for all animals. A veterinary pathologist was available for consultation during normal working hours.

9.13.4. Organ Weights

The organs identified in Text Table 8 were weighed at necropsy for all animals. Paired organs were weighed and are reported together. Organs were weighed before fixation unless otherwise noted. Terminal body weights were used for organ weight analysis.
### Text Table 8

| Organs Weighed at Necropsy |
|----------------------------|
| **Brain**                  |
| **Epididymis**             |
| **Gland, adrenal**         |
| **Gland, pituitary**       |
| **Gland, prostate**        |
| **Gland, thyroid**         |
| **Heart**                  |
| **Kidney**                 |
| **Liver**                  |
| **Lung**                   |
| **Ovary**                  |
| **Spleen**                 |
| **Testis**                 |
| **Thymus**                 |
| **Uterus**                 |

* Paired organ weight.

### 9.13.5. Tissue Collection and Preservation

Representative samples of the tissues identified in Text Table 9 were collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated.

### Text Table 9

| Tissue Collection and Preservation |
|-----------------------------------|
| **Administration sites**          |
| **Animal identification**         |
| **Artery, aorta**                 |
| **Bone marrow smear**             |
| **Bone marrow, femur**            |
| **Bone marrow, sternum**          |
| **Bone, femur**                   |
| **Bone, sternum**                 |
| **Brain**                         |
| **Cervix**                        |
| **Epididymis**                    |
| **Eye**                           |
| **Gall Bladder**                  |
| **Gland, adrenal**                |
| **Gland, lacrimal**               |
| **Gland, mammary**                |
| **Gland, parathyroid**            |
| **Gland, pituitary**              |
| **Gland, prostate**               |
| **Gland, salivary**               |
| **Gland, seminal vesicle**        |
| **Gland, thyroid**                |
| **Gross lesions/masses**          |
| **Gut-associated lymphoid tissue**|
| **Heart**                         |
| **Kidney**                        |
| **Large intestine, appendix**     |
| **Large intestine, caecum**       |
| **Large intestine, colon**        |
| **Large intestine, rectum**       |
| **Large intestine, saccalus rotundus**|
| **Liver**                         |
| **Lung**                          |
| **Lymph node, mandibular**        |
| **Lymph node, mesenteric**        |
| **Lymph node, lumbar**            |
| **Lymph node, inguinal**          |
| **Muscle, skeletal**              |
| **Nerve, optic**                  |
| **Nerve, sciatic**                |
| **Oesophagus**                    |
| **Ovary**                         |
| **Oviduct**                       |
| **Pancreas**                      |
| **Skin**                          |
| **Small intestine, duodenum**     |
| **Small intestine, ileum**        |
| **Small intestine, jejunum**      |
| **Spinal cord**                   |
| **Spleen**                        |
| **Stomach**                       |
| **Testis**                        |
| **Thymus**                        |
| **Tongue**                        |
| **Trachea**                       |
| **Ureter**                        |
| **Urinary bladder**               |
| **Uterus**                        |
| **Vagina**                        |

* Preserved in Davidson’s fixative.

* Preserved in Modified Davidson’s fixative.

### 9.13.6. Histology

Tissues identified in Text Table 9 (except animal identification and bone marrow smears) were embedded in paraffin, sectioned, mounted on glass slides, and stained with haematoxylin and eosin.
Bone marrow smears were collected at necropsy. The smears were retained but not evaluated.

**9.13.7. Histopathology**

Histopathological evaluation was performed by a veterinary pathologist with training and experience in laboratory animal pathology.

**9.13.8. Peer Review**

A pathology peer review was conducted by a second pathologist at Charles River Laboratories, Preclinical Services, Tranent, Edinburgh, EH33 2NE, UK as per the appropriate SOP of the Pathology Department.

**10. COMPUTERISED SYSTEMS**

Critical computerised systems used in the study are listed below. All computerised systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data. The computer systems used by the Responsible Scientist are detailed in the phase report (Appendix 19).

| System Name     | Version No. | Description of Data Collected and/or Analysed                                      |
|-----------------|-------------|-------------------------------------------------------------------------------------|
| Dispense        | 7.0.3.7     | Test item control                                                                   |
| Provantis       | Release 14  | In-life data collection                                                              |
| Nautilus 2003   | Release 2   | Clinical Pathology Laboratory Information Management System (LIMS)                   |
| PLACES 2000     | 1.1         | Histopathology/Organ Weights                                                        |

**11. STATISTICAL ANALYSIS**

All statistical tests were two-sided and performed at the 5% significance level using in-house software. Males and females were analysed separately.

Pairwise comparisons were only performed against the control group (Group 1). The following pairwise comparisons were performed:

- Control Group vs Group 2
- Control Group vs Group 3

Body weight, food consumption, haematology, coagulation and clinical chemistry were analysed for homogeneity of variance using the ‘F-Max’ test. If the group variances appeared homogenous, a parametric ANOVA was used and pairwise comparisons were made using Fisher’s F protected LSD method via Student’s t test, i.e. pairwise comparisons were made only if the overall F-test was significant. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a Kruskal-Wallis non-parametric ANOVA was used and pairwise comparisons were made using chi squared protection (via z tests, the non-parametric equivalent of Student’s t test).

In circumstances where it was not possible to perform the F-Max test due to zero standard deviation in at least one group, the non-parametric ANOVA results were reported.
Organ weights were analysed using ANOVA as above and by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. In addition, organ weights as a percentage of terminal body weight were analysed using ANOVA.

In circumstances where the variances in the ANCOVA remained heterogeneous following log or square root transformations, the data was subjected to rank transformation prior to analysis. Where it was not possible to perform the F-Max test due to the small sample size (less than 3 animals in any group), the untransformed parametric ANCOVA results are reported.

In the ANOVA and ANCOVA summary tables, the results of the analysis are reported indicating the level of statistical significance (p<0.05, p<0.01 and p<0.001) of each pairwise comparison.

Actual p-values are not reported in the summary tables for these analyses.

12. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, documentation, samples, specimens and final reports from this study are the property of the Sponsor. These materials will be available at the Test Facility during the progress of the study. When the Final Report is issued, all study-specific raw data, documentation, protocol, samples, specimens and final reports will be archived by the Test Facility for a period of 2 years. After this period, the Sponsor will be contacted to determine the disposition of these materials.

Electronic data generated by the Test Facility will be archived and the software and hardware required to produce it in a readable form will be maintained and available.

All records, and reports generated from phases or segments performed by the Test Site will be returned to the Test Facility for archiving. Residue samples, specimens will be retained at the Test Site for research purposes.
13. RESULTS

13.1. Mortality
There were no unscheduled deaths during the observation period.

13.2. Clinical Observations
(Appendices 3 and 4)
There was no signs indicative of systemic toxicity noted during the observation period.
There were local signs recorded at injection sites of 3 animals. Animal 32F (25µg/dose; Group 2) on Days 23-28 had a scab recorded at the injection site and discoloured skin from Days 29-49. Animal 6M (25 µg/dose; Group 2) had a lesion on the injection site on Day 50 and discoloured skin on Day 57 and 64. Animal 27M (50µg/dose; Group 3) had a lesion/scab at the injection site on left hind limb from Days 22-29 and discoloured skin on Day 36.
These local signs were considered minor, transient, had no relationship with dosage and for one animal (Animal 27M) were related to the small injury caused at clipping of injection sites. Overall it was difficult to relate these observations to MenPF-1.

13.3. Dermal Scoring
(Appendix 5)
There was no irritation noted at injection sites that were considered to be related to administration of MenPF-1.
The few instances of very slight erythema that were recorded were sporadic, transient and there was no evidence of a relationship with dosage.

13.4. Body Weight and Body Weight Changes
(Tables 1-2 and Appendices 6-7)
Bodyweight or body weight change was unaffected by treatment with MenPF-1.

13.5. Food Consumption
(Tables 3-4 and Appendices 8-9)
Food consumed was unaffected by treatment with MenPF-1.
There were occasions where a statistically significant difference in food consumption was recorded. These differences were noted in females receiving 25 µg/dose, where food consumed was lower, when compared with controls (p>0.05). This lower food consumed was recorded on day 45 of the treatment period and Days 73, 87 and 91 of the recovery period. Inspection of the individual animal data indicated that there was individual variation within the data and that 2 animals (Animals 23F and 24F) consumed less food than others within the group. These differences were isolated, did not result in any difference in body weight and there was no evidence of a relationship with dosage. These differences were considered not to be related to administration with MenPF-1.

13.6. Ophthalmic Examinations
(Appendix 10)
There were no changes in the eye that were related to administration with MenPF-1.
13.7. Body Temperature
(Appendix 11)
Body temperature was unaffected by treatment with MenPF-1.

13.8. Haematology
(Tables 5-7 and Appendices 13-15)
There was an effect on the number of white blood cells in males on Day 66, 48 hours after dosing, that was considered to be related to treatment with MenPF-1.

On Day 66, the number of circulating neutrophils was approximately 2x higher in males receiving 50 µg/dose, when compared with controls (p<0.01). The group mean for the controls was $1.29 \times 10^9$/L with an individual range of 0.88-2.34 $\times 10^9$/L and for the males receiving 50 µg/dose the group mean was $2.43 \times 10^9$/L with an individual range of 1.58-3.41 $\times 10^9$/L. All of the individual values for the males receiving the vaccine were higher than the group mean of the controls and values were also higher than those recorded pretrial. The number of monocytes was higher in males receiving 25 or 50 µg/dose, when compared with controls (p<0.01 or p<0.05, respectively).

On Day 66, the mean cell haemoglobin concentration was higher in females receiving 25µg/dose, when compared with controls (p<0.05). There was no effect on any other red blood cell index and this minor difference was considered not to be related to treatment with MenPF-1.

At Day 92, haematology was considered to be unaffected by treatment with MenPF-1.

13.9. Coagulation
(Table 5-7 and Appendices 13-15)
There was an effect on fibrinogen in males and females receiving MenPF-1.

On Day 66, fibrinogen was higher in males and females receiving MenPF-1, when compared with controls (p<0.001). The group mean values (mg/dL) are summarised.

|                | Males          | Females         |
|----------------|----------------|-----------------|
| Treatment Pretrial Day 66 | Treatment Pretrial Day 66 |
| Control        | 226 199        | Control         | 168 129        |
| 25 µg/dose     | 225 279        | 25 µg/dose      | 171 215        |
| 50 µg/dose     | 230 335        | 50 µg/dose      | 179 222        |

There was also a shorter activated partial thromboplastin time (~8%), which achieved statistical significance, noted in males receiving 50 µg/dose, when compared with controls (p<0.05). One of the control values (Animal 21) was longer than the others in the group and this may be in some part due to this value being from a repeat blood collection. If this value is excluded, inspection of the individual data indicated that although there was variation within the data, broadly between the groups the values were similar. Four of the 6 values recorded for males receiving the vaccine are within the control range. There was no evidence of a relationship with dosage with males receiving 25 µg/dose having a longer activated partial thromboplastin time recorded. This small difference was considered not to be related to treatment with MenPF-1. The shorter prothrombin times recorded in males receiving 25µg/dose and females receiving 50 µg/dose, when compared with controls, was considered to be unrelated to treatment with the vaccine given the small magnitude of change and lack of a relationship with dosage.
On Day 92, coagulation was unaffected by treatment with MenPF-1.

13.10. Clinical Chemistry
(Tables 8-10 and Appendices 16-18)
There was an effect on plasma proteins in males and females receiving MenPF-1.
On Day 66, globulin (p<0.001) and total protein (p<0.01) was higher in males receiving 25 µg/dose and males and females receiving 50 µg/dose, when compared with controls. There was a lower albumin:globulin ratio in these groups. The protein levels were also higher than those recorded pretrial.
There were other statistically significant differences recorded, for example, lower potassium in males receiving 50 µg/dose, when compared with controls, however these differences were considered to be unrelated to treatment with the vaccine given the small magnitude of change and lack of a relationship with dosage.
On Day 92, there were no plasma chemistry differences that were considered to be related to treatment with MenPF-1. There were statistically significant differences recorded, for example, higher aspartate aminotransferase activity in females receiving 25 µg/dose when compared with controls, however these differences were considered to be unrelated to treatment with the vaccine given the small magnitude of change, similar values recorded pretrial and lack of a relationship with dosage.

13.11. Antibody Analysis
(Appendix 19)
The data provided by the Sponsor-designated Responsible Scientist indicated the presence of specific IgG to dosages of 25 or 50 µg MenPF-1/dose. An increase in titre was generally observed with increasing dose and time in both males and females. Although the group mean for each of the groups receiving MenPF-1 was lower on Day 92, inspection of the individual data indicated that 7/12 animals had a similar or higher titre then those recorded on Day 64.

13.12. Gross Pathology
(Tables 11-12; Appendices 20-21 and 26)
13.12.1. Scheduled Euthanasia (Day 66)
There were enlarged lumbar lymph nodes (left) recorded in 2/3 males and 1/3 females that received 50 µg/dose.
Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1.
13.12.2. Scheduled Euthanasia (Day 92)
Test article-related gross findings noted at the terminal euthanasia were not observed at the end of the recovery period. Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1.
13.13. **Organ Weights**  
(Tables 13-18 and Appendices 22-25)  
Organ weights were considered to be unaffected by treatment with MenPF-1.  
On Day 66, absolute adrenal gland weights were statistically higher in females that received 25 or 50 µg/dose, when compared with controls (p<0.05). No dose-response was evident. There was no difference noted after adjustment for terminal body weight and as analysis as a percentage of terminal body weight (relative). This difference was considered not to be related to treatment with MenPF-1.  
On Day 92, there were statistically significant differences in males and females that received 25 or 50 µg/dose, when compared with controls; lower absolute and relative liver weight in females (25 µg/dose), lower covariant thymus weight in females (50 µg/dose) and a higher liver weight in females (50 µg/dose). There was non histological correlate and no relationship with dosage, consequently these differences were considered not to be related to treatment with MenPF-1.

13.14. **Histopathology**  
(Tables 19-20; Appendices 20-21 and 26)  

13.14.1. **Scheduled Euthanasia (Day 66)**  
There was accumulation of macrophages, observed both in the injection sites and lumbar lymph nodes, which was characterised by aggregates of macrophages containing an abundant, pale basophilic, amorphous cytoplasmic material considered to be aluminium hydroxide. These macrophages were admixed with variable numbers of multinucleated giant cells.  
Lymphoid hyperplasia was also recorded which correlated with the enlarged lumbar lymph nodes observed at necropsy.  
Other microscopic findings at this dose level observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1.  
A number of changes were observed in the clinical chemistry, haematology and coagulation group mean values, when compared to their respective controls: there were increased total proteins and globulins, and decreased albumin/globulin ratio in all treated males and in females given 50 µg/dose; increased neutrophil counts in males given 50 µg/dose; increased monocyte counts in all treated male groups; and increased fibrinogen in treated groups from both sexes. These differences correlated with the inflammatory reaction observed in the injection sites.

13.14.2. **Scheduled Euthanasia (Day 92)**  
Some of the microscopic findings noted at the terminal euthanasia (Day 66) were observed at the end of the period off dose (Day 92), however were of a lesser severity and frequency. No treatment related findings were noted in Injection Site 2 (Animal 27).  
Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1.
14. DISCUSSION

Intramuscular administration of up to 50 µg/dose of MenPF-1 was associated with an expected, minor physiological response and findings of macrophages, giant cells and polymorphonuclear inflammation at the injection sites and lymph node enlargement at the draining lymph node. These findings were noted 4 weeks after the last injection, however, they were of a lesser severity indicating recovery.

The presence of antibodies to MenPF-1 indicated immunogenicity in rabbits, and confirmed that this species was a suitable selection for this study.

The local inflammatory response noted histologically with the findings of myofibre necrosis and/or regeneration, interstitial fibrosis and/or mineralisation at the injection sites and lymphoid hyperplasia at the injection site draining lymph node correlated systemically with higher fibrinogen and higher levels of the acute phase protein globulin noted after four injections. These minor differences in protein levels are considered to be a physiological response and of little toxicological significance.

The accumulation of the macrophages noted at the injection sites and lymph nodes was considered to be related to the aluminium hydroxide. This response is not unusual where an aluminium based adjuvant has been administered.
15. CONCLUSION

In conclusion, administration of the vaccine, MenPF-1, when given by intramuscular injection for 4 occasions over a 9 week period, was well tolerated in rabbits up to 50 µg/dose. There was only an expected, minor inflammatory response which was associated with vaccine administration, characterised by macrophages, giant cells and polymorphonuclear and mononuclear inflammation at the injection sites with on-going recovery noted. There was no evidence of systemic toxicity.
16. REFERENCES

Nøkleby et al (2007). Safety review: Two outer membrane vesicle (OMV) vaccines against systemic Neisseria meningitidis serogroup B disease. Vaccine 25 (2007) 3080-3084
Figures
Treatment Period

Animal Rack 1

|   | Group No. | Animal No. | Cage No. |
|---|-----------|------------|----------|
| 1 |           | 1          | 1        |
| 1 |           | 2          | 2        |
| 1 |           | 3          | 3        |
| 1 |           | 4          | 4        |

Animal Rack 2

|   | Group No. | Animal No. | Cage No. |
|---|-----------|------------|----------|
| 1 |           | 10         | 10       |
| 1 |           | 11         | 11       |
| 1 |           | 12         | 12       |
| 1 |           | 13         | 13       |

Animal Rack 3

|   | Group No. | Animal No. | Cage No. |
|---|-----------|------------|----------|
| 2 |           | 2          | 2        |
| 2 |           | 3          | 3        |
| 2 |           | 4          | 4        |
| 2 |           | 5          | 5        |

Animal Rack 4

|   | Group No. | Animal No. | Cage No. |
|---|-----------|------------|----------|
| 1 |           | 13         | 13       |
| 1 |           | 14         | 14       |
| 1 |           | 15         | 15       |
| 1 |           | 16         | 16       |

Animal Rack 5

|   | Group No. | Animal No. | Cage No. |
|---|-----------|------------|----------|
| 3 |           | 7          | 7        |
| 3 |           | 8          | 8        |
| 3 |           | 9          | 9        |
| 3 |           | 10         | 10       |

Animal Rack 6

|   | Group No. | Animal No. | Cage No. |
|---|-----------|------------|----------|
| 3 |           | 16         | 16       |
| 3 |           | 17         | 17       |
| 3 |           | 18         | 18       |
| 3 |           | 19         | 19       |

Figure 1  Cage Plan
### Figure 1  Cage Plan (continued)

#### Recovery Period

| Animal Rack 1 |  | Animal Rack 2 |  | Animal Rack 3 |  |
|---------------|---|---------------|---|---------------|---|
| 1             | 19| 2             | 22| 1             | 28|
| 2             | 19| 22            | 23| 2             | 29|
| 3             | 21| 24            | 25| 3             | 30|
| 1             | 21| 24            | 25| 2             | 30|
| 3             | 26| 26            | 27| 3             | 30|

| Animal Rack 4 |  | Animal Rack 5 |  | Animal Rack 6 |  |
|---------------|---|---------------|---|---------------|---|
| 2             | 31| 2             | 32| 1             | 36|
| 2             | 31| 32            | 34| 2             | 36|
| 3             | 33| 33            | 34| 3             | 36|

| Animal Rack 6 |  | Animal Rack 6 |  |
|---------------|---|---------------|---|
| 1             | 31| 32            | 34|
| 2             | 33| 34            | 36|
| 3             | 35| 36            | 36|
Figure 2
Body Weights (kg): Group Mean Values: Males
Figure 2  (continued)
Body Weights (kg): Group Mean Values: Females
Tables
### Table 1

**Body Weights with Change (kg): Group Mean Values: Treatment Period**

| Group / sex | Test Item | Dosage (µg/dose) | Day | Mean | SD | n |
|-------------|-----------|------------------|-----|------|----|---|
| 1M          | Control   | 0                | -7  | 2.6  | 0.1 | 6 |
|             | MenPF-1   | 25               | 0   | 2.8  | 0.1 | 6 |
|             | MenPF-1   | 50               | 3   | 2.8  | 0.0 | 6 |
|             |           |                  | 7   | 2.8  | 0.1 | 6 |
|             |           |                  | 10  | 2.9  | 0.1 | 6 |
|             |           |                  | 14  | 2.9  | 0.1 | 6 |
|             |           |                  | 17  | 3.0  | 0.1 | 6 |
|             |           |                  | 21  | 3.0  | 0.1 | 6 |
|             |           |                  | 24  | 3.1  | 0.1 | 6 |
|             |           |                  | 28  | 3.1  | 0.1 | 6 |
|             |           |                  | 31  | 3.1  | 0.1 | 6 |
|             |           |                  | 35  | 3.1  | 0.1 | 6 |
|             |           |                  | 38  | 3.1  | 0.1 | 6 |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 1  (continued)

**Body Weights with Change (kg): Group Mean Values: Treatment Period**

| Group / sex | Day 42 | Day 45 | Day 49 | Day 52 | Day 56 | Day 59 | Day 63 | Change 0 - 63 |
|-------------|--------|--------|--------|--------|--------|--------|--------|--------------|
| **1M**      | Mean   | 3.1    | 3.1    | 3.2    | 3.3    | 3.3    | 3.3    | 0.5         |
|             | SD     | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    | 0.1         |
|             | n      | 6      | 6      | 6      | 6      | 6      | 6      | 6           |
| **2M**      | Mean   | 3.3    | 3.3    | 3.3    | 3.3    | 3.4    | 3.4    | 0.6         |
|             | SD     | 0.1    | 0.1    | 0.2    | 0.2    | 0.1    | 0.1    | 0.1         |
|             | n      | 6      | 6      | 6      | 6      | 6      | 6      | 6           |
| **3M**      | Mean   | 3.2    | 3.3    | 3.3    | 3.3    | 3.3    | 3.3    | 0.6         |
|             | SD     | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2         |
|             | n      | 6      | 6      | 6      | 6      | 6      | 6      | 6           |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
## Table 1  
### (continued)  
**Body Weights with Change (kg): Group Mean Values: Treatment Period**

| Test Item | Dosage (µg/dose) | Group / sex | Day |
|-----------|------------------|-------------|-----|
|           |                  | 1 Control   | 2 MenPF-1 | 3 MenPF-1 |
|           |  0               |  25         |  50       |
|           | -7               |  0          |  3        |
|           | 0                |  10         |  14       |
|           | 7                |  21         |  24       |
|           | 10               |  28         |  31       |
|           | 14               |  35         |  38       |

| Group / sex | Mean  | SD    | n   |
|-------------|-------|-------|-----|
| 1F          | 2.7   | 0.1   | 6   |
|             | 2.9   | 0.1   | 6   |
|             | 3.0   | 0.2   | 6   |
|             | 3.1   | 0.2   | 6   |
|             | 3.2   | 0.2   | 6   |
|             | 3.3   | 0.2   | 6   |
|             | 3.4   | 0.2   | 6   |
|             | 3.5   | 0.2   | 6   |
|             | 3.6   | 0.2   | 6   |
|             | 3.7   | 0.2   | 6   |

| 2F          | 2.6   | 0.1   | 6   |
|             | 2.8   | 0.1   | 6   |
|             | 2.9   | 0.2   | 6   |
|             | 3.0   | 0.2   | 6   |
|             | 3.1   | 0.2   | 6   |
|             | 3.2   | 0.2   | 6   |
|             | 3.3   | 0.2   | 6   |
|             | 3.4   | 0.2   | 6   |
|             | 3.5   | 0.2   | 6   |
|             | 3.6   | 0.2   | 6   |
|             | 3.7   | 0.2   | 6   |

| 3F          | 2.7   | 0.2   | 6   |
|             | 2.9   | 0.2   | 6   |
|             | 3.0   | 0.2   | 6   |
|             | 3.1   | 0.2   | 6   |
|             | 3.2   | 0.2   | 6   |
|             | 3.3   | 0.2   | 6   |
|             | 3.4   | 0.2   | 6   |
|             | 3.5   | 0.2   | 6   |
|             | 3.6   | 0.2   | 6   |
|             | 3.7   | 0.2   | 6   |

| Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001 |
|---------------------------------------------------------------|
### Table 1 (continued)

**Body Weights with Change (kg): Group Mean Values: Treatment Period**

| Group / sex | 42 | 45 | 49 | 52 | 56 | 59 | 63 | Change 0 - 63 |
|-------------|----|----|----|----|----|----|----|----------------|
| 1F          |    |    |    |    |    |    |    |                |
| Mean        | 3.8| 3.8| 3.9| 3.9| 4.0| 4.1| 4.1| 1.2            |
| SD          | 0.3| 0.3| 0.3| 0.3| 0.3| 0.3| 0.3| 0.2            |
| n           | 6  | 6  | 6  | 6  | 6  | 6  | 6  |                |
| 2F          |    |    |    |    |    |    |    |                |
| Mean        | 3.6| 3.6| 3.6| 3.6| 3.7| 3.8| 3.8| 1.0            |
| SD          | 0.3| 0.2| 0.3| 0.3| 0.3| 0.3| 0.3| 0.3            |
| n           | 6  | 6  | 6  | 6  | 6  | 6  | 6  |                |
| 3F          |    |    |    |    |    |    |    |                |
| Mean        | 3.8| 3.8| 3.9| 3.9| 4.0| 4.0| 4.0| 1.1            |
| SD          | 0.3| 0.3| 0.4| 0.4| 0.4| 0.4| 0.3|                |
| n           | 6  | 6  | 6  | 6  | 6  | 6  | 6  |                |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
| Group / sex | Day | Change |
|------------|-----|--------|
| 1M Mean    |     | 3.4    |
|            | SD  | 0.2    |
|            | n   | 3      |
| 2M Mean    |     | 3.4    |
|            | SD  | 0.2    |
|            | n   | 3      |
| 3M Mean    |     | 3.2    |
|            | SD  | 0.2    |
|            | n   | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 2  (continued)

**Body Weights with Change (kg): Group Mean Values: Recovery Period**

| Group / sex | Day 66 | Day 70 | Day 73 | Day 77 | Day 80 | Day 84 | Day 87 | Day 91 | Change 66 - 91 |
|-------------|--------|--------|--------|--------|--------|--------|--------|--------|---------------|
| 1F          | Mean   | 4.2    | 4.3    | 4.3    | 4.4    | 4.4    | 4.5    | 4.5    | 4.5           |
|             | SD     | 0.4    | 0.4    | 0.4    | 0.4    | 0.4    | 0.5    | 0.4    | 0.1           |
|             | n      | 3      | 3      | 3      | 3      | 3      | 3      | 3      | 3             |
| 2F          | Mean   | 3.7    | 3.7    | 3.7    | 3.8    | 3.8    | 3.8    | 3.8    | 3.8           |
|             | SD     | 0.2    | 0.3    | 0.3    | 0.3    | 0.3    | 0.3    | 0.3    | 0.1           |
|             | n      | 3      | 3      | 3      | 3      | 3      | 3      | 3      | 3             |
| 3F          | Mean   | 4.2    | 4.1    | 4.2    | 4.3    | 4.3    | 4.3    | 4.3    | 4.3           |
|             | SD     | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2           |
|             | n      | 3      | 3      | 3      | 3      | 3      | 3      | 3      | 3             |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 3
**Food Consumption (g/animal/day): Group Mean Values: Treatment Period**

| Group / sex | 0       | 3       | 7       | 10      | 14      | 17      | 21      | 24      | 28      | 31      | 35      | 38      | 42      |
|-------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1M Mean     | 128.9   | 123.4   | 124.8   | 119.7   | 138.5   | 127.2   | 139.8   | 124.5   | 132.1   | 124.9   | 133.3   | 127.9   | 131.8   |
| SD          | 16.9    | 14.1    | 10.6    | 17.6    | 14.4    | 16.1    | 13.2    | 18.3    | 17.1    | 20.2    | 14.9    | 21.2    | 17.1    |
| n           | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       |
| 2M Mean     | 140.8   | 132.5   | 140.1   | 134.4   | 149.1   | 131.6   | 145.6   | 130.0   | 135.1   | 134.4   | 135.1   | 130.6   | 137.7   |
| SD          | 13.9    | 22.1    | 17.7    | 25.8    | 24.9    | 18.3    | 22.8    | 19.4    | 25.1    | 21.5    | 20.0    | 15.2    | 20.8    |
| n           | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       |
| 3M Mean     | 129.1   | 122.6   | 132.8   | 128.1   | 137.8   | 131.2   | 137.5   | 122.1   | 128.7   | 126.4   | 126.8   | 126.1   | 131.1   |
| SD          | 11.2    | 5.8     | 11.8    | 11.4    | 14.8    | 16.1    | 13.9    | 16.2    | 9.0     | 13.6    | 10.0    | 16.4    | 20.0    |
| n           | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       | 6       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 3 (continued)

**Food Consumption (g/animal/day): Group Mean Values: Treatment Period**

| Group / sex | Test Item | Dosage (µg/dose) | Day | 1   | 2   | 3   |
|-------------|-----------|------------------|-----|-----|-----|-----|
| 1M          | Control   | 0                | 45  | 128.7 | 135.8 | 141.7 |
|             | MenPF-1   | 25               | 49  | 135.0 | 131.6 | 140.9 |
|             |           |                  | 52  |       |      |      |
|             | MenPF-1   | 50               | 56  |       |      |      |
|             |           |                  | 59  |       |      |      |
|             |           |                  | 63  |       |      |      |
| 2M          | Control   | 0                | 45  | 125.2 | 131.3 | 137.5 |
|             | MenPF-1   | 25               | 49  | 136.3 | 145.8 | 139.1 |
|             |           |                  | 52  |       |      |      |
|             | MenPF-1   | 50               | 56  |       |      |      |
|             |           |                  | 59  |       |      |      |
|             |           |                  | 63  |       |      |      |
| 3M          | Control   | 0                | 45  | 123.2 | 129.0 | 133.2 |
|             | MenPF-1   | 25               | 49  | 130.0 | 130.2 | 122.0 |
|             |           |                  | 52  |       |      |      |
|             | MenPF-1   | 50               | 56  |       |      |      |
|             |           |                  | 59  |       |      |      |
|             |           |                  | 63  |       |      |      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
## Table 3  (continued)

### Food Consumption (g/animal/day): Group Mean Values: Treatment Period

| Group / sex | 1   | 2   | 3   |
|-------------|-----|-----|-----|
| Test Item   | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| Day | 0 | 3 | 7 | 10 | 14 | 17 | 21 | 24 | 28 | 31 | 35 | 38 | 42 |
|-----|---|---|---|----|----|----|----|----|----|----|----|----|----|
| 1F  | 154.5 | 139.0 | 160.0 | 144.1 | 166.2 | 153.7 | 168.3 | 152.6 | 161.2 | 155.7 | 161.3 | 159.5 | 167.7 |
| SD  | 17.3 | 21.4 | 28.9 | 27.6 | 25.0 | 28.4 | 27.4 | 25.5 | 29.1 | 22.2 | 24.8 | 27.3 | 29.1 |
| n   | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 2F  | 149.1 | 136.1 | 151.7 | 144.7 | 160.9 | 144.1 | 162.9 | 140.4 | 147.9 | 147.2 | 163.4 | 148.5 | 150.7 |
| SD  | 10.0 | 12.4 | 11.3 | 15.8 | 15.4 | 15.6 | 12.8 | 17.2 | 15.0 | 25.3 | 14.1 | 21.9 | 25.3 |
| n   | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 3F  | 164.7 | 157.0 | 176.4 | 162.3 | 184.7 | 168.3 | 180.8 | 163.7 | 175.3 | 172.8 | 180.5 | 174.6 | 176.0 |
| SD  | 25.9 | 25.4 | 34.1 | 25.6 | 45.0 | 27.5 | 37.2 | 32.7 | 36.0 | 38.5 | 38.1 | 35.5 | 40.2 |
| n   | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 3  (continued)
Food Consumption (g/animal/day): Group Mean Values: Treatment Period

| Group / Test Item | Dosage (µg/dose) | Day 45 | Day 49 | Day 52 | Day 56 | Day 59 | Day 63 |
|-------------------|------------------|--------|--------|--------|--------|--------|--------|
| Control           | 0                | 166.1  | 170.3  | 167.9  | 180.1  | 176.0  | 169.4  |
|                   | SD               | 20.5   | 27.8   | 26.7   | 28.7   | 23.7   | 25.6   |
|                   | n                | 6      | 6      | 6      | 6      | 6      | 6      |

1F Mean 136.9

| Group / Test Item | Dosage (µg/dose) | Day 45 | Day 49 | Day 52 | Day 56 | Day 59 | Day 63 |
|-------------------|------------------|--------|--------|--------|--------|--------|--------|
| MenPF-1           | 25               | 134.2  | 132.5  | 150.5  | 153.5  | 152.1  |
|                   | SD               | 22.5   | 47.8   | 37.7   | 29.6   | 28.0   |
|                   | n                | 6      | 6      | 6      | 6      | 6      | 6      |

2F Mean 167.6

| Group / Test Item | Dosage (µg/dose) | Day 45 | Day 49 | Day 52 | Day 56 | Day 59 | Day 63 |
|-------------------|------------------|--------|--------|--------|--------|--------|--------|
| MenPF-1           | 50               | 169.2  | 170.7  | 164.3  | 153.3  |
|                   | SD               | 15.4   | 25.7   | 39.3   | 51.8   | 23.9   | 36.4   |
|                   | n                | 6      | 6      | 6      | 6      | 6      | 6      |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
Table 4
Food Consumption (g/animal/day): Group Mean Values: Recovery Period

| Group / sex | Test Item | Dosage (µg/dose) | 1     | 2     | 3     | Day |
|-------------|-----------|------------------|-------|-------|-------|-----|
| Test Item   | Control   | 0                | 148.6 | 133.4 | 121.8 | 66  |
|             | MenPF-1   | 25               | 131.4 | 114.2 | 139.8 | 70  |
|             | MenPF-1   | 50               | 114.2 | 139.8 | 129.7 | 73  |
|             |           |                  | 131.4 | 114.2 | 139.8 | 77  |
|             |           |                  | 131.4 | 114.2 | 139.8 | 80  |
|             |           |                  | 131.4 | 114.2 | 139.8 | 84  |
|             |           |                  | 131.4 | 114.2 | 139.8 | 87  |
|             |           |                  | 131.4 | 114.2 | 139.8 | 91  |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 4  (continued)
Food Consumption (g/animal/day): Group Mean Values: Recovery Period

| Group / sex | Group 1 | Group 2 | Group 3 |
|------------|---------|---------|---------|
| Dosage (µg/dose) | Control | MenPF-1 | MenPF-1 |
| 1F | 66 | 70 | 73 | 77 | 80 | 84 | 87 | 91 |
| Mean | 164.0 | 172.3 | 172.0 | 177.7 | 161.3 | 172.9 | 165.8 | 171.0 |
| SD | 29.4 | 34.2 | 26.3 | 25.8 | 33.3 | 24.5 | 28.0 | 20.2 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 2F | 66 | 70 | 73 | 77 | 80 | 84 | 87 | 91 |
| Mean | 117.8 | 131.0 | 111.9* | 145.4 | 112.1 | 123.4 | 110.8* | 126.4* |
| SD | 25.7 | 21.4 | 22.4 | 28.9 | 21.1 | 34.2 | 20.4 | 17.3 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 3F | 66 | 70 | 73 | 77 | 80 | 84 | 87 | 91 |
| Mean | 146.3 | 154.2 | 166.0 | 183.8 | 152.0 | 172.4 | 160.1 | 167.8 |
| SD | 37.0 | 15.9 | 8.6 | 19.1 | 8.4 | 14.6 | 16.8 | 16.9 |
| n | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
Table 5

Haematology and Coagulation: Group Mean Values: Pretrial

| Group / sex | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------------|-----------|-----------------|---|---|---|
|             | Control   |                 | 0 | 25 | 50 |
|             | MenPF-1   |                 | 25 |    |    |
|             | MenPF-1   |                 | 50 |    |    |

| Group / sex | Hb (g/dL) | RBC (x10^12/L) | Hct (L/L) | MCH (pg) | MCV (fl) | MCHC (g/dL) | RDW (%) | Reti (%) | Ret (x10^12/L) | WBC | Neut | Lymph | Mono | Eos | Baso |
|-------------|-----------|----------------|-----------|----------|---------|-------------|---------|---------|---------------|-----|------|--------|------|-----|------|
| 1M          | Mean      | 13.0           | 6.18      | 0.377    | 21.1    | 61.1        | 34.5    | 12.7    | 2.5           | 150 | 6.50 | 1.08   | 4.67 | 0.12| 0.17 | 0.46 |
|             | SD        | 0.4            | 0.33      | 0.014    | 1.0     | 2.1         | 0.7     | 0.9     | 1.4           | 75  | 1.09 | 0.28   | 1.18 | 0.07| 0.05 | 0.06 |
|             | n         | 6              | 6         | 6         | 6       | 6           | 6       | 6       | 6             | 6   | 6    | 6      | 6    | 6   | 6    |
| 2M          | Mean      | 12.8           | 6.15      | 0.369    | 20.8    | 60.2        | 34.5    | 12.5    | 1.9           | 113 | 6.35 | 1.67   | 3.92 | 0.09| 0.15 | 0.51 |
|             | SD        | 0.4            | 0.35      | 0.009    | 1.0     | 2.2         | 0.7     | 0.2     | 0.3           | 14  | 1.06 | 0.04   | 0.44 | 0.05| 0.05 | 0.08 |
|             | n         | 6              | 6         | 6         | 6       | 6           | 6       | 6       | 6             | 6   | 6    | 6      | 6    | 6   | 6    |
| 3M          | Mean      | 12.8           | 6.14      | 0.371    | 20.8    | 60.5        | 34.4    | 12.6    | 2.1           | 127 | 6.63 | 1.63   | 4.17 | 0.10| 0.15 | 0.57 |
|             | SD        | 0.6            | 0.16      | 0.010    | 0.8     | 1.2         | 0.7     | 0.9     | 0.5           | 29  | 1.21 | 0.55   | 0.50 | 0.03| 0.08 | 0.19 |
|             | n         | 6              | 6         | 6         | 6       | 6           | 6       | 6       | 6             | 6   | 6    | 6      | 6    | 6   | 6    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 5  (continued)
Haematology and Coagulation : Group Mean Values: Pretrial

| Group / sex | LUC x10⁹/L | Platelet x10⁹/L | PT s | APTT s | Fib mg/dL |
|-------------|------------|-----------------|------|--------|----------|
| 1M Mean     | 0.01       | 409             | 6.3  | 60.7   | 226      |
| SD          | 0.00       | 68              | 0.2  | 3.1    | 15       |
| n           | 6          | 6               | 6    | 6      | 6        |
| 2M Mean     | 0.01       | 402             | 6.4  | 62.5   | 225      |
| SD          | 0.00       | 98              | 0.3  | 4.5    | 19       |
| n           | 5          | 6               | 6    | 6      | 6        |
| 3M Mean     | 0.02       | 444             | 6.2  | 58.9   | 230      |
| SD          | 0.01       | 38              | 0.2  | 6.0    | 20       |
| n           | 6          | 6               | 6    | 6      | 6        |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 5 (continued)
**Haematology and Coagulation: Group Mean Values: Pretrial**

| Group / sex | 1  | 2  | 3  |
|-------------|----|----|----|
| Test Item   | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| Group / sex | Hb g/dL | RBC x10^{12}/L | Hct L/L | MCH pg | MCV fl | MCHC g/dL | RDW % | Reti % | Ret x10^{12}/L | WBC x10^{9}/L | Neut | Lymph | Mono | Eos | Baso |
|-------------|---------|----------------|---------|--------|--------|-----------|-------|-------|---------------|--------------|------|-------|------|-----|------|
| 1F Mean     | 11.9    | 5.58           | 0.349   | 21.3   | 62.6   | 34.0      | 12.4  | 2.6   | 145           | 6.51         | 2.03 | 3.73  | 0.07 | 0.14 | 0.54 |
| SD          | 0.6     | 0.40           | 0.017   | 0.6    | 1.6    | 0.5       | 0.5   | 0.5   | 22            | 1.12         | 0.90 | 0.80  | 0.04 | 0.08 | 0.22 |
| n           | 6       | 6              | 6       | 6      | 6      | 6         | 6     | 6     | 6             | 6            | 6    | 6     | 6    | 6    | 6    |
| 2F Mean     | 12.4    | 5.82           | 0.361   | 21.4   | 62.0   | 34.4      | 12.7  | 3.0   | 173           | 6.87         | 1.82 | 4.20  | 0.08 | 0.15 | 0.60 |
| SD          | 0.2     | 0.19           | 0.007   | 0.8    | 2.6    | 0.3       | 0.8   | 0.5   | 31            | 1.19         | 0.66 | 0.49  | 0.02 | 0.04 | 0.12 |
| n           | 6       | 6              | 6       | 6      | 6      | 6         | 6     | 6     | 6             | 6            | 6    | 6     | 6    | 6    | 6    |
| 3F Mean     | 12.2    | 5.67           | 0.354   | 21.6   | 62.6   | 34.5      | 12.5  | 2.5   | 141           | 6.74         | 1.87 | 4.03  | 0.12 | 0.17 | 0.55 |
| SD          | 0.8     | 0.38           | 0.021   | 0.8    | 2.2    | 0.8       | 0.5   | 0.4   | 21            | 0.82         | 0.54 | 0.95  | 0.08 | 0.04 | 0.13 |
| n           | 6       | 6              | 6       | 6      | 6      | 6         | 6     | 6     | 6             | 6            | 6    | 6     | 6    | 6    | 6    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
| Group / sex | LUC x10^9/L | Plat x10^9/L | PT s | APTT s | Fib mg/dL |
|------------|-------------|--------------|------|--------|----------|
| 1F Mean    | 0.01        | 431          | 6.2  | 55.5   | 168      |
| SD         | 0.00        | 73           | 0.3  | 2.3    | 16       |
| n          | 4           | 6            | 6    | 6      | 6        |
| 2F Mean    | 0.02        | 463          | 6.4  | 55.5   | 171      |
| SD         | 0.01        | 73           | 0.1  | 3.6    | 18       |
| n          | 6           | 6            | 5    | 5      | 5        |
| 3F Mean    | 0.02        | 454          | 6.2  | 51.4   | 179      |
| SD         | 0.01        | 69           | 0.2  | 7.2    | 15       |
| n          | 6           | 6            | 6    | 6      | 6        |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
| Group / sex | Hb g/dL | RBC x10¹²/L | Hct L/L | MCH pg | MCV fl | MCHC g/dL | RDW % | Reti % | Ret x10⁹/L | WBC | Neut | Lymph | Mono | Eos | Baso |
|------------|--------|-------------|--------|--------|-------|----------|-------|-------|------------|-----|------|-------|------|-----|------|
| 1M Mean    | 13.1   | 6.42        | 0.397  | 20.4   | 61.9  | 33.0     | 12.0  | 2.5   | 162        | 7.39| 1.29 | 5.35  | 0.05 | 0.18| 0.52 |
| SD         | 0.4    | 0.10        | 0.007  | 0.7    | 1.4   | 0.5     | 0.6   | 0.4   | 26         | 1.62| 0.53 | 1.43  | 0.02 | 0.05| 0.11 |
| n          | 6      | 6           | 6      | 6      | 6     | 6       | 6     | 6     | 6          | 6   | 6    | 6     | 6    | 6   | 6    |
| 2M Mean    | 13.3   | 6.42        | 0.398  | 20.7   | 62.2  | 33.3     | 12.2  | 2.7   | 174        | 6.64| 1.74 | 4.18  | 0.13 | 0.15| 0.44 |
| SD         | 0.6    | 0.40        | 0.015  | 0.8    | 2.1   | 0.5     | 0.5   | 0.6   | 36         | 1.50| 0.44 | 1.13  | 0.10 | 0.03| 0.14 |
| n          | 5      | 5           | 5      | 5      | 5     | 5       | 5     | 5     | 5          | 5   | 5    | 5     | 5    | 5   | 5    |
| 3M Mean    | 13.1   | 6.44        | 0.399  | 20.3   | 62.1  | 32.8     | 12.1  | 2.5   | 162        | 8.31| 2.43 | 5.03  | 0.09 | 0.19| 0.56 |
| SD         | 0.6    | 0.26        | 0.015  | 0.7    | 1.3   | 0.7     | 0.4   | 0.6   | 42         | 1.53| 0.73 | 0.84  | 0.02 | 0.08| 0.18 |
| n          | 6      | 6           | 6      | 6      | 6     | 6       | 6     | 6     | 6          | 6   | 6    | 6     | 6    | 6   | 6    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 6  (continued)
Haematology and Coagulation : Group Mean Values: Day 66

| Group / sex | LUC (x10^9/L) | Plat | PT (s) | APTT (s) | Fib (mg/dL) |
|-------------|---------------|------|--------|----------|-------------|
| 1M Mean     | 0.01          | 364  | 6.1    | 56.2     | 199         |
| SD          | 0.00          | 37   | 0.3    | 4.5      | 35          |
| n           | 6             | 6    | 6      | 6        | 6           |
| 2M Mean     | 0.01          | 414  | 5.8a   | 58.8     | 279c        |
| SD          | 0.01          | 102  | 0.0    | 2.5      | 29          |
| n           | 4             | 5    | 5      | 5        | 5           |
| 3M Mean     | 0.01          | 382  | 5.9    | 51.8b    | 335c        |
| SD          | 0.01          | 49   | 0.1    | 2.4      | 30          |
| n           | 6             | 6    | 6      | 6        | 6           |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
### Table 6  (continued)

**Haematology and Coagulation : Group Mean Values: Day 66**

| Test Item | Dosage (µg/dose) | 1  | 2  | 3  |
|-----------|------------------|----|----|----|
|           | Group            | 0  | 25 | 50 |

| Group / sex | Hb g/dL | RBC x10¹²/L | Hct L/L | MCH pg | MCHC g/dL | MCV fL | RDW % | Reti % | Ret x10¹²/L | WBC | Neut | Lymph | Mono | Eos | Baso |
|-------------|---------|-------------|---------|--------|-----------|--------|-------|--------|-------------|-----|------|-------|------|-----|------|
| 1F Mean     | 12.2    | 5.89        | 0.374   | 20.7   | 63.7      | 32.5   | 12.7  | 3.1    | 179         | 7.91| 1.51 | 5.58  | 0.06 | 0.18| 0.56 |
| SD          | 0.8     | 0.53        | 0.025   | 0.6    | 1.9       | 0.3    | 0.4   | 0.5    | 18          | 4.34| 0.55 | 4.06  | 0.05 | 0.06| 0.19 |
| n           | 6       | 6           | 6       | 6      | 6         | 6      | 6     | 6      | 6           | 6   | 6    | 6     | 6    | 6   | 6    |
| 2F Mean     | 12.8    | 6.20        | 0.386   | 20.7   | 62.3      | 33.2   | 12.4  | 2.9    | 178         | 6.89| 1.76 | 4.34  | 0.08 | 0.17| 0.53 |
| SD          | 0.4     | 0.30        | 0.013   | 0.9    | 2.3       | 0.4    | 0.6   | 0.4    | 28          | 0.70| 0.57 | 0.70  | 0.03 | 0.05| 0.12 |
| n           | 6       | 6           | 6       | 6      | 6         | 6      | 6     | 6      | 6           | 6   | 6    | 6     | 6    | 6   | 6    |
| 3F Mean     | 12.2    | 5.90        | 0.370   | 20.7   | 62.7      | 32.9   | 12.3  | 3.1    | 184         | 6.61| 1.62 | 4.18  | 0.13 | 0.15| 0.51 |
| SD          | 0.3     | 0.33        | 0.014   | 0.8    | 1.8       | 0.6    | 0.9   | 1.1    | 72          | 1.38| 0.47 | 1.04  | 0.09 | 0.06| 0.08 |
| n           | 6       | 6           | 6       | 6      | 6         | 6      | 6     | 6      | 6           | 6   | 6    | 6     | 6    | 6   | 6    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 6  (continued)
Haematology and Coagulation : Group Mean Values: Day 66

| Group   | Test Item | Dosage (µg/dose) | 1   | 2   | 3   |
|---------|-----------|------------------|-----|-----|-----|
|         | Control   | 0                |     |     |     |
|         | MenPF-1   | 25               |     |     |     |
|         | MenPF-1   | 50               |     |     |     |

|        |          | F              | SD  | n   |      |      |
|--------|----------|----------------|-----|-----|------|------|
| LUC    | x10⁹/L  | PT (s)         | APTT (s) | Fib (mg/dL) |
| 1F     | Mean     | 0.02           | 6.1 | 55.6 | 129  |
|        | SD       | 0.01           | 0.2 | 4.2  | 16   |
|        | n        | 5              | 6   | 6    | 6    |
| 2F     | Mean     | 0.01           | 5.9 | 56.4 | 215⁵ |
|        | SD       | 0.01           | 0.2 | 5.2  | 35   |
|        | n        | 5              | 6   | 6    | 6    |
| 3F     | Mean     | 0.01           | 5.8³ | 58.6 | 222⁶³ |
|        | SD       | 0.01           | 0.1 | 8.8  | 49   |
|        | n        | 6              | 6   | 6    | 6    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 7

**Haematology and Coagulation: Group Mean Values: Day 92**

| Group / sex | Test Item | Dosage (µg/dose) | Hb (g/dL) | RBC (x10^12/L) | Hct (%) | MCH (pg) | MCV (fl) | MCHC (g/dL) | RDW (%) | Reti (x10^4/L) | Ret (x10^4/L) | WBC (x10^9/L) | Neut | Lymph | Mono | Eos | Baso |
|-------------|-----------|-----------------|-----------|----------------|--------|----------|----------|-------------|--------|---------------|---------------|--------------|------|-------|------|-----|------|
| 1M Mean     | Control   | 0               | 13.6      | 6.40           | 0.408  | 21.2     | 63.7     | 33.2        | 11.9   | 2.7           | 170           | 6.64         | 1.15 | 4.73  | 0.03 | 0.21| 0.51 |
|             |           |                 | 0.7       | 0.25           | 0.018  | 0.6      | 0.6      | 0.6         | 0.8    | 0.4           | 22            | 1.06         | 0.38 | 0.64  | 0.02 | 0.02| 0.05 |
|             |           |                 | 3         | 3              | 3      | 3        | 3        | 3           | 3      | 3             | 3             | 3             | 3     | 3     | 3    | 3   | 3    |
| 2M Mean     | MenPF-1   | 25              | 13.5      | 6.44           | 0.410  | 21.0     | 63.7     | 33.0        | 11.9   | 2.1           | 131           | 6.03         | 1.26 | 4.10  | 0.05 | 0.15| 0.48 |
|             |           |                 | 1.1       | 0.72           | 0.030  | 0.6      | 2.5      | 0.4         | 0.1    | 0.4           | 37            | 1.46         | 0.70 | 0.49  | 0.01 | 0.06| 0.21 |
|             |           |                 | 2         | 2              | 2      | 2        | 2        | 2           | 2      | 2             | 2             | 2             | 2     | 2     | 2    | 2   | 2    |
| 3M Mean     | MenPF-1   | 50              | 13.4      | 6.40           | 0.403  | 21.0     | 62.9     | 33.3        | 12.2   | 2.2           | 142           | 6.48         | 1.09 | 4.62  | 0.04 | 0.19| 0.52 |
|             |           |                 | 0.8       | 0.28           | 0.014  | 0.6      | 1.0      | 0.8         | 1.0    | 0.1           | 13            | 1.24         | 0.46 | 0.72  | 0.01 | 0.05| 0.10 |
|             |           |                 | 3         | 3              | 3      | 3        | 3        | 3           | 3      | 3             | 3             | 3             | 3     | 3     | 3    | 3   | 3    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 7  (continued)
#### Haematology and Coagulation: Group Mean Values: Day 92

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|------------------|
|       |           | 1                |
|       |           | 2                |
|       |           | 3                |

| Test Item | Dosage (µg/dose) | 1  | 2  | 3  |
|------------|------------------|----|----|----|
| Control    |                  | 0  | 25 | 50 |
| MenPF-1    |                  | 25 |    |    |
| MenPF-1    |                  | 50 |    |    |

| Group / sex | LUC x10⁹/L | Plat x10⁹/L | PT s | APTT s | Fib mg/dL |
|-------------|------------|-------------|------|--------|-----------|
| 1M          | Mean       | 0.01        | 354  | 6.1    | 56.9      | 176       |
|             | SD         | 0.01        | 85   | 0.3    | 3.7       | 11        |
|             | n          | 3           | 3    | 3      | 3         | 3         |
| 2M          | Mean       | 0.02        | 396  | 5.9    | 57.4      | 184       |
|             | SD         | 0.01        | 63   | 0.2    | 1.9       | 24        |
|             | n          | 2           | 2    | 3      | 3         | 3         |
| 3M          | Mean       | 0.01        | 398  | 6.0    | 56.5      | 180       |
|             | SD         | 0.01        | 26   | 0.3    | 1.7       | 26        |
|             | n          | 3           | 3    | 3      | 3         | 3         |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
| Group / sex | Hb (g/dL) | RBC x10^6/L | Hct L/L | MCH pg | MCV fL | MCHC g/dL | RDW % | Reti % | Ret x10^5/L | WBC | Neut | Lymph | Mono | Eos | Baso |
|-------------|-----------|-------------|---------|--------|--------|-----------|-------|--------|-------------|-----|------|-------|------|-----|------|
| 1F Mean     | 12.6      | 5.98        | 0.385   | 21.2   | 64.4   | 32.8      | 11.6  | 2.4    | 143         | 5.78| 1.26 | 3.80  | 0.05 | 0.20| 0.46 |
| 1F SD       | 0.7       | 0.43        | 0.021   | 0.4    | 1.1    | 0.3       | 0.4   | 0.1    | 17          | 0.82| 0.65 | 0.60  | 0.03 | 0.10| 0.13 |
| 1F n        | 3         | 3           | 3       | 3      | 3      | 3         | 3     | 3      | 3           | 3   | 3    | 3     | 3    | 3   | 3    |
| 2F Mean     | 12.9      | 6.03        | 0.387   | 21.4   | 64.2   | 33.3      | 11.9  | 2.1    | 129         | 5.04| 0.78 | 3.67  | 0.03 | 0.14| 0.42 |
| 2F SD       | 0.9       | 0.57        | 0.032   | 1.2    | 3.6    | 0.6       | 1.0   | 0.4    | 31          | 2.45| 0.55 | 1.77  | 0.02 | 0.06| 0.18 |
| 2F n        | 3         | 3           | 3       | 3      | 3      | 3         | 3     | 3      | 3           | 3   | 3    | 3     | 3    | 3   | 3    |
| 3F Mean     | 12.4      | 5.87        | 0.377   | 21.2   | 64.5   | 33.0      | 12.0  | 2.3    | 135         | 5.36| 1.19 | 3.48  | 0.06 | 0.14| 0.47 |
| 3F SD       | 0.5       | 0.46        | 0.016   | 1.0    | 3.0    | 0.1       | 0.7   | 0.4    | 33          | 0.94| 0.21 | 0.67  | 0.03 | 0.01| 0.18 |
| 3F n        | 3         | 3           | 3       | 3      | 3      | 3         | 3     | 3      | 3           | 3   | 3    | 3     | 3    | 3   | 3    |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 7 (continued)
**Haematology and Coagulation: Group Mean Values: Day 92**

| Group / sex | LUC x10^9/L | PT s | APTT s | Fib mg/dL |
|-------------|-------------|------|--------|-----------|
| 1F Mean     | 0.01        | 5.9  | 56.4   | 133       |
| SD          | 0.01        | 0.1  | 1.1    | 19        |
| n           | 3           | 3    | 3      | 3         |
| 2F Mean     | 0.01        | 5.8  | 58.0   | 132       |
| SD          | 0.01        | 0.0  | 9.3    | 1         |
| n           | 3           | 3    | 2      | 2         |
| 3F Mean     | 0.01        | 5.8  | 53.6   | 157       |
| SD          | 0.01        | 0.2  | 3.9    | 26        |
| n           | 3           | 3    | 3      | 3         |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 8
Clinical Chemistry: Group Mean Values: Pretrial

| Group / sex | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R Na mmol/L | K mmol/L |
|-------------|---------|---------|---------|---------|---------|-------------|------------|-------------|-------------|--------|--------|--------|----------------|---------|
| 1M Mean     | 164     | 26      | 11      | 70      | 633     | 7.1         | 8.01       | 1.7         | 0.8         | 54     | 42     | 13     | 3.3            | 142     |
| SD          | 43      | 8       | 2       | 17      | 203     | 0.5         | 0.40       | 0.0         | 0.3         | 3      | 2      | 1      | 0.3             | 2       |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6      | 6      | 6               | 6       |
| 2M Mean     | 153     | 29      | 11      | 60      | 496     | 7.4         | 8.15       | 1.7         | 0.7         | 58^a   | 44     | 14     | 3.1            | 141     |
| SD          | 13      | 7       | 1       | 9       | 134     | 0.5         | 0.17       | 0.0         | 0.2         | 2      | 1      | 1      | 0.2             | 1       |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6      | 6      | 6               | 6       |
| 3M Mean     | 192     | 25      | 11      | 62      | 598     | 7.1         | 7.98       | 1.7         | 0.9         | 56     | 43     | 14     | 3.2            | 143     |
| SD          | 33      | 7       | 1       | 17      | 165     | 0.4         | 0.33       | 0.0         | 0.2         | 2      | 1      | 1      | 0.2             | 2       |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6      | 6      | 6               | 6       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 8  (continued)

Clinical Chemistry : Group Mean Values: Pretrial

| Group / sex | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|-----------|-------------|-----------|-------------|
| 1M          | 104       | 1.86        | 3.63      | 64          |
| Mean        |           |             |           |             |
| SD          | 2         | 0.15        | 0.09      | 12          |
| n           | 6         | 6           | 6         | 6           |
| 2M          | 100       | 1.87        | 3.75      | 60          |
| Mean        |           |             |           |             |
| SD          | 2         | 0.15        | 0.04      | 10          |
| n           | 6         | 6           | 6         | 6           |
| 3M          | 102       | 1.94        | 3.69      | 64          |
| Mean        |           |             |           |             |
| SD          | 2         | 0.18        | 0.09      | 3           |
| n           | 6         | 6           | 6         | 6           |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
Table 8  (continued)
Clinical Chemistry : Group Mean Values: Pretrial

| Group / sex | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bi μmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R | Na mmol/L | K mmol/L |
|-------------|---------|---------|---------|---------|---------|-------------|------------|-------------|-------------|--------|--------|---------|------|-----------|---------|
| 1F Mean     | 232     | 23      | 11      | 70      | 961     | 7.0         | 7.71       | 1.7         | 1.3         | 55     | 42     | 14      | 3.1  | 142       | 4.4     |
| SD          | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6      | 6       | 6    | 6         | 6       |
| 2F Mean     | 219     | 29      | 11      | 69      | 555     | 6.7         | 8.56       | 1.7         | 1.3         | 54     | 42     | 13      | 3.2  | 141       | 4.2     |
| SD          | 39      | 9       | 2       | 17      | 199     | 0.5         | 0.45       | 0.0         | 0.3         | 4      | 3      | 1       | 0.2  | 1         | 0.3     |
| 3F Mean     | 174     | 29      | 12      | 58      | 879     | 7.1         | 8.64       | 1.7         | 1.6         | 59     | 45     | 14      | 3.3  | 141       | 4.3     |
| SD          | 23      | 9       | 2       | 6       | 392     | 1.1         | 0.57       | 0.0         | 0.4         | 2      | 1      | 1       | 0.3  | 1         | 0.3     |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6      | 6       | 6    | 6         | 6       |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
### Table 8 (continued)

**Clinical Chemistry : Group Mean Values: Pretrial**

| Group | Test Item | Dosage (µg/dose) | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------|-----------|-----------------|-----------|------------|-----------|-------------|
| 1     | Control   | 0               | 102       | 1.95       | 3.66      | 60          |
|       |           |                  | 6         | 0.29       | 0.05      | 6           |
|       |           |                  | 6         | 6          | 6         | 6           |
| 2     | MenPF-1   | 25              | 100       | 2.04       | 3.61      | 63          |
|       |           |                  | 6         | 0.11       | 0.15      | 2           |
|       |           |                  | 6         | 6          | 6         | 6           |
| 3     | MenPF-1   | 50              | 100       | 1.99       | 3.72      | 59          |
|       |           |                  | 6         | 0.26       | 0.05      | 9           |
|       |           |                  | 6         | 6          | 6         | 6           |

Significantly different from Group 1:
a=p<0.05, b=p<0.01, c=p<0.001
## Table 9

**Clinical Chemistry: Group Mean Values: Day 66**

| Group / sex | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R | Na mmol/L | K mmol/L |
|-------------|---------|---------|---------|---------|---------|-------------|------------|--------------|-------------|--------|--------|---------|-------|-----------|---------|
| 1M Mean     | 62      | 37      | 14      | 56      | 610     | 7.5         | 7.52       | 1.7          | 0.4         | 57     | 45     | 12      | 3.9   | 144       | 4.6     |
| SD          | 11      | 17      | 4       | 7       | 159     | 1.1         | 0.57       | 0.0          | 0.1         | 3      | 2      | 1       | 0.3   | 1         | 0.3     |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6            | 6           | 6      | 6      | 6       | 6     | 6         | 6       |
| 2M Mean     | 62      | 39      | 13      | 59      | 528     | 7.6         | 7.26       | 1.7          | 0.6         | 61³    | 46     | 15⁶     | 3.0⁵  | 144       | 4.6     |
| SD          | 9       | 12      | 4       | 18      | 164     | 1.1         | 0.30       | 0.0          | 0.2         | 1      | 2      | 2       | 0.5   | 2         | 0.2     |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6            | 6           | 6      | 6      | 6       | 6     | 6         | 6       |
| 3M Mean     | 71      | 42      | 14      | 84      | 760     | 6.8         | 7.50       | 1.7          | 0.5         | 61³    | 46     | 15⁶     | 3.0⁵  | 146       | 4.3⁵   |
| SD          | 16      | 22      | 4       | 56      | 576     | 0.5         | 0.45       | 0.0          | 0.1         | 2      | 2      | 1       | 0.1   | 1         | 0.2     |
| n           | 6       | 6       | 6       | 6       | 6       | 6           | 6          | 6            | 6           | 6      | 6      | 6       | 6     | 6         | 6       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 9 (continued)

**Clinical Chemistry : Group Mean Values: Day 66**

| Group / sex | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea μmol/L |
|-------------|-----------|-------------|-----------|-------------|
| 1M          | Mean      | 107         | 1.27      | 3.60        | 67          |
|             | SD        | 1           | 0.10      | 0.09        | 13          |
|             | n         | 6           | 6         | 6           |
| 2M          | Mean      | 105         | 1.37      | 3.70        | 62          |
|             | SD        | 2           | 0.10      | 0.04        | 16          |
|             | n         | 6           | 6         | 6           |
| 3M          | Mean      | 105         | 1.36      | 3.65        | 68          |
|             | SD        | 2           | 0.11      | 0.10        | 6           |
|             | n         | 6           | 6         | 6           |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
Table 9  (continued)
Clinical Chemistry : Group Mean Values: Day 66

| Group / sex | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R | Na mmol/L | K mmol/L |
|-------------|---------|---------|---------|---------|---------|-------------|------------|-------------|-------------|--------|---------|----------|------|-----------|---------|
| 1F          | Mean    | 104     | 35      | 10      | 47      | 764         | 7.8        | 7.09        | 1.7         | 1.3    | 58      | 46       | 13   | 3.7       | 45      |
|             | SD      | 49      | 21      | 2       | 12      | 356         | 1.8        | 0.49        | 0.0         | 0.1    | 1       | 1        | 1    | 0.4       | 0.2     |
|             | n       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6       | 6        | 6    | 6         | 6       |
| 2F          | Mean    | 76      | 45      | 14      | 60      | 651         | 8.0        | 7.58        | 1.7         | 1.2    | 58      | 45       | 14   | 3.3       | 145     |
|             | SD      | 16      | 15      | 2       | 17      | 264         | 0.9        | 0.39        | 0.0         | 0.2    | 3       | 2        | 2    | 0.3       | 1       |
|             | n       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6       | 6        | 6    | 6         | 6       |
| 3F          | Mean    | 84      | 59      | 17      | 44      | 623         | 7.6        | 7.41        | 1.8         | 1.8    | 62      | 46       | 16   | 3.0       | 144     |
|             | SD      | 17      | 33      | 8       | 11      | 159         | 1.2        | 0.44        | 0.2         | 0.8    | 2       | 2        | 1    | 0.2       | 2       |
|             | n       | 6       | 6       | 6       | 6       | 6           | 6          | 6           | 6           | 6      | 6       | 6        | 6    | 6         | 6       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 9  (continued)
Clinical Chemistry : Group Mean Values: Day 66

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
|       | 1         | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

| Group / sex | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|-----------|-------------|-----------|-------------|
| 1F Mean     | 104       | 1.44        | 3.70      | 74          |
| SD          | 2         | 0.16        | 0.05      | 10          |
| n           | 6         | 6           | 6         | 6           |
| 2F Mean     | 103       | 1.55        | 3.62      | 82          |
| SD          | 2         | 0.21        | 0.18      | 16          |
| n           | 6         | 6           | 6         | 6           |
| 3F Mean     | 103       | 1.45        | 3.64      | 76          |
| SD          | 2         | 0.17        | 0.08      | 8           |
| n           | 6         | 6           | 6         | 6           |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 10
#### Clinical Chemistry: Group Mean Values: Day 92

| Group / sex | ALP  | ALT  | AST  | LDH  | CPK  | Urea | Glu   | T.Bil | Chol  | TP   | Alb  | Glob | AG-R | Na   | K   |
|-------------|------|------|------|------|------|------|-------|-------|-------|------|------|------|------|------|-----|
| 1M Mean     | 55   | 26   | 14   | 64   | 506  | 7.0  | 6.62  | 1.7   | 0.3   | 56   | 44   | 12   | 3.8  | 145  | 4.0 |
| SD          | 8    | 12   | 4    | 5    | 88   | 0.8  | 0.93  | 0.0   | 0.1   | 1    | 1    | 1    | 0.3  | 2    | 0.5 |
| n           | 3    | 3    | 3    | 3    | 3    | 3    | 3     | 3     | 3     | 3    | 3    | 3    | 3    | 3    | 3   |
| 2M Mean     | 50   | 39   | 14   | 73   | 481  | 8.2  | 6.58  | 1.7   | 0.5   | 59   | 47   | 12   | 4.0  | 145  | 4.6 |
| SD          | 6    | 6    | 3    | 21   | 95   | 1.4  | 0.04  | 0.0   | 0.1   | 2    | 2    | 1    | 0.2  | 2    | 0.0 |
| n           | 3    | 3    | 3    | 3    | 3    | 3    | 3     | 3     | 3     | 3    | 3    | 3    | 3    | 3    | 3   |
| 3M Mean     | 102  | 32   | 15   | 72   | 441  | 6.6  | 6.98  | 1.7   | 0.5   | 57   | 45   | 12   | 3.7  | 144  | 4.3 |
| SD          | 68   | 17   | 7    | 10   | 51   | 0.7  | 0.42  | 0.0   | 0.2   | 1    | 1    | 1    | 0.3  | 1    | 0.1 |
| n           | 3    | 3    | 3    | 3    | 3    | 3    | 3     | 3     | 3     | 3    | 3    | 3    | 3    | 3    | 3   |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 10  (continued)
Clinical Chemistry : Group Mean Values: Day 92

| Group / sex | Test Item | Dosage (µg/dose) | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|-----------|-----------------|-----------|-------------|-----------|-------------|
| 1M Mean     | Control   | 1               | 105       | 1.19        | 3.60      | 67          |
| SD          |           | 1               | 1         | 0.14        | 0.13      | 8           |
| n           |           | 3               | 3         | 3           | 3         |             |
| 2M Mean     | MenPF-1   | 25              | 105       | 1.13        | 3.88<sup>b</sup> | 75          |
| SD          |           | 2               | 2         | 0.11        | 0.07      | 24          |
| n           |           | 3               | 3         | 3           | 3         |             |
| 3M Mean     | MenPF-1   | 50              | 103       | 1.23        | 3.60      | 74          |
| SD          |           | 2               | 2         | 0.03        | 0.04      | 4           |
| n           |           | 3               | 3         | 3           | 3         |             |

Significantly different from Group 1:a=p<0.05, b=p<0.01, c=p<0.001
Table 10  (continued)
Clinical Chemistry : Group Mean Values: Day 92

| Group / sex | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R mmol/L | Na mmol/L | K mmol/L |
|-------------|--------|--------|--------|--------|--------|------------|------------|-------------|------------|--------|--------|--------|-----------|---------|---------|
| 1F Mean     | 82     | 33     | 11     | 58     | 551    | 9.2        | 6.32       | 1.7         | 1.3        | 60     | 48     | 12     | 4.0       | 144     | 4.4     |
| n           | 3      | 3      | 3      | 3      | 3      | 3          | 3          | 3           | 3          | 3      | 3      | 3      | 3         | 3       | 3       |
| 2F Mean     | 62     | 39     | 17b    | 79     | 493    | 9.0        | 7.26b      | 1.7         | 1.0        | 55b    | 43b    | 12     | 3.7       | 143     | 4.3     |
| SD          | 4      | 10     | 3      | 71     | 102    | 1.0        | 0.28       | 0.0         | 0.2        | 2      | 1      | 1      | 0.2       | 2       | 0.1     |
| n           | 3      | 3      | 3      | 3      | 3      | 3          | 3          | 3           | 3          | 3      | 3      | 3      | 3         | 3       | 3       |
| 3F Mean     | 87     | 32     | 14     | 42     | 393    | 8.7        | 6.51       | 1.7         | 1.6        | 62     | 47     | 14a    | 3.3       | 143     | 4.2     |
| SD          | 25     | 9      | 1      | 7      | 112    | 1.0        | 0.19       | 0.0         | 1.1        | 1      | 2      | 1      | 0.3       | 1       | 0.2     |
| n           | 3      | 3      | 3      | 3      | 3      | 3          | 3          | 3           | 3          | 3      | 3      | 3      | 3         | 3       | 3       |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
### Table 10 (continued)
#### Clinical Chemistry: Group Mean Values: Day 92

| Group | Test Item | Dosage (µg/dose) | Control | MenPF-1 | MenPF-1 |
|-------|-----------|------------------|---------|---------|---------|
|       |           |                  | 0       | 25      | 50      |

| Group / sex | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|-----------|-------------|-----------|-------------|
| 1F Mean     | 104       | 1.16        | 3.78      | 83          |
| SD          | 3         | 0.15        | 0.04      | 9           |
| n           | 3         | 3           | 3         | 3           |
| 2F Mean     | 104       | 1.35        | 3.49<sup>b</sup> | 100         |
| SD          | 3         | 0.16        | 0.02      | 22          |
| n           | 3         | 3           | 3         | 3           |
| 3F Mean     | 104       | 1.18        | 3.70      | 81          |
| SD          | 1         | 0.15        | 0.15      | 13          |
| n           | 3         | 3           | 3         | 3           |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
## Table 11
### Summary of Necropsy Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| NECROPSY FINDINGS | GROUP TOTALS | Males | Females |
|-------------------|--------------|-------|---------|
|                   | Grp 1 | Grp 2 | Grp 3 | Grp 1 | Grp 2 | Grp 3 |
| GENERAL COMMENTS  |       |       |       |       |       |       |
| Number of animals necropsied | 3 | 3 | 3 | 3 | 3 | 3 |
| LUNG              |       |       |       |       |       |       |
| Spongy           |       |       |       | 1 | 1 | |
| Discolouration   | 3 | 3 | 2 | 3 | 2 | 2 |
| LYMPH NODE (LUMBAR) |       |       |       |       |       |       |
| Discolouration, one/both | 1 | 1 | 1 | |
| Enlargement, left | 2 | 1 | 1 |
| LYMPH NODE (MESENTERIC) |       |       |       |       |       |       |
| Discolouration   |       |       |       | 1 | 1 | 1 |
| OVIDUCT          |       |       |       |       |       |       |
| Cyst, right      |       |       |       | 1 | | |

The absence of a numeral indicates that the lesion specified was not identified.

Pathology File Ref.: PLAFOR_520419_MACMAIN_LL_KEEP2.SPL
Table 11  (continued)
Summary of Necropsy Findings: Day 66

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
| 1     | Control   | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

| Group   | Males | Females |
|---------|-------|---------|
| TESTIS  | Grp 1 | Grp 2   | Grp 3 | Grp 1 | Grp 2 | Grp 3 |
| Small, right | 2     |         |       |       |       |       |
| THYMUS  |       |         |       |       |       |       |
| Discolouration | 1     |         |       |       |       |       |

The absence of a numeral indicates that the lesion specified was not identified.

Pathology File Ref.: PLAFOR_520419_MACMAIN_LL_KEEP2.SPL
### Table 12
Summary of Necropsy Findings: Day 92

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
| 1     | Control   | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

| NECROPSY FINDINGS | INCIDENCE |
|-------------------|-----------|
|                   | Males     | Females |
|                   | Grp 1 | Grp 2 | Grp 3 | Grp 1 | Grp 2 | Grp 3 |
| GENERAL COMMENTS  |       |       |       |       |       |       |
| Number of animals necropsied | 3 | 3 | 3 | 3 | 3 | 3 |
| ADRENAL GLAND     |       |       |       |       |       |       |
| Discolouration, both | 1 |
| LUNG              |       |       |       |       |       |       |
| Spongy           | 1 | 1 | 2 | 2 | 2 |
| Discolouration   | 2 | 3 | 2 | 3 | 2 |
| LYMPH NODE (INGUINAL) |       |       |       |       |       |       |
| Discolouration, right | 1 |
| LYMPH NODE (LUMBAR) |       |       |       |       |       |       |
| Discolouration, one/both | 1 | 3 |

The absence of a numeral indicates that the lesion specified was not identified.
Table 12
Summary of Necropsy Findings: Day 92

| Group : | 1 | 2   | 3   |
|--------|---|-----|-----|
| Test Item : | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) : | 0 | 25  | 50  |

| NECROPSY FINDINGS | Group | Males | Females |
|-------------------|-------|-------|---------|
|                   | Grp 1 | Grp 2 | Grp 3 | Grp 1 | Grp 2 | Grp 3 |
| LYMPH NODE (MANDIBULAR) |       |       |       |       |       |       |
| Discolouration     | 1     |       |       | 1     |       |       |
| OVARY              |       |       |       |       | 1     |       |
| Foci, dark, both   |       |       |       |       |       | 1     |
| OVIDUCT            |       |       |       |       |       |       |
| Cyst, right        |       |       |       |       |       | 1     |
| THYROID GLAND      |       |       |       |       |       |       |
| Small, right       |       |       |       |       |       | 1     |
| TRACHEA            |       |       |       |       |       |       |
| Fluid accumulation | 2     | 1     | 1     | 2     | 2     | 2     |

The absence of a numeral indicates that the lesion specified was not identified.

Pathology File Ref.: PLAFOR_520419_MACREC_LL_KEEP1.SPL
# Table 13

**Absolute Organ Weights (g): Group Mean Values: Day 66**

| Group / sex | Body Weight (kg) | Adrenals | Brain | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate |
|-------------|------------------|----------|-------|--------------|-------|---------|-------|------|-----------|----------|
| 1M Mean     | 3.2              | 0.2992   | 10.19 | 2.1413       | 8.96  | 18.87   | 98.37 | 22.54| 0.030     | 0.97     |
| SD          | 0.1              | 0.0543   | 0.54  | 0.1608       | 1.13  | 2.12    | 25.11 | 1.60 | 0.001     | 0.49     |
| n           | 3                | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        |
| 2M Mean     | 3.4              | 0.3112   | 9.72  | 2.4714       | 9.85  | 21.88   | 140.20| 27.85| 0.039     | 1.30     |
| SD          | 0.1              | 0.0348   | 0.23  | 0.5107       | 0.91  | 2.64    | 24.51 | 3.39 | 0.006     | 0.26     |
| n           | 3                | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        |
| 3M Mean     | 3.4              | 0.2538   | 9.71  | 2.4485       | 8.79  | 19.20   | 112.52| 22.51| 0.026     | 0.88     |
| SD          | 0.2              | 0.0017   | 0.16  | 0.4247       | 0.66  | 1.29    | 20.63 | 6.36 | 0.001     | 0.15     |
| n           | 3                | 2        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 13 (continued)  
Absolute Organ Weights (g) : Group Mean Values: Day 66

| Group / sex | Spleen | Testes | Thymus | Thyroid |
|-------------|--------|--------|--------|---------|
| 1M Mean     | 1.053  | 4.40   | 2.863  | 0.406   |
| SD          | 0.137  | 1.32   | 0.777  | 0.108   |
| n           | 3      | 3      | 3      | 3       |
| 2M Mean     | 1.065  | 5.63   | 3.076  | 0.253   |
| SD          | 0.063  | 0.78   | 0.209  | 0.042   |
| n           | 3      | 3      | 3      | 3       |
| 3M Mean     | 1.295  | 5.56   | 3.532  | 0.311   |
| SD          | 0.136  | 0.74   | 0.673  | 0.071   |
| n           | 3      | 3      | 3      | 3       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 13  (continued)
Absolute Organ Weights (g) : Group Mean Values: Day 66

| Group / sex | Body Weight (kg) | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen |
|-------------|------------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|
| 1F          | Mean             | 4.0      | 0.2487| 10.29 | 9.74    | 21.80 | 130.57| 27.93   | 0.380     | 0.039   | 1.870  |
|             | SD               | 0.1      | 0.0443| 0.57  | 1.79    | 2.64  | 16.28 | 0.60    | 0.026     | 0.015   | 0.413  |
|             | n                 | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3       | 3      |
| 2F          | Mean             | 3.9      | 0.3442| 9.68  | 9.97    | 20.27 | 123.04| 21.51   | 0.438     | 0.036   | 1.800  |
|             | SD               | 0.4      | 0.0331| 0.36  | 1.11    | 3.54  | 47.05 | 8.16    | 0.114     | 0.005   | 0.273  |
|             | n                 | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3       | 3      |
| 3F          | Mean             | 3.8      | 0.3295| 9.57  | 9.09    | 22.36 | 129.88| 21.31   | 0.504     | 0.031   | 1.387  |
|             | SD               | 0.6      | 0.0390| 0.26  | 1.90    | 5.57  | 48.84 | 10.72   | 0.144     | 0.010   | 0.354  |
|             | n                 | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3       | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 13  (continued)  
Absolute Organ Weights (g) : Group Mean Values: Day 66

| Group / sex | Thymus | Thyroid | Uterus |
|-------------|--------|---------|--------|
| 1F Mean     | 3.100  | 0.363   | 8.05   |
| SD          | 0.790  | 0.083   | 0.76   |
| n           | 3      | 3       | 3      |
| 2F Mean     | 3.542  | 0.426   | 10.28  |
| SD          | 0.722  | 0.050   | 1.58   |
| n           | 3      | 3       | 3      |
| 3F Mean     | 3.613  | 0.403   | 8.36   |
| SD          | 1.302  | 0.042   | 1.26   |
| n           | 3      | 3       | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 14
Organ Weights (Covariance Analysis): Group Mean Values: Day 66

| Group / sex | Adrenals    | Brain   | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate | Spleen |
|-------------|-------------|---------|--------------|-------|---------|-------|------|-----------|----------|--------|
| 1M Mean     | 0.2968      | 10.12   | 2.2396       | 8.94  | 19.66   | 106.23| 21.44| 0.031     | 1.07     | 1.03   |
| SE          | 0.0322      | 0.26    | 0.2893       | 0.70  | 1.46    | 16.70 | 3.12 | 0.004     | 0.24     | 0.09   |
| n           | 3           | 3       | 3            | 3     | 3       | 3     | 3    | 2         | 3        | 3      |
| 2M Mean     | 0.3136      | 9.76    | 2.4223       | 9.86  | 21.48   | 136.27| 28.40| 0.038     | 1.25     | 1.08   |
| SE          | 0.0322      | 0.23    | 0.2537       | 0.61  | 1.28    | 14.65 | 2.73 | 0.003     | 0.21     | 0.08   |
| n           | 3           | 3       | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 3M Mean     | 0.2538      | 9.75    | 2.3994       | 8.80  | 18.80   | 108.59| 23.06| 0.026     | 0.83     | 1.31   |
| SE          | 0.0322      | 0.23    | 0.2537       | 0.61  | 1.28    | 14.65 | 2.73 | 0.003     | 0.21     | 0.08   |
| n           | 2           | 3       | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 14 (continued)
**Organ Weights (Covariance Analysis): Group Mean Values: Day 66**

| Group / sex | Test Item | Dosage (µg/dose) | 1   | 2   | 3   |
|-------------|-----------|------------------|-----|-----|-----|
| 1M          | Control   | 0                | 4.04| 3.09| 0.423|
|             |           | SE               | 0.69| 0.43| 0.058|
|             |           | n                | 3   | 3   | 3   |
| 2M          | MenPF-1   | 25               | 5.81| 2.96| 0.245|
|             |           | SE               | 0.60| 0.37| 0.051|
|             |           | n                | 3   | 3   | 3   |
| 3M          | MenPF-1   | 50               | 5.74| 3.42| 0.303|
|             |           | SE               | 0.60| 0.37| 0.051|
|             |           | n                | 3   | 3   | 3   |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
## Table 14 (continued)

Organ Weights (Covariance Analysis): Group Mean Values: Day 66

| Group / sex | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen | Thymus |
|-------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|--------|
| 1F Mean     | 0.2505   | 10.29 | 9.56  | 21.22   | 124.35| 26.98| 0.367   | 0.040     | 1.88   | 2.99   |
| SE          | 0.0238   | 0.26  | 0.77  | 1.28    | 7.77  | 3.26 | 0.045   | 0.006     | 0.22   | 0.42   |
| n           | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      | 3      |
| 2F Mean     | 0.3442   | 9.68  | 9.97  | 20.27   | 123.04| 21.51| 0.438   | 0.036     | 1.80   | 3.54   |
| SE          | 0.0237   | 0.26  | 0.76  | 1.27    | 7.72  | 3.24 | 0.045   | 0.006     | 0.22   | 0.42   |
| n           | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      | 3      |
| 3F Mean     | 0.3277   | 9.58  | 9.27  | 22.94   | 136.11| 22.26| 0.517   | 0.031     | 1.38   | 3.73   |
| SE          | 0.0238   | 0.26  | 0.77  | 1.28    | 7.77  | 3.26 | 0.045   | 0.006     | 0.22   | 0.42   |
| n           | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 14 (continued)
#### Organ Weights (Covariance Analysis): Group Mean Values: Day 66

| Group / sex | Test Item | Dosage (µg/dose) | Thyroid | Uterus |
|-------------|-----------|------------------|---------|--------|
| 1F Mean     | Control   | 0                | 0.368   | 7.99   |
|             | SE        | 0.033            | 0.75    |        |
| n           |           | 3                | 3       |        |
| 2F Mean     | MenPF-1   | 25               | 0.426   | 10.28  |
|             | SE        | 0.033            | 0.75    |        |
| n           |           | 3                | 3       |        |
| 3F Mean     | MenPF-1   | 50               | 0.398   | 8.43   |
|             | SE        | 0.033            | 0.75    |        |
| n           |           | 3                | 3       |        |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 15
Relative Organ Weights (% Body Weight): Group Mean Values: Day 66

| Group / sex | Adrenals | Brain  | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate | Spleen |
|-------------|----------|--------|--------------|-------|---------|-------|------|-----------|----------|--------|
| 1M Mean     | 0.0093   | 0.319  | 0.06697      | 0.281 | 0.589   | 3.073 | 0.706| 0.0010    | 0.030    | 0.0330 |
| SD          | 0.0016   | 0.027  | 0.00552      | 0.044 | 0.061   | 0.775 | 0.072| 0.0001    | 0.014    | 0.0045 |
| n           | 3        | 3      | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 2M Mean     | 0.0092   | 0.286  | 0.07293      | 0.290 | 0.643   | 4.132 | 0.818| 0.0111    | 0.038    | 0.0313 |
| SD          | 0.0012   | 0.007  | 0.01633      | 0.030 | 0.075   | 0.792 | 0.087| 0.0002    | 0.008    | 0.0022 |
| n           | 3        | 3      | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 3M Mean     | 0.0063   | 0.287  | 0.07171      | 0.259 | 0.565   | 3.293 | 0.667| 0.0008    | 0.026    | 0.0382 |
| SD          | 0.0024   | 0.016  | 0.00832      | 0.007 | 0.024   | 0.415 | 0.214| 0.0000    | 0.003    | 0.0051 |
| n           | 3        | 3      | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 15 (continued)
Relative Organ Weights (% Body Weight): Group Mean Values: Day 66

| Group / sex | Test Item | Dosage (µg/dose) | Testes | Thymus | Thyroid |
|-------------|-----------|-----------------|--------|--------|---------|
| 1M Mean     | Control   | 0               | 0.137  | 0.090  | 0.0127  |
| SD          |           |                 | 0.041  | 0.024  | 0.0030  |
| n           |           |                 | 3      | 3      | 3       |
| 2M Mean     | MenPF-1   | 25              | 0.164  | 0.090  | 0.0074  |
| SD          |           |                 | 0.028  | 0.007  | 0.0014  |
| n           |           |                 | 3      | 3      | 3       |
| 3M Mean     | MenPF-1   | 50              | 0.164  | 0.104  | 0.0092  |
| SD          |           |                 | 0.029  | 0.017  | 0.0020  |
| n           |           |                 | 3      | 3      | 3       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 15 (continued)
Relative Organ Weights (% Body Weight): Group Mean Values: Day 66

| Group | Test Item | Dosage (µg/dose) | 1      | 2      | 3      |
|-------|-----------|------------------|--------|--------|--------|
| Control |          | 0                | 0.0063 | 0.260  | 0.245  |
| 1F     | MenPF-1   | 25               | 0.549  | 0.256  | 0.519  |
|        |           | 50               | 3.291  | 0.549  | 3.097  |
| 2F     |           |                  | 0.291  | 0.038  | 0.180  |
|        |           |                  | 0.011  | 0.018  | 0.823  |
|        |           |                  | 0.001  | 0.030  | 0.006  |
| 3F     |           |                  | 0.0088 | 0.578  | 3.306  |
|        |           |                  | 0.0019 | 0.237  | 0.540  |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
| Group / sex | Thyroid   | Uterus  |
|-------------|-----------|---------|
| 1F          | 0.0092    | 0.203   |
|             | 0.0023    | 0.020   |
|             | 3         | 3       |
| 2F          | 0.0111    | 0.266   |
|             | 0.0023    | 0.055   |
|             | 3         | 3       |
| 3F          | 0.0107    | 0.219   |
|             | 0.0024    | 0.012   |
|             | 3         | 3       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 16
Absolute Organ Weights (g) : Group Mean Values: Day 92

| Group / sex | Body Weight (kg) | Adrenals | Brain | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate |
|-------------|------------------|----------|-------|--------------|-------|---------|-------|------|-----------|----------|
| 1M Mean     | 3.5              | 0.2622   | 10.11 | 2.1997       | 9.68  | 20.37   | 108.86| 23.40| 0.020     | 0.99     |
| 1M SD       | 0.2              | 0.0381   | 0.38  | 0.2994       | 0.85  | 1.14    | 4.18  | 7.25 | 0.007     | 0.12     |
| 1M n        | 3                | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        |
| 2M Mean     | 3.5              | 0.3237   | 9.75  | 1.9825       | 9.20  | 17.95   | 115.60| 28.66| 0.022     | 0.80     |
| 2M SD       | 0.2              | 0.0305   | 0.60  | 0.3467       | 0.70  | 4.12    | 29.19 | 4.39 | 0.002     | 0.13     |
| 2M n        | 3                | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        |
| 3M Mean     | 3.3              | 0.3486   | 10.17 | 2.1297       | 8.37  | 16.75   | 92.85 | 23.18| 0.030     | 1.06     |
| 3M SD       | 0.3              | 0.0588   | 0.24  | 0.3844       | 0.24  | 0.75    | 13.30 | 9.49 | 0.015     | 0.36     |
| 3M n        | 3                | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        |

Significantly different from Group 1:a=p<0.05, b=p<0.01, c=p<0.001
Table 16  (continued)
Absolute Organ Weights (g) : Group Mean Values: Day 92

| Group / sex | Spleen | Testes | Thymus | Thyroid |
|-------------|--------|--------|--------|---------|
| 1M Mean     | 1.006  | 6.35   | 3.437  | 0.252   |
| SD          | 0.432  | 0.63   | 0.927  | 0.094   |
| n           | 3      | 3      | 3      | 3       |
| 2M Mean     | 1.185  | 5.14   | 3.175  | 0.276   |
| SD          | 0.083  | 0.82   | 1.546  | 0.084   |
| n           | 3      | 3      | 3      | 3       |
| 3M Mean     | 1.151  | 5.42   | 2.733  | 0.207   |
| SD          | 0.324  | 0.34   | 0.887  | 0.024   |
| n           | 3      | 3      | 3      | 3       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 16  (continued)
Absolute Organ Weights (g) : Group Mean Values: Day 92

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|------------------|
|       |           | 1                | 2                | 3                |
|       | Control   | 0                | 25               | 50               |

| Group / sex | Body Weight (kg) | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen |
|-------------|------------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|
| 1F Mean     | 4.5              | 0.3051   | 9.72  | 9.90  | 22.16   | 129.33| 31.06| 0.492   | 0.026     | 1.828  |
| SD          | 0.4              | 0.0517   | 0.77  | 1.27  | 2.95    | 14.95 | 8.26 | 0.093   | 0.009     | 0.319  |
| n           | 3                | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      |
| 2F Mean     | 3.9              | 0.2566   | 9.49  | 8.61  | 18.25   | 95.22 | 22.69| 0.395   | 0.031     | 1.808  |
| SD          | 0.4              | 0.0403   | 0.68  | 0.36  | 1.57    | 7.81  | 5.28 | 0.134   | 0.012     | 0.547  |
| n           | 3                | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      |
| 3F Mean     | 4.3              | 0.3020   | 9.47  | 10.53 | 20.53   | 139.77| 30.24| 0.538   | 0.021     | 1.310  |
| SD          | 0.2              | 0.0507   | 0.76  | 1.16  | 1.88    | 9.23  | 8.48 | 0.180   | 0.015     | 0.209  |
| n           | 3                | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 16 (continued)
Absolute Organ Weights (g) : Group Mean Values: Day 92

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|------------------|
| 1     | Control   | 0                |
| 2     | MenPF-1   | 25               |
| 3     | MenPF-1   | 50               |

| Group / sex | Thymus | Thyroid | Uterus |
|-------------|--------|---------|--------|
| 1F Mean     | 4.497  | 0.354   | 9.25   |
| SD          | 1.202  | 0.073   | 2.12   |
| n           | 3      | 3       | 3      |
| 2F Mean     | 2.728  | 0.350   | 9.70   |
| SD          | 1.017  | 0.081   | 1.93   |
| n           | 3      | 3       | 3      |
| 3F Mean     | 3.079  | 0.370   | 9.75   |
| SD          | 0.482  | 0.114   | 1.88   |
| n           | 3      | 3       | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
### Table 17
**Organ Weights (Covariance Analysis): Group Mean Values: Day 92**

| Group / sex |   | Adrenals | Brain | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate | Spleen |
|-------------|---|----------|-------|--------------|-------|---------|-------|------|-----------|----------|--------|
| 1M Mean     |   | 0.2741   | 10.07 | 2.2842       | 9.59  | 19.60   | 102.70| 21.54| 0.020     | 1.04     | 1.10   |
| 1M SE       |   | 0.0244   | 0.29  | 0.1986       | 0.41  | 1.29    | 9.08  | 4.17 | 0.006     | 0.14     | 0.16   |
| 1M n        |   | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 2M Mean     |   | 0.3285   | 9.74  | 2.0163       | 9.16  | 17.64   | 113.14| 27.92| 0.022     | 0.82     | 1.22   |
| 2M SE       |   | 0.0233   | 0.27  | 0.1897       | 0.40  | 1.23    | 8.68  | 3.99 | 0.006     | 0.13     | 0.16   |
| 2M n        |   | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 3M Mean     |   | 0.3319   | 10.22 | 2.0114       | 8.51  | 17.83   | 101.47| 25.78| 0.030     | 0.98     | 1.02   |
| 3M SE       |   | 0.0256   | 0.30  | 0.2082       | 0.43  | 1.35    | 9.52  | 4.38 | 0.007     | 0.14     | 0.17   |
| 3M n        |   | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 17 (continued)
Organ Weights (Covariance Analysis): Group Mean Values: Day 92

| Group / sex | Test Item | Dosage (µg/dose) |
|-------------|-----------|------------------|
| 1M          | Control   | 0                |
| 2M          | MenPF-1   | 25               |
| 3M          | MenPF-1   | 50               |

| Group / sex | Testes | Thymus | Thyroid |
|-------------|--------|--------|---------|
| 1M          | Mean   | 6.48   | 0.253   |
|             | SE     | 0.38   | 0.049   |
|             | n      | 3      | 3       |
| 2M          | Mean   | 5.19   | 0.277   |
|             | SE     | 0.36   | 0.047   |
|             | n      | 3      | 3       |
| 3M          | Mean   | 5.24   | 0.206   |
|             | SE     | 0.40   | 0.052   |
|             | n      | 3      | 3       |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 17  (continued)
Organ Weights (Covariance Analysis): Group Mean Values: Day 92

| Group / sex | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen | Thymus |
|-------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|--------|
| 1F Mean     | 0.2828   | 10.03 | 9.53  | 20.71   | 122.40| 33.90| 0.530   | 0.030     | 1.89   | 3.81   |
| SE          | 0.0272   | 0.44  | 0.64  | 0.80    | 4.59  | 4.66 | 0.094   | 0.008     | 0.27   | 0.23   |
| n           | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      | 3      |
| 2F Mean     | 0.2873   | 9.07  | 9.12  | 20.25   | 104.74| 18.78| 0.342   | 0.025     | 1.72   | 3.67   |
| SE          | 0.0298   | 0.49  | 0.70  | 0.88    | 5.03  | 5.11 | 0.103   | 0.009     | 0.29   | 0.25   |
| n           | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      | 3      |
| 3F Mean     | 0.2936   | 9.59  | 10.39 | 19.99   | 137.17| 31.30| 0.552   | 0.022     | 1.34   | 2.82   |
| SE          | 0.0244   | 0.40  | 0.58  | 0.72    | 4.12  | 4.19 | 0.084   | 0.007     | 0.24   | 0.21   |
| n           | 3        | 3     | 3     | 3       | 3     | 3    | 3       | 3         | 3      | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 17 (continued)
Organ Weights (Covariance Analysis): Group Mean Values: Day 92

| Group                      | 1     | 2    | 3    |
|----------------------------|-------|------|------|
| Test Item                  | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose)           | 0     | 25   | 50   |

| Group / sex | Thyroid | Uterus |
|-------------|---------|--------|
| 1F Mean     | 0.306   | 9.31   |
|             | SE      | 0.047  |
|             | n       | 3      |
| 2F Mean     | 0.416   | 9.61   |
|             | SE      | 0.052  |
|             | n       | 3      |
| 3F Mean     | 0.352   | 9.77   |
|             | SE      | 0.042  |
|             | n       | 3      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 18
Relative Organ Weights (% Body Weight): Group Mean Values: Day 92

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| Group / sex | Adrenals | Brain | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate | Spleen |
|-------------|----------|-------|--------------|-------|---------|-------|------|-----------|----------|--------|
| 1M          | 0.0075   | 0.286 | 0.06248      | 0.274 | 0.578   | 3.090 | 0.662| 0.0005    | 0.028    | 0.0289 |
| SD          | 0.0015   | 0.007 | 0.01013      | 0.026 | 0.038   | 0.235 | 0.206| 0.0002    | 0.003    | 0.0136 |
| n           | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 2M          | 0.0094   | 0.282 | 0.05753      | 0.266 | 0.515   | 3.310 | 0.828| 0.0006    | 0.023    | 0.0343 |
| SD          | 0.0009   | 0.025 | 0.01246      | 0.010 | 0.085   | 0.633 | 0.128| 0.0000    | 0.004    | 0.0043 |
| n           | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |
| 3M          | 0.0108   | 0.313 | 0.06591      | 0.258 | 0.514   | 2.835 | 0.700| 0.0009    | 0.033    | 0.0356 |
| SD          | 0.0026   | 0.029 | 0.01558      | 0.027 | 0.028   | 0.255 | 0.259| 0.0006    | 0.014    | 0.0111 |
| n           | 3        | 3     | 3            | 3     | 3       | 3     | 3    | 3         | 3        | 3      |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
## Table 18  (continued)
### Relative Organ Weights (% Body Weight): Group Mean Values: Day 92

| Group / sex | Test Item | Dosage (µg/dose) | 1   | 2   | 3   |
|-------------|-----------|------------------|-----|-----|-----|
| 1M          | Control   | 0                | 0.180 | 0.097 | 0.0072 |
|             | MenPF-1   | 25               |       |      |      |
|             | MenPF-1   | 50               |       |      |      |
| 2M          | Control   | 0                | 0.149 | 0.091 | 0.0079 |
|             | MenPF-1   | 25               |       |      |      |
|             | MenPF-1   | 50               |       |      |      |
| 3M          | Control   | 0                | 0.167 | 0.083 | 0.0064 |
|             | MenPF-1   | 25               |       |      |      |
|             | MenPF-1   | 50               |       |      |      |
|             |           |                  |       |      |      |

Significantly different from Group 1: a=p<0.05, b=p<0.01, c=p<0.001
Table 18  (continued)
Relative Organ Weights (% Body Weight): Group Mean Values: Day 92

| Group / sex | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------------|-----------|-----------------|---|---|---|
| 1F          | Control   | 0               | 0.218 | 0.219 | 0.491 | 2.871 | 0.705 | 0.0110 | 0.0006 | 0.0406 | 0.099 |
|             | MenPF-1   | 25              | 0.0068 | 0.0005 | 0.0003 | 0.0125 | 0.0046 | 0.0023 | 0.0003 | 0.0057 | 0.017 |

1F Mean

| Group / sex | Dosage (µg/dose) | 1 | 2 | 3 |
|-------------|-----------------|---|---|---|
| 2F          | MenPF-1         | 50 | 0.247 | 0.223 | 0.473 | 2.470 | 0.583 | 0.0104 | 0.0008 | 0.0477 | 0.069 |
|             | 0.0009          | 0.037 | 0.012 | 0.033 | 0.206 | 0.094 | 0.0044 | 0.0004 | 0.0174 | 0.020 |

2F Mean

| Group / sex | Dosage (µg/dose) | 1 | 2 | 3 |
|-------------|-----------------|---|---|---|
| 3F          | MenPF-1         | 50 | 0.219 | 0.244 | 0.473 | 3.225 | 0.705 | 0.0125 | 0.0005 | 0.0304 | 0.071 |
|             | 0.0012          | 0.020 | 0.035 | 0.030 | 0.105 | 0.226 | 0.0046 | 0.0003 | 0.0057 | 0.008 |

3F Mean

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
Table 18  (continued)
Relative Organ Weights (% Body Weight): Group Mean Values: Day 92

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|------------------|
|       |           | 1                | 2        | 3        |
|       | Control   | Control          | MenPF-1  | MenPF-1  |
| Thyroid | Mean    | 0.0078          | 0.0014  | 0.0090   |
|        | SD       | 0.0014          | 0.043    | 0.0013   |
|        | n        | 3               | 3        | 3        |
| Uterus | Mean    | 0.205           | 0.254    | 0.226    |
|        | SD       | 0.043           | 0.067    | 0.052    |
|        | n        | 3               | 3        | 3        |

Significantly different from Group 1:a=p<0.05,b=p<0.01,c=p<0.001
### Table 19
**Summary of Histological Findings: Day 66**

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|-----------------|---|---|---|
|       | Control   | 0               |   |   |   |
|       | MenPF-1   | 25              |   |   |   |
|       | MenPF-1   | 50              |   |   |   |

**GROUP TOTALS**

| HISTOLOGICAL FINDINGS | GROUP | Males | Females |
|-----------------------|-------|-------|---------|
|                       | Grp 1 | Grp 3 | Grp 1   | Grp 3   |
| ADRENAL GLAND         |       |       |         |
| No abnormality detected | 3     | 3     | 2       | 2       |
| Diffuse cortical cell hypertrophy | 0     | 0     | 0       | 1       |
| Cortical vacuolated cell focus | 0     | 0     | 1       | 0       |
| AORTA                 | (3)   | (3)   | (3)     | (3)     |
| No abnormality detected | 3     | 3     | 3       | 2       |
| Mineralisation, medial | 0     | 0     | 0       | 1       |
| APPENDIX              | (3)   | (3)   | (3)     | (3)     |
| No abnormality detected | 3     | 3     | 3       | 3       |
| BRAIN                 | (3)   | (3)   | (3)     | (3)     |
| No abnormality detected | 3     | 3     | 3       | 3       |
| CAECUM                | (3)   | (3)   | (3)     | (3)     |
| No abnormality detected | 3     | 3     | 3       | 3       |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | 1  | 2  | 3  |
|-------|----|----|----|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS | Males | Females |
|-----------------------|--------------|-------|---------|
|                       |              | Grp 1 | Grp 3  | Grp 1 | Grp 3  |
| CERVIX                |              | (3)   | (3)    |       |        |
| No abnormality detected |              | 3     | 3      |       |        |
| COLON                 |              | (3)   | (3)   | (3)  | (3)   |
| No abnormality detected |              | 3     | 3     | 3    | 3     |
| DUODENUM              |              | (3)   | (3)   | (3)  | (3)   |
| No abnormality detected |              | 3     | 3     | 3    | 3     |
| EPIDIDYMIS            |              | (3)   | (3)   |       |        |
| No abnormality detected |              | 1     | 3     |       |        |
| Aspermia, unilateral  |              | 2     | 0     |       |        |
| EYE                   |              | (3)   | (3)   | (3)  | (3)   |
| No abnormality detected |              | 3     | 3     | 3    | 3     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_530419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|------------------|---|---|---|
|       | Control   |                  |   |   |   |
|       | MenPF-1   | 25               |   |   |   |
|       | MenPF-1   | 50               |   |   |   |

| GROUP TOTALS |
|---|---|---|---|---|
| Males | Females | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
|---|---|---|---|---|---|
| FEMUR | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 3 | 3 |
| GALL BLADDER | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 3 | 3 |
| HEART | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 2 | 3 |
| Inflammatory cell foci, myocardial | 0 | 0 | 1 | 0 |
| ILEUM | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 3 | 3 |
| INJECTION SITE 1 | (3) | (3) | (3) | (3) |
| Macrophage accumulation, intramuscular | | | | |
| minimal | 0 | 0 | 0 | 2 |
| mild | 0 | 1 | 2 | 0 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| GROUP TOTALS | Males | Females |
|--------------|-------|---------|
| GROUP | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| INJECTION SITE 1 | (3) | (3) | (3) | (3) |
| Macrophage accumulation, intramuscular | 3 | 2 | 1 | 1 |
| moderate | 3 | 3 | 3 | 3 |
| Total Incidence | 0 | 0 | 0 | 1 |
| Inflammation, polymorphonuclear leukocytic | 0 | 1 | 0 | 1 |
| mild | 0 | 1 | 0 | 0 |
| moderate | 0 | 1 | 0 | 0 |
| Total Incidence | 0 | 2 | 0 | 2 |
| Inflammation, polymorphonuclear leukocytic, dermal, focal | 0 | 0 | 1 | 0 |
| minimal | 0 | 0 | 1 | 0 |
| Total Incidence | 0 | 0 | 1 | 0 |
| Inflammation, mononuclear cell | 0 | 0 | 0 | 1 |
| minimal | 1 | 1 | 2 | 0 |
| mild | 1 | 0 | 0 | 0 |
| moderate | 2 | 1 | 2 | 1 |
| Myofibre necrosis | 0 | 0 | 1 | 0 |
| minimal | 1 | 1 | 0 | 1 |
| mild | 0 | 1 | 0 | 0 |
| moderate | 0 | 1 | 0 | 0 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|-----------------------|--------------|
|                       | Males | Females |
|                       | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| INJECTION SITE 1      | (3)   | (3)   | (3)   | (3)   |
| Myofibre necrosis     |       |       |       |       |
| Total Incidence       | 1     | 2     | 1     | 1     |
| Regeneration, myofibre|       |       |       |       |
| minimal               | 0     | 0     | 1     | 2     |
| mild                  | 0     | 1     | 0     | 0     |
| Total Incidence       | 0     | 1     | 1     | 2     |
| Fibrosis, interstitial|       |       |       |       |
| minimal               | 0     | 0     | 0     | 1     |
| mild                  | 0     | 1     | 0     | 1     |
| marked                | 0     | 1     | 0     | 1     |
| Total Incidence       | 0     | 2     | 0     | 2     |
| Mineralisation        | 0     | 0     | 0     | 1     |
| JEJUNUM               | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected | 3   | 3     | 3     | 3     |
| KIDNEY                | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected | 1   | 1     | 3     | 1     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|------------------------|--------------|
|                        | Males | Females |
|                        | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| KIDNEY                 | (3)   | (3)   | (3)   | (3)   |
| Nephropathy, focal     | 0     | 1     | 0     | 0     |
| Basophilic tubules     | 2     | 1     | 0     | 2     |
| Tubular mineralisation | 0     | 1     | 0     | 2     |
| LACRIMAL GLAND         | (3)   | (2)   | (2)   | (3)   |
| No abnormality detected| 3     | 2     | 2     | 3     |
| LIVER                  | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected| 3     | 3     | 3     | 1     |
| Oval cell hyperplasia  | 0     | 0     | 0     | 1     |
| Inflammatory cell infiltration, periportal | 0 | 0 | 0 | 1 |
| LUNG                   | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected| 3     | 0     | 3     | 2     |
| Inflammatory cell foci | 0     | 2     | 0     | 1     |
| Osseous metaplasia, focal | 0 | 1 | 0 | 0 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MKMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | Test Item   | Dosage (µg/dose) | 1  | 2  | 3  |
|-------|-------------|------------------|----|----|----|
|       | Control     |                  | 0  |    |    |
|       | MenPF-1     | 25               |    |    |    |
|       | MenPF-1     | 50               |    |    |    |

**GROUP TOTALS**

| HISTOLOGICAL FINDINGS | GROUP | Males | Females |
|-----------------------|-------|-------|---------|
|                       | Grp 1 | Grp 3 | Grp 1   | Grp 3 |
| LYMPH NODE (INGUINAL) |       |       |         |       |
| No abnormality detected | 3     | 3     | 3       | 3     |
| MACROPHAGE ACCUMULATION |       |       |         |       |
| Minimal               | 1     | 2     | 0       | 2     |
| Mild                  | 0     | 0     | 1       | 0     |
| Total Incidence       | 1     | 2     | 1       | 2     |
| LYMPH NODE (LUMBAR)  |       |       |         |       |
| No abnormality detected | 1     | 1     | 1       | 0     |
| LYMPH NODE (MANDIBULAR) |     |       |         |       |
| No abnormality detected | 3     | 3     | 3       | 3     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| GROUP TOTALS | Males | Females |
|--------------|-------|---------|
| Grp 1 | Grp 3 | Grp 1 | Grp 3 |

| HISTOLOGICAL FINDINGS | GROUP |
|------------------------|-------|
| LYMPH NODE (MESENTERIC) | (3) (3) (2) (3) |
| No abnormality detected | 3 2 1 2 |
| Erythrocytosis/erythrophagocytosis | 0 1 1 1 |
| MAMMARY GLAND | (3) (3) |
| No abnormality detected | 2 3 |
| Duct ectasia | 1 0 |
| OESOPHAGUS | (3) (3) (3) (3) |
| No abnormality detected | 3 3 3 3 |
| OPTIC NERVE | (3) (3) (3) (3) |
| No abnormality detected | 3 3 3 3 |
| OVARY | (3) (3) |
| No abnormality detected | 3 3 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)  
Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|------------------------|--------------|
|                        | Males | Females |
|                        | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| OVIDUCT | (3) | (3) | 3 | 2 |
| No abnormality detected | 0 | 1 | |
| Cyst | |
| PANCREAS (ENDOCRINE) | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 3 | 3 |
| PANCREAS (EXOCRINE) | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 3 | 3 |
| PARATHYROID GLAND | (2) | (1) | (1) | (2) |
| No abnormality detected | 2 | 1 | 1 | 2 |
| PITUITARY GLAND | (2) | (3) | (3) | (3) |
| No abnormality detected | 2 | 3 | 3 | 3 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | Test Item   | Dosage (µg/dose) |
|-------|-------------|------------------|
| 1     | Control     | 0                |
| 2     | MenPF-1     | 25               |
| 3     | MenPF-1     | 50               |

|               | GROUP TOTALS |
|---------------|--------------|
|               | Males        | Females       |
| HISTOLOGICAL FINDINGS | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| PROSTATE      | (3)         | (3)         |       |
| No abnormality detected | 3   | 3         |       |
| RECTUM        | (3)         | (3)         | (3)   | (3)   |
| No abnormality detected | 3   | 3         | 3     | 3     |
| SALIVARY GLAND (SUBMAXILLARY) | (3) | (3) | (3) | (3) |
| No abnormality detected | 3   | 3         | 3     | 3     |
| SCIATIC NERVE | (3)         | (3)         | (3)   | (3)   |
| No abnormality detected | 3   | 3         | 3     | 3     |
| SEMINAL VESICLE | (3)   | (3)       |       |
| No abnormality detected | 3   | 3         |       |

Figures in brackets represent the number of animals from which this tissue was examined microscopically
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | Test Item     | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|---------------|------------------|---|---|---|
|       | Control       |                  |   |   |   |
|       | MenPF-1       | 25               |   |   |   |
|       | MenPF-1       | 50               |   |   |   |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|-----------------------|--------------|
|                       | Males | Females |
|                       | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| SKELETAL MUSCLE       |       |       |       |
| No abnormality detected | (3)  | (3)  | (3)  | (3)  |
| Inflammatory cell foci | 3    | 2    | 3    | 0    |
|                       | 0    | 1    | 0    | 3    |
| SKIN AND SUBCUTIS     |       |       |       |
| No abnormality detected | (3)  | (3)  | (3)  | (3)  |
|                       | 3    | 3    | 3    | 3    |
| SPINAL CORD           |       |       |       |
| No abnormality detected | (3)  | (3)  | (3)  | (3)  |
|                       | 3    | 3    | 3    | 3    |
| SPLEEN                |       |       |       |
| No abnormality detected | (3)  | (3)  | (3)  | (3)  |
|                       | 3    | 3    | 3    | 3    |
| STERNUM               |       |       |       |
| No abnormality detected | (3)  | (3)  | (3)  | (3)  |
|                       | 3    | 3    | 3    | 3    |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_L8E_KEEP1.SPL
Table 19  (continued)
Summary of Histological Findings: Day 66

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|-----------------|---|---|---|
| 1     | Control   |                 |   |   |   |
| 2     | MenPF-1   |                 | 0 | 25| 50|
| 3     | MenPF-1   |                 |   |   |   |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|-----------------------|--------------|
|                       | Males | Females |
|                       | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| STOMACH               | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected| 3 3 3 3 |
| TESTIS                | (3)   | (3)   |
| No abnormality detected| 1 2 |
| Seminiferous epithelial degeneration, unilateral| 2 0 |
| Segmental hypoplasia, focal| 0 1 |
| Immaturity, unilateral| 1 0 |
| THYMUS                | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected| 3 3 3 3 |
| THYROID GLAND         | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected| 3 2 3 3 |
| Inflammatory cell foci| 0 1 0 0 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
Table 19 (continued)
Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |     |     |     |     |
|------------------------|--------------|-----|-----|-----|-----|
|                        | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| TONGUE                 | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected | 3     | 3     | 3     | 3     |
| TRACHEA                | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected | 3     | 3     | 3     | 3     |
| URETER                 | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected | 3     | 3     | 3     | 3     |
| URINARY BLADDER        | (3)   | (3)   | (3)   | (3)   |
| No abnormality detected | 3     | 2     | 3     | 3     |
|                        |       |       | 0     | 1     |
|                        |       |       |       | 0     |
| UTERUS                 | (3)   | (3)   |     |     |
| No abnormality detected | 3     | 3     |     |     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICMAIN_LBE_KEEP1.SPL
### Table 19 (continued)
#### Summary of Histological Findings: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|------------------------|--------------|
|                        | Males | Females |
|                        | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| VAGINA                 |       |       |       |
| No abnormality detected| 3     | 3     | 3     |
| GUT ASSOCIATED LYMPHOID TISSUE |       |       |       |
| (3)                    | (3)   | (3)   | (3)   |
| No abnormality detected| 3     | 3     | 3     | 2     |
| Inflammation, Peyer's patch, focal | 0     | 0     | 0     | 1     |
| SACCULUS ROTUNDUS      |       |       |       |
| (3)                    | (3)   | (3)   | (3)   |
| No abnormality detected| 3     | 3     | 3     | 3     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MKMAIN_LBE_KEEP1.SPL
### Table 20
Summary of Histological Findings: Day 92

| Group   | 1 | 2 | 3 |
|---------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS                  | GROUP TOTALS |
|----------------------------------------|--------------|
|                                        | Males | Females |
|                                        | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| **INJECTION SITE 1**                   |       |       |       |       |
| No abnormality detected                | 0     | 1     | 2     | 1     |
| Macrophage accumulation, intramuscular |       |       |       |       |
| minimal                               | 0     | 0     | 0     | 1     |
| mild                                  | 1     | 0     | 0     | 0     |
| moderate                              | 2     | 2     | 1     | 1     |
| Total Incidence                       | 3     | 2     | 1     | 2     |
| **Inflammation, with necrosis**       |       |       |       |       |
| minimal                               | 0     | 1     | 0     | 0     |
| mild                                  | 0     | 1     | 0     | 1     |
| Total Incidence                       | 0     | 2     | 0     | 1     |
| **Inflammation, mononuclear cell**    |       |       |       |       |
| minimal                               | 0     | 0     | 0     | 1     |
| Total Incidence                       | 0     | 0     | 0     | 1     |
| **Myofibre necrosis**                 |       |       |       |       |
| minimal                               | 0     | 1     | 0     | 1     |
| Total Incidence                       | 0     | 1     | 0     | 1     |
| **Regeneration, myofibre**            |       |       |       |       |
| minimal                               | 0     | 2     | 0     | 1     |
| Total Incidence                       | 0     | 2     | 0     | 1     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICREC_LBE_KEEP1.SPL
Table 20 (continued)
Summary of Histological Findings: Day 92

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| HISTOLOGICAL FINDINGS | GROUP TOTALS | | | |
|-----------------------|--------------|---|---|---|
| | Males | | Females | |
| | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| INJECTION SITE 1 | (3) | (3) | (3) | (3) |
| Fibrosis, interstitial | | | | |
| minimal | 0 | 0 | 0 | 1 |
| mild | 0 | 2 | 0 | 0 |
| Total Incidence | 0 | 2 | 0 | 1 |
| INJECTION SITE 2 | (1) | | | |
| No abnormality detected | | | | |
| | 1 | | | |
| LYMPH NODE (INGUINAL) | (3) | (3) | (3) | (3) |
| No abnormality detected | 3 | 3 | 3 | 2 |
| Macrophage accumulation | | | | |
| minimal | 0 | 0 | 0 | 1 |
| Total Incidence | 0 | 0 | 0 | 1 |
| LYMPH NODE (LUMBAR) | (3) | (3) | (2) | (1) |
| No abnormality detected | 0 | 0 | 1 | 1 |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.

Pathology File Ref.: PLAFOR_520419_MICREC_LBE_KEEP1.SPL
Table 20 (continued)
Summary of Histological Findings: Day 92

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|-----------------|---|---|---|
|       | Control   | 0               |   |   |   |
|       | MenPF-1   | 25              |   |   |   |
|       | MenPF-1   | 50              |   |   |   |

| HISTOLOGICAL FINDINGS | GROUP TOTALS |
|-----------------------|--------------|
|                       | Males | Females |
|                       | Grp 1 | Grp 3 | Grp 1 | Grp 3 |
| LYMPH NODE (LUMBAR)   |       |       |       |       |
| Macrophage accumulation |       |       |       |       |
| minimal               | 1     | 1     | 1     | 0     |
| mild                  | 2     | 0     | 0     | 0     |
| Total Incidence       | 3     | 1     | 1     | 0     |
| Erythrocytosis/erythrophagocytosis | 1     | 3     | 0     | 0     |

Figures in brackets represent the number of animals from which this tissue was examined microscopically.
Appendices
Appendix 1
Protocol, Amendments and Deviations

FINAL PROTOCOL

Test Facility Study No. 520419

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

SPONSOR:
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Oxford, OX3 9DU
UK

TEST FACILITY:
Charles River Laboratories
Preclinical Services, Tranent (PCS-EDI)
Edinburgh, EH33 2NE
UK

03 August 2011
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Protocol, Amendments and Deviations

1. OBJECTIVE(S)
A prophylactic vaccine for the prevention of infection from bacterial meningitis is under development by the Sponsor. The objective of this study is to determine the potential toxicity of MenPF-1 Vaccine when given by intramuscular injection for 4 occasions over a 9 week period to rabbits to evaluate the potential reversibility of any findings, and to provide data to support the use of MenPF-1 in humans. In addition immunogenicity will be characterised.

2. PROPOSED STUDY SCHEDULE
Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Experimental Start Date: 10 Aug 2011
(First date of study-specific data collection)

Experimental Completion Date: Nov 2011
(Last date data are collected from the study)

Animal Arrival/Transfer: 02 Aug 2011

Initiation of Dosing: 17 Aug 2011

Completion of In-life: 16 Nov 2011
(Last date of necropsy)

Unaudited Draft Report: 09 Dec 2011

Final Report: 10 Feb 2012
(Expected date of Study Director signature)

3. GUIDELINES FOR STUDY DESIGN
The design of this study was based on the study objective(s), the overall product development strategy for the test item, and the following study design guidelines:

- Committee for Medicinal Products for Human Use (CHMP). Note for Guidance on Repeated Dose Toxicity. CPMP/SWP/1042/99rev1.
- ICH Harmonised Tripartite Guideline S6. Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.
- CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/ICH/302/95). December 1997.
- WHO guidelines on nonclinical evaluation of vaccines (WHO Technical report series No. 927, 2005)
- CPMP Note for Guidance on Non-Clinical Local Tolerance Testing of Medicinal Products (CPMP/SWP/2145/00). March 2001.
4. REGULATORY COMPLIANCE

This study will be performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as accepted by Regulatory Authorities throughout the European Community, United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI).

The test site for antibody determination is not a member of the UK GLP Compliance Programme, however, it is the responsibility of Charles River to implement adequate study management, monitoring and QAU mechanisms to ensure work undertaken at this test site is conducted in accordance with the principles of GLP.

5. QUALITY ASSURANCE

5.1. Test Facility

The Test Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the protocol, conduct inspections at intervals adequate to assure the integrity of the study, and audit the Final Report to assure that it accurately describes the methods and standard operating procedures and that the reported results accurately reflect the raw data of the study.

5.2. Test Site

The test facility QAU conducted a pre-study facility inspection of the test site (National Institute of Biological Standards and Controls).

The conduct of the following study phase will be audited by the Test Facility QAU:

- Antibody determination

For the study phase inspected by the Test Facility QAU, copies of each inspection report will be made available to the Study Director and Test Facility Management. The Test Facility QAU will also audit the data generated and relevant sections of the report for this phase of the study.

6. SPONSOR

Sponsor Representatives

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Appendix 1  (continued)
Protocol, Amendments and Deviations

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7. RESPONSIBLE PERSONNEL

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Appendix 1 (continued)
Protocol, Amendments and Deviations

Test Facility-designated Individual Scientists (IS)

Pathologist          TBC
Charles River Laboratories
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E-mail: name@crl.com

Each IS is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. Each IS will provide a report addressing their assigned phase of the study, which will be included as an appendix to the Final Report.

Sponsor-designated Responsible Scientist (RS)

Antibody Analysis    Caroline Vipond PhD
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National Institute of Biological Standards and Control
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E-mail: caroline.vipond@nibsc.hpa.org.uk

The RS is required to report any deviations or other circumstances that could affect the quality or integrity of the study to the Study Director in a timely manner. The RS will provide results in table format (QC checked), including description of methods used addressing their assigned phase of the study, which will be included as an appendix to the Final Report.

All records from the antibody determination (copy of protocol and amendments, raw data, original QC’d data, calibration records etc.) will be returned to Charles River for archiving with remaining study data.

8. TEST AND CONTROL ITEMS

8.1. Test Item

Identification: MenPF-1
Supplier: Department for Biopharmaceutical production. Norwegian Institute of Public Health, Oslo. Norway

Batch (Lot) Number: FMOX1102
Expiration Date: Concomitant assessment. ongoing
Physical Description: Opaque, even. milky suspension; easily redispersed
Appendix 1 (continued)
Protocol, Amendments and Deviations

Purity: The active pharmaceutical ingredient (API), formulated as outer membrane vesicles, is a mixture of *Neisseria meningitidis* serogroup B outer membrane proteins that shows >93% adsorption degree to aluminium hydroxide adjuvant. The API contains 8.0% 70kD Feta F3-3 variant protein, 21.7% Class 1 P1.7.16 variant protein and 32.6% Class 3 P3.15 protein. The test item batch (i.e., vaccine product) contains 1.0 mg/mL aluminium. Dose calculations will not be corrected for purity.

Correction Factor: Not applicable

Concentration: 25 μg protein/dose of 0.5 mL

Storage Conditions: In a refrigerator set to maintain 4°C

8.2. Control Item

Identification: MOX Control

Supplier: Department for Biopharmaceutical production, Norwegian Institute of Public Health. Oslo, Norway

Batch (Lot) Number: FMOX1103

Expiration Date: Concomitant assessment. ongoing

Physical Description: Opaque, even milky suspension, easily redispersed

Purity: The product contains Alhydrogel, specifically containing 1.1 mg/mL aluminium. Dose calculations will not be corrected for purity.

Concentration: 0.333 % w/v Alhydrogel in 3% sucrose solution

Storage Conditions: In a refrigerator set to maintain 4°C

8.3. Test and Control Item Characterisation

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity, composition, and stability for the test and control item(s). A Certificate of Analysis (CoA) or equivalent documentation will be provided for inclusion in the Final Report. The Sponsor will also provide information concerning the regulatory standard that was followed for these evaluations. Potency data (immunogenicity) will not be provided as part of the CoA on delivery of the test item.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test and control items, and this information is available to the appropriate regulatory agencies should it be requested.

8.4. Reserve Samples

For each batch (lot) of test and control item, a reserve sample (approximately 1 vial) will be collected and maintained under the appropriate storage conditions by the Test Facility.
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8.5.  Test and Control Item Inventory and Disposition

Records of the receipt, distribution, and storage of test and control items will be maintained. With the exception of reserve samples, all unused Sponsor-supplied test and control items will be returned to the Sponsor after finalisation of the study report.

Shipping Contact
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9.  SAFETY

Safety instructions for this study are provided on the Sponsor supplied safety data sheet. An internal COSHH safety sheet will be prepared at Charles River.

10. DOSE FORMULATION AND ANALYSIS

10.1. Preparation of Control Item

The control item, MOX control, is provided in single dose vials for administration to Group 1 control animals. No aliquoting of the control item is required and 0.5 mL will be withdrawn from each vial. The control item vials will be stored in a refrigerator set to maintain 4°C until use. The aliquots will be removed from the refrigerator and allowed to warm to room temperature for at least 30 minutes before dosing. To ensure homogeneity, the vials must be shaken before drawing the volume intended for injecting.

Any residual volumes will be discarded before issuance of the Final Report.

10.2. Preparation of Test Item

The test item, MenPF-1 Vaccine, is provided in single dose vials and will be administered as received. No aliquoting of the test item is required. An adequate amount of the test item will be dispensed; 0.5 mL of suspension for 25 microgrammes of protein. The vials will be removed
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from the refrigerator and allowed to warm to room temperature for at least 30 minutes before dosing. To ensure homogeneity, the vials must be shaken before drawing the volume intended for injecting.

Any residual volumes will be discarded before issuance of the Final Report.

10.3.   Sample Collection and Analysis
The test and control items will be used as received from the Sponsor: therefore, samples for dose formulation analysis will not be collected by the Test Facility.

11.   TEST SYSTEM
Species: Rabbit
Strain: New Zealand White
Source: Harlan UK
Number of Males Ordered: 18
Number of Females Ordered: 18
Target Age at the Initiation of Dosing: 12 weeks
Target Weight at the Initiation of Dosing: 2.5 kg
The actual age, weight, and number of animals received will be listed in the Final Report.

11.1.   Justification of Test System and Number of Animals
At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist.

The rabbit has been selected by the Study Director in consultation with the Sponsor as the test model:
- to satisfy regulatory requirements for toxicity testing.
- because of the availability of background data and proven suitability in toxicology studies.
Immunogenicity can also be investigated in this species.

The number of animals chosen for this study is the smallest number considered necessary to provide sufficient data.

11.2.   Animal Identification
Each animal will receive a unique ear tag which will identify it individually within the study and which corresponds to that animal's number.
Appendix 1  (continued)
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11.3. Environmental Acclimation
The animals will be allowed to acclimate to the Charles River rabbit toxicology accommodation for a period of up to 2 weeks before the commencement of dosing.

11.4. Selection, Assignment, Replacement and Disposition of Animals
Animals will be removed in random order from their transport boxes and allocated to dose group on arrival by placing them in separate cages. Cages will be housed on racks according to treatment and labelled with the study number, animal number and group number.

Control animals will be housed on a separate rack.

Animals suspected of being diseased will be culled from the study. If significant numbers of animals are unsuitable, the entire batch will be rejected by the Study Director and a new batch obtained.

During the week before the commencement of dosing, the animals will be approved for entry into the experiment on the basis of satisfactory clinical observation records and body weight profile.

The disposition of all animals will be documented in the study records.

12. HUSBANDRY

12.1. Housing
Cage type: Stainless steel cages containing an automatic watering valve, mesh tops and a metal food hopper with a ‘Noryl’ dual level interior and perforated floor. Beneath each cage will be a suspended tray containing absorbent paper. Paper will be changed at least once each week.

Cage size: Approximate dimensions 77 x 70 x 48 cm.
Cage rack: Cages will be suspended on movable racks
Animal housing: Individually

Cages and racks will be changed as necessary throughout the course of the study as detailed in Charles River SOPs.

The animal room floor and work surfaces will be washed as necessary with disinfectant solution.

12.2. Environmental Conditions
The targeted conditions for animal room environment will be as follows:

Temperature: 16°- 20°C
Humidity: 40%-85%
Ventilation: A minimum of 15 air changes per hour
Appendix 1 (continued)
Protocol, Amendments and Deviations

Light Cycle: 12 hours light and 12 hours dark (except when interrupted by study procedures/activities)

There will be automatic control of temperature which will be continuously monitored and recorded. Humidity will be continuously monitored and recorded. Deviations from target temperature and humidity ranges will be presented in the study report.

There will be automatic control of light cycle.

12.3. Food
Each animal will receive Harlan Diet supplied by Harlan, UK.

The food will be available to the animals ad libitum. Each animal will also be offered a supplement of hay at least 3 times per week.

The diet used is considered not to contain any additional substances, in sufficient concentration, to have any influence on the outcome of the study.

The diet will be supplied with a batch analysis for major nutritive components and significant contaminants and will be used within the manufacturers' designated shelf-life. The hay is not analysed.

An analytical certificate for each batch of diet used will be retained at Charles River, Edinburgh.

12.4. Water
The animals will have access to water ad libitum from the public supply.

The water used by Charles River Edinburgh is analysed at regular intervals for dissolved materials, heavy metals, pesticide residues, pH, nitrates and nitrites. Microbiological screening is also conducted. An analytical certificate for each analysis will be retained at Charles River, Edinburgh.

The water used is considered not to contain any additional substances, in sufficient concentration, to have any influence on the outcome of the study.

12.5. Animal Enrichment
Wooden chewsticks and bunny blocks will be provided with a certificate of analysis for significant contaminants. An analytical certificate for each batch of chewsticks and bunny blocks used will be retained at Charles River, Edinburgh.

Other items may be included to enrich the cage environment. Details will be given in the study report.

12.6. Veterinary Care
All animals are under the care of Charles River clinical veterinary surgeons, who are available at all times to provide advice and assistance. All treatment used to prevent or control intercurrent diseases will be implemented at the discretion of the Study Director, and where possible after consultation with the Sponsor. Records will be maintained for all affected individual animals
13. EXPERIMENTAL DESIGN

Experimental Design

| Group Number | Animal Numbers | Test Item | Dosage (ug/dose) | Conc. (ug/mL) | Dose Volume (mL/dose) |
|--------------|----------------|-----------|------------------|---------------|----------------------|
|              | Main Study     | Recovery  |                  |               |                      |
| 1            | 1-3            | 19-21     | 4-6              | 28-30         | MOX Control          | 0                     | 0                     | 0.5 mL               |
| 2            | 7-9            | 22-24     | 10-12            | 31-33         | MenPF-1              | 25                    | 50                   | 0.5 mL               |
| 3            | 13-15          | 25-27     | 16-18            | 34-36         | MenPF-1              | 50                    | 50                   | 2 x 0.5 mL            |

13.1. Administration of Test and Control Items

The test and control items will be administered to the appropriate rabbits by intramuscular injection on Days 1, 22, 43 and 64. The dose volume will be 0.5 mL or 2 x 0.5 mL. The first day of dosing for each animal will be designated as Day 1. The injection site will be the left hind limb (Injection Site 1). The same site will be used each injection. The site will be clipped free from hair and marked afterwards.

Vaccine vials will be inverted before dosing.

For necropsy the site will be clipped free from hair and marked.

13.2. Justification of Route and Dosage Levels

The intramuscular route of administration has been selected for this study as this route has been defined by the Sponsor as the route of clinical application/human exposure.

The dose levels have been agreed with the Sponsor and took into account the maximum tolerated dose in the test model and other factors such as anticipated therapeutic dose. The test item has been produced with a similar methodology as for the vaccine product MenBvac (Norwegian Institute of Public Health), based on deoxycholate extracted outer membrane vesicles from *Neisseria meningitidis*. MenBvac is known to be moderately reactogenic but safe in humans (Nøkleby et al. Vaccine 2007: 16: 3080-4).

Clinical injections are planned every 6 weeks, with three doses intended. In this preclinical study injections will be given over a shorter period and one more injection (n + 1) will be given. The intended clinical dose is 25 µg/dose. This amount and 2x this amount is being given in full in this preclinical study and based on body weight of rabbit 3 kg: human 60 kg and the administration of an additional injection this is considered to provide adequate safety data.
14. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

The in-life procedures, observations, and measurements listed below will be performed for all main study and recovery animals.

14.1. Mortality/Moribundity Checks

Frequency: All animals will be checked early morning and as late as possible each day for viability.

Procedure: Any animal showing signs of severe debility or intoxication and if determined to be moribund or suffering excessively will be euthanised.

14.2. Clinical Observations

14.2.1. Detailed Clinical Observations

Frequency: Once weekly commencing during the last week of the prestudy period.

Procedure: Animals removed from the cage for examination.

14.2.2. Postdose Observations

Frequency: Dosing days - Regularly throughout the day. Non-dosing days - Once each day.

Procedure: All the animals will be examined for reaction to treatment. The onset, intensity and duration of these signs will be recorded (if appropriate), particular attention being paid to the animals during and for the first hour after dosing.

14.3. Dermal Scoring

Frequency: At each injection: 0 h (before dosing), 24 h, 48 h after dosing.

Procedure: Skin will be assessed for erythema and eschar formation, oedema formation, skin thickening, desquamation and any other reaction to treatment.

| Erythema and Eschar Formation                                      | Grade |
|-------------------------------------------------------------------|-------|
| No erythema                                                      | 0     |
| Very slight erythema (barely perceptible)                        | 1     |
| Well defined erythema                                            | 2     |
| Moderate to severe erythema                                      | 3     |
| Severe erythema (beet redness) to slight eschar formation         | 4     |
| (injuries in depth)                                              |       |

| Oedema Formation                                                  | Grade |
|-------------------------------------------------------------------|-------|
| No oedema                                                        | 0     |
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14.4.  Body Weights
Frequency:  
  Pretrial – Once  
  Dosing Period – Twice weekly  
  Recovery Period – Twice weekly

Procedure:  Animals showing weight loss or deterioration in condition will be weighed more frequently as necessary.

14.5.  Food Consumption
Frequency:  
  Pretrial – Twice weekly  
  Dosing Period – Twice weekly  
  Recovery Period – Twice weekly

Procedure:  The quantity of food consumed by each animal will be measured and recorded.

14.6.  Water Consumption
Procedure:  Water consumption will not be measured as all animals are on an automatic watering system.

14.7.  Ophthalmic Examinations
Frequency:  
  Pretrial – Once  
  Dosing Period – At end of dosing period

Procedure:  The eyes will be examined using an indirect ophthalmoscope after the application of a mydriatic agent (1% Tropicamide, Mydriacyl®). The anterior, lenticular and fundic areas will be examined.

14.8.  Body Temperature
Frequency:  
  Pretrial – All animals once  
  Dosing Period –0 h (before dosing), 1 h, 3 h, 24 h and 48 h after dosing

Procedure:  Measured by digital thermometer inserted into ear.
Appendix 1  (continued)
Protocol, Amendments and Deviations

15. LABORATORY EVALUATIONS

15.1. Clinical Pathology

15.1.1. Sample Collection

Blood will be collected from an auricular artery. Additional blood samples may be obtained (e.g. due to clotting of non-serum samples) if permissible sampling frequency and blood volume are not exceeded. After collection, samples will be transferred to the appropriate laboratory for processing.

Animals will not be fasted. Samples will be collected according to the following table.

| Group Nos. | Time Point  | Haematology | Coagulation | Clinical Chemistry |
|------------|-------------|-------------|-------------|-------------------|
| 1-3        | Prettrial   | X           | X           | X                 |
| 1-3        | Day 66      | X           | X           | X                 |
| 1-3        | Day 92      | X           | X           | X                 |
| Unscheduled euthanasia (when possible) Before euthanasia | X | X | X |

X = sample to be collected.

Any residual/retained clinical pathology samples will be discarded before issuance of the Final Report.

15.1.2. Haematology

Target Volume: 0.5 mL
Anticoagulant: EDTA

| Haematology Parameters | White blood cell count |
|------------------------|------------------------|
| Red blood cell count   | Neutrophils            |
| Haemoglobin            | Lymphocytes            |
| Haematocrit            | Monocytes              |
| Mean cell volume       | Eosinophils            |
| Mean cell haemoglobin  | Basophils              |
| Reticulocytes          | Large unstained cells  |
| Reticocyte count (absolute) | Other cells (as appropriate) |
| Red blood cell distribution width | |
| Platelets              |                         |
| Blood Smear (see ^ below) |                       |

^ A blood smear will be prepared from each haematology specimen. Blood smears will be labelled, stained, stored and archived. The smears may be subsequently evaluated and this will be described in a protocol amendment with approval of the Study Director and Sponsor.
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15.1.3. Coagulation
Target Volume: 0.9 mL
Anticoagulant: 3.8% (w/v) trisodium citrate
Processing: To plasma

| Activated partial thromboplastin time | Prothrombin time |
|--------------------------------------|------------------|
| Fibrinogen                           |                  |

15.1.4. Clinical Chemistry
Target Volume: 1.5 mL
Anticoagulant: Lithium Heparin
Processing: To plasma

| Urea | Total protein |
|------|--------------|
| Glucose | Albumin |
| Aspartate aminotransferase | Globulin |
| Alanine aminotransferase | Albumin/globulin ratio |
| Alkaline phosphatase | Cholesterol |
| Creatine phosphokinase | Creatinine |
| Lactate dehydrogenase | Total bilirubin |
| Sodium | Calcium |
| Potassium | Inorganic phosphate |
| Chloride | |

15.1.5. Bone Marrow Smear Evaluation
Bone marrow smears will be collected as described in the Tissue Collection and Preservation table (Section 16.5). Evaluation of stained smears may be added by amendment at the discretion of the Study Director in consultation with the pathologist and the Sponsor.

15.2. Antibody Sample Collection, Processing, and Analysis
Blood will be collected from all animals from an auricular artery.
Time Points: Pretrial, before dosing on Day 22 and Day 64 and on Day 92.
Target Volume: 2 mL
Anticoagulant: None
Processing: To serum – centrifugation at least 1500 g/2°C-8°C/10 min
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The serum samples will be stored in a freezer set to maintain -80°C and then shipped on dry ice. All samples must remain frozen (temperature required -80°C) and temperature must be recorded during transportation.

Shipping Contact
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National Institute of Biological Standards and Control
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UK
Tel: 01707 641567
E-mail: caroline.vipond@nibsc.hpa.org.uk

The immunology laboratory will be notified before shipment of the samples. Upon receipt at the immunology laboratory, the samples will be stored ≤-20°C.

The samples will be analysed for antibodies against MenPF-1 using a validated ELISA analytical method. No validation has been performed for the plate reader software, however the calibration performed by the service engineer confirms Operational Qualification (OQ) and standards, and QC samples run with each batch of samples confirm Performance Qualification (PQ) of the reader.

Any residual/retained anti-therapeutic antibody samples will be retained for research purposes. The results from any subsequent analyses of these samples will not be covered in this study.

16. TERMINAL PROCEDURES

Terminal procedures are summarised in the following table:

| Group Number | Number of Animals | Scheduled Euthanasia Day | Necropsy Procedures | Histology | Histopathology |
|--------------|-------------------|--------------------------|---------------------|-----------|---------------|
|              | M | F | Necropsy | Tissue Collection | Organ Weights |           |                |
| 1            | 3 | 3 | X         | X                  | X            | Full Tissue  | Full Tissue*  |
| 2            | 3 | 3 | X         | X                  | X            | None         | None          |
| 3            | 3 | 3 | X         | X                  | X            | Full Tissue  | Full Tissue*  |
| 1            | 3 | 3 | X         | X                  | X            | Select Tissues | Select Tissues* |
| 2            | 3 | 3 | X         | X                  | X            | None         | None          |
| 3            | 3 | 3 | X         | X                  | X            | Select Tissues | Select Tissues* |
| Unscheduled Deaths | X | X | -         | Full Tissue       | Full Tissue |

X = procedure to be conducted; * = not applicable.
* See Tissue Collection and Preservation table for listing of tissues.
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b Injection site and lumbar and inguinal lymph node.

16.1. Unscheduled Deaths
If a main study or recovery animal dies on study, a necropsy will be conducted and specified tissues will be saved. If necessary, the animal will be refrigerated to minimise autolysis.

Main study or recovery animals may be euthanised for humane reasons as per Test Facility SOPs. The body weight will be recorded and samples for evaluation of clinical pathology parameters and antibody analysis will be obtained if possible as specified in Section 15. These animals will undergo necropsy, and specified tissues will be retained. If necessary, the animal will be refrigerated to minimise autolysis.

16.2. Scheduled Euthanasia
Main study and recovery animals surviving until scheduled euthanasia will have a terminal body weight recorded, and will be euthanised by an intravenous overdose of a barbiturate, followed by exsanguination. When possible, the animals will be euthanised rotating across dose groups such that similar numbers of animals from each group, including controls will be necropsied throughout the day. Animals will not be fasted before their scheduled necropsy.

16.3. Necropsy
Main study and recovery animals will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Necropsy examinations will be conducted by a trained technician and will consist of an external and internal examination and recording of observations for all animals. A veterinary pathologist will be available for consultation during normal working hours.

At the discretion of the necropsy supervising pathologist, images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented and communicated to the Study Director. Images and associated documentation will be retained and archived.

16.4. Organ Weights
The organs identified for weighing in the Tissues Collection and Preservation table will be weighed at necropsy for all scheduled euthanasia animals. Organ weights will not be recorded for animals found dead or euthanised in poor condition or in extremis. Paired organs will be reported together. Terminal body weights will be used for organ weight analysis.

16.5. Tissue Collection and Preservation
Representative samples of the tissues identified in the Tissue Collection and Preservation table will be collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated. Additional tissue samples may be collected to elucidate abnormal findings.
## Tissue Collection and Preservation

| Tissue                        | Weigh | Collect | Microscopic Evaluation | Comment                                                                                     |
|-------------------------------|-------|---------|------------------------|---------------------------------------------------------------------------------------------|
| Administration site           | -     | X       | X                      | Injection Site 1. Collect additional muscle around marked area as contingency.               |
| Animal identification         | -     | X       | -                      | -                                                                                           |
| Artery, aorta                 | -     | X       | X                      | From thoracic segment.                                                                       |
| Bone marrow smear             | -     | X       | -                      | One bone marrow smear will be collected from the femur at scheduled necropsies only (for possible examination). Smears will not be collected from animals that are found dead. Bone marrow smears are allowed to air dry and are not fixed in formalin. |
| Bone marrow, femur            | -     | X       | X                      | Collect with bone, femur                                                                     |
| Bone marrow, sternum          | -     | X       | X                      | Collect with bone, sternum                                                                   |
| Bone, femur with articulating surface | -   | X       | X                      | Collect distal end to include femoral tibial joint.                                           |
| Bone, sternum                 | -     | X       | -                      | -                                                                                           |
| Brain                         | X     | X       | X                      | Forebrain, midbrain, cerebellum. and medulla oblongata.                                      |
| Cervix                        | -     | X       | X                      | Collect with uterus.                                                                         |
| Epididymis                    | X     | X       | X                      | Separate weights and examination.                                                            |
| Eye                           | -     | X       | X                      | Separate examination: Preserve in Davidson's fixative.                                       |
| Gallbladder                   | -     | X       | X                      | -                                                                                           |
| Gland, adrenal                | X     | X       | X                      | Separate weights and examination.                                                            |
| Gland, lacrimal               | -     | X       | X                      | Only 1 required for examination.                                                             |
| Gland, mammary                | -     | X       | X                      | Collect with thoracic skin and include nipple; mammary gland will be examined in females only |
| Gland, parathyroid            | -     | X       | X                      | Collect with thyroid: Examine only if present in the routine section of thyroid.             |
| Gland, pituitary              | X     | X       | X                      | -                                                                                           |
| Gland, prostate               | X     | X       | X                      | -                                                                                           |
| Gland, salivary               | -     | X       | X                      | Submandibular: Only 1 required for examination.                                              |
| Gland, seminal vesicle        | -     | X       | X                      | -                                                                                           |
| Gland, thyroid                | X     | X       | X                      | Separate weights and examination; weight includes parathyroid.                               |
| Gross lesions/masses          | -     | X       | X                      | -                                                                                           |
| Gut-associated lymphoid tissue| -     | X       | X                      | Collect with small intestine.                                                                |
| Heart                         | X     | X       | X                      | -                                                                                           |
| Kidney                        | X     | X       | X                      | Separate weights and examination.                                                            |
| Large intestine, appendix     | -     | X       | X                      | -                                                                                           |
| Large intestine, caecum        | -     | X       | X                      | -                                                                                           |
| Large intestine, colon         | -     | X       | X                      | -                                                                                           |
| Large intestine, rectum        | -     | X       | X                      | -                                                                                           |
| Large intestine: sacculus rotundus | -   | X       | X                      | Drain gallbladder before weighing                                                            |
| Liver                         | X     | X       | X                      | Infuse with 10% neutral buffered formalin after weighing.                                     |
| Lung                          | X     | X       | X                      | Only 1 required for examination.                                                             |
| Lymph node, mandibular        | -     | X       | X                      | -                                                                                           |
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| Tissue                           | Weigh | Collect | Microscopic Evaluation | Comment                                                   |
|----------------------------------|-------|---------|------------------------|-----------------------------------------------------------|
| Lymph node, mesenteric          | -     | X       | X                      | -                                                         |
| Lymph node, lumbar              | -     | X       | X                      | Identify left and right.                                  |
| Lymph node, inguinal            | -     | X       | X                      | Identify left and right.                                  |
| Muscle, skeletal                 | -     | X       | X                      | From thigh                                                |
| Nerve, optic                    | -     | X       | X                      | Preserve in Davidson’s fixative; Examine only if present in the routine section of the eye. |
| Nerve, sciatic                   | -     | X       | X                      | Only 1 required for examination.                          |
| Oesophagus                       | -     | X       | X                      | -                                                         |
| Ovary                            | X     | X       | X                      | Separate weights and examination.                         |
| Oviduct                          | -     | X       | X                      | Only 1 required for examination. Collect with uterus     |
| Pancreas                         | -     | X       | X                      | -                                                         |
| Skin                             | -     | X       | X                      | Collect with mammary gland.                               |
| Small intestine, duodenum        | -     | X       | X                      | -                                                         |
| Small intestine, ileum           | -     | X       | X                      | -                                                         |
| Small intestine, jejunum         | -     | X       | X                      | -                                                         |
| Spinal cord                      | -     | X       | X                      | Cervical, thoracic, lumbar.                               |
| Spleen                           | X     | X       | X                      | -                                                         |
| Stomach                          | -     | X       | X                      | Fundus and pylorus                                        |
| Testis                           | X     | X       | X                      | Separate weights and examination; Preserve in Modified Davidson’s fixative. |
| Thymus                           | X     | X       | X                      | -                                                         |
| Tongue                           | -     | X       | X                      | -                                                         |
| Trachea                          | -     | X       | X                      | -                                                         |
| Ureter                           | -     | X       | X                      | Only 1 required for examination.                          |
| Urinary bladder                  | -     | X       | X                      | -                                                         |
| Uterus                           | X     | X       | X                      | -                                                         |
| Vagina                           | -     | X       | X                      | -                                                         |

X = procedure to be conducted; - = not applicable.

### 17. HISTOLOGY AND HISTOPATHOLOGY

#### 17.1. Histology

Tissues in the Tissue Collection and Preservation table from animals identified in the Terminal Procedures table will be embedded in paraffin, sectioned, mounted on glass slides, and stained with haematoxylin and eosin.

#### 17.2. Histopathology

Histopathological evaluation will be performed by a veterinary pathologist with training and experience in laboratory animal pathology. Any additional stains or evaluations, if deemed necessary by the pathologist, will be added by protocol amendment following discussion with the Study Director and in consultation with the Sponsor.

At the discretion of the study pathologist and after acknowledgement by the study director, images may be captured for consultation purposes.
17.3. Pathology Peer Review

A pathology peer review, as per the appropriate SOP of the Pathology Department, will be conducted by a second pathologist at:

Charles River Laboratories
Preclinical Services
Tranent
Edinburgh, EH33 2NE
UK

The peer review statement or equivalent documentation will be included as an appendix to the Final Report.

18. COMPUTERISED SYSTEMS

The following critical computerised systems will be used in the study. Any additional critical computerised systems used during the course of the study will be added by protocol amendment. The actual critical computerised systems used will be specified in the Final Report.

Data for parameters not required by protocol, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by protocol and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

| System Name | Description of Data Collected and/or Analysed |
|-------------|-----------------------------------------------|
| Dispense    | Dose Formulation                             |
| Provanis    | In-life data collection and reporting         |
| Nautilus 2003 | Clinical Pathology Laboratory Information Management System (LIMS) |
| PLACES 2000 | Histopathology/Organ Weights                  |

19. STATISTICAL ANALYSIS

Unless otherwise stated, all statistical tests will be two-sided and performed at the 5% significance level using in-house software. Males and females will be analysed separately.

Pairwise comparisons will only be performed against the control group (Group 1). The following pairwise comparisons will be performed:

- Control Group v Group 2
- Control Group v Group 3

Body weight, food consumption, haematology, coagulation and clinical chemistry will be analysed for homogeneity of variance using the ‘F-Max’ test. If the group variances appear homogeneous, a parametric ANOVA will be used and pairwise comparisons will be made using Fisher’s F protected LSD method via Student’s t test i.e. pairwise comparisons will be made only
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if the overall F-test is significant. If the variances are heterogeneous, log or square root transformations will be used in an attempt to stabilise the variances. If the variances remain heterogeneous, then a Kruskal-Wallis non-parametric ANOVA will be used and pairwise comparisons will be made using chi squared protection (via z tests. the non-parametric equivalent of Student’s t test).

In circumstances where it is not possible to perform the F Max test due to zero standard deviation in at least one group, the non-parametric ANOVA results will be reported.

Organ weights will be analysed using ANOVA as above and by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. In addition, organ weights as a percentage of terminal body weight will be analysed using ANOVA.

In circumstances where the variances in the ANCOVA remain heterogeneous following log or square root transformations, the data will be subjected to a rank transformation prior to analysis. Where it is not possible to perform the F-Max test due to the small sample size (less than 3 animals in any group), the untransformed parametric ANCOVA results will be reported.

In the ANOVA and ANCOVA summary tables, the results of the analysis will be reported indicating the level of statistical significance (p<0.05, p<0.01 and p<0.001) of each pairwise comparison.

Actual p-values will not be reported in the summary tables for these analyses.

More extensive analysis will be carried out after consultation with the sponsor and will involve additional costs.

20. AMENDMENTS AND DEVIATIONS

Changes to the approved protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary protocol changes in advance with the Sponsor.

All protocol and SOP deviations will be documented in the study records. Deviations from the protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the RS, and reported to the Study Director for authorisation/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

21. RETENTION OF RECORDS, SAMPLES AND SPECIMENS

All study-specific raw data, documentation, protocol, samples, specimens, and interim (if applicable) and final reports from this study are the property of the Sponsor. These materials will be available at the Test Facility during the study and will be transferred to the Test Facility archive by no later than the date of final report issuance and will be archived for a period of 2 years. After this period, the Sponsor will be contacted to determine the disposition of these materials.

Electronic data generated by the Test Facility will be archived and the software and hardware required to produce it in a readable form will be maintained and available.
Appendix 1  (continued)  
Protocol, Amendments and Deviations

All records, samples, specimens and reports generated from phases or segments performed by Test Facility-designated subcontractors and the Test Site will be returned to the Test Facility for archiving.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Protocol, protocol amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test and control item receipt, identification, preparation, and analysis
- In-life measurements and observations
- Clinical pathology sample collection and evaluation
- Bioanalytical sample collection and evaluation
- Gross and microscopic observations and related data (including internal peer review notes)
- Organ weight measurements
- Statistical analysis results

22. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalised following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (bookmarked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility’s handwritten signatures will be retained.

23. ANIMAL WELFARE

The UK Home Office controls scientific procedures on animals in the UK and does so by the issue of licences under the Animals (Scientific Procedures) Act 1986. The regulations conform to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, Council of Europe) and achieve the standard of care required by the US Department of Health and Human Services’ Guide for the Care and Use of Laboratory Animals.
Appendix 1 (continued)
Protocol, Amendments and Deviations

The Home Office licence governing this study strictly specifies the limits of severity of effects on the animals. From the available information, the procedures described in the protocol are not anticipated to cause any effects which exceed the severity limit of the procedure. Any animal which shows unacceptable reactions may be euthanised or other actions taken as required by the Home Office to alleviate distress.

23.1. Home Office Project Licence No.
PPL 60/4185, Toxicology of Pharmaceuticals, Protocol 1.
Appendix 1  (continued)
Protocol, Amendments and Deviations

24. REFERENCES
None.
25. TEST FACILITY APPROVAL

The signature below indicates that Test Facility Management approves the Study Director identified in this protocol.

[Signature]
Date: 03 Aug 2011

Andy Danks, BSc
Test Facility Management

The signature below indicates that the Study Director approves the study protocol.

[Signature]
Date: 05 Aug 2011

Bruce Robertson, BSc
Study Director
26. SPONSOR APPROVAL

The signature of the Sponsor Representative below indicates approval of this protocol. The protocol was approved by the Sponsor on 02 Aug 2011.

[Signature]

Date: 2/8/11.

Andrew J Pollard, FRCPCH PhD
Sponsor Representative
Appendix 1  (continued)
Protocol, Amendments and Deviations

PROTOCOL AMENDMENT NO. 1

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

Test Facility Study No. 520419

Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.

1. Section 13. Experimental Design

Experimental Design

| Group Number | Animal Numbers | Test Item | Dosage (ug/dose) | Conc. (ug/mL) | Dose Volume (mL/dose) |
|--------------|----------------|-----------|------------------|---------------|-----------------------|
|              | Main Study     | Recovery  |                  |               |                       |
|              | M  F           | M  F      |                  |               |                       |
| 1            | 1-3            | 10-21     | 4-6              | 28-30         | 0.5 mL                |
|              | 1-3            | 10-12     | 19-21            | 28-30         |                       |
| 2            | 7-9            | 22-24     | 10-22            | 31-33         | 0.5 mL                |
|              | 4-6            | 13-15     | 22-24            | 31-33         |                       |
| 3            | 13-15          | 23-27     | 16-18            | 34-36         | 0.5 mL                |
|              | 7-9            | 16-18     | 25-27            | 34-36         |                       |

Justification(s):
To amend animal numbers to a more suitable order.

2. Section 15.1.1. Sample Collection

Blood will be collected from an auricular artery. Additional blood samples may be obtained (e.g. due to clotting of non-serum samples) if permissible sampling frequency and blood volume are not exceeded. After collection, samples will be transferred to the appropriate laboratory for processing.

Animals will not be fasted. Samples will be collected according to the following table.

Samples for Clinical Pathology Evaluation

| Group Nos. | Time Point | Haematology | Coagulation | Clinical Chemistry |
|------------|------------|-------------|-------------|--------------------|
| 1-3        | Pretrial   | X           | X           | X                  |
| 1-3        | Day 64     | X           | X           |                    |
| 1-3        | Day 66     | X           | X           |                    |
| 1-3        | Day 92     | X           | X           |                    |
Protocol Amendment No. 1

| Group Nos.                  | Time Point          | Haematology | Coagulation | Clinical Chemistry |
|-----------------------------|---------------------|-------------|-------------|--------------------|
| Unscheduled euthanasia (when possible) | Before euthanasia   | X           | X           | X                  |

X = sample to be collected.

Any residual/retained clinical pathology samples will be discarded before issuance of the Final Report.

Justification(s):

To allow animal unit to take blood sample for Clinical Pathology and Anti-Antibody analysis at the same collection time.
Appendix 1 (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 1

Amendment Approval:

[Signature]

Bruce Robertson, BSc
Study Director

Date: 05 April 2011

[Signature]

Andrew J. Pullard, FRCPCH PhD
Sponsor Representative

Date: 20/1/12
Appendix 1  (continued)
Protocol, Amendments and Deviations

PROTOCOL AMENDMENT NO. 2

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

Test Facility Study No. 520419

Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.

1. Amendment 1, Item 2, Section 15.1.1. Sample Collection

Blood will be collected from an auricular artery. Additional blood samples may be obtained (e.g. due to clotting of non-serum samples) if permissible sampling frequency and blood volume are not exceeded. After collection, samples will be transferred to the appropriate laboratory for processing.

Animals will not be fasted. Samples will be collected according to the following table.

| Group Nos.      | Time Point     | Haematology | Coagulation | Clinical Chemistry |
|-----------------|----------------|-------------|-------------|--------------------|
| 1-3             | Pretrial       | X           | X           | X                  |
| 1-3             | Day 64 66      | X           | X           | X                  |
| 1-3             | Day 92         | X           | X           | X                  |
| Unscheduled euthanasia (when possible) | Before euthanasia | X      | X           | X                  |

X = sample to be collected.

Any residual/retained clinical pathology samples will be discarded before issuance of the Final Report.

Justification(s):

Amendment 1 incorrectly changed the day of clinical pathology sampling at Day 66 to Day 64. A bleed on Day 64 would not be considered fit for purpose as animals would not have received their final dose. Following discussions with the Sponsor, the bleed was re-instated on Day 66.
Appendix 1 (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 2

Amendment Approval:

[Signature]
Date: 15/8/19

Bruce Robertson, BSc
Study Director

Andrew J Pollard, FRCPCH, PhD
Sponsor Representative
Appendix 1 (continued)
Protocol, Amendments and Deviations

PROTOCOL AMENDMENT NO. 3

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

Test Facility Study No. 520419

Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.

1. Section 7. Responsible Personnel

Study Director
Bruce Robertson, BSc
Charles River Laboratories
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Justification(s):
To change Study Director to cover for a period of temporary absence.
Appendix 1  (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 3

Amendment Approval:
The signature below indicates that Test Facility Management approves the Study Director identified in this protocol amendment.

Andy Danks, BSc
Test Facility Management

Elizabeth Donald, BSc
Study Director

Andrew J Pollard, FRCPCH PhD
Sponsor Representative
Appendix 1 (continued)
Protocol, Amendments and Deviations

PROTOCOL AMENDMENT NO. 4

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

Test Facility Study No. 520419

Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.

1. Section: Various

At the second injection Animal 27M (Group 3) received a skin nick during clipping of the injection site, which resulted in the injection being given into the right hind limb. The animal was inspected by the veterinary surgeon and any treatment was recorded and will be reported.

The right hind limb injection site will be designated Injection site 2 and clipping and marking will be as is occurring for Injection site 1. The skin nick has healed and subsequent injections will be given in the left hind limb (Injection site 1).

For this animal, both injection sites will be collected at necropsy and examined histologically.

The Study Director agreed this change with the technical staff at the time of injection and the Sponsor was informed.

Justification(s):

To formally document an agreed change and to inform all parties.

2. Section 7. Responsible Personnel

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Elizabeth Donald, BSc
Charles River Laboratories
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Appendix 1  (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 4

Fax: +44 (0) 1875 614555
E-mail: bruce.robertson@crl.com

Justification(s):
The original Study Director has returned to work following a period of temporary absence.
Appendix 1 (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 4

Amendment Approval:
The signature below indicates that Test Facility Management approves the Study Director identified in this protocol amendment.

_________________________________  Date: 27 Sep 2011
Andy Danks, BSc
Test Facility Management

_________________________________  Date: 23 Sep 2011
Bruce Robertson, BSc
Study Director

_________________________________  Date: 28/9/11
Andrew J Pollard, FRCPCH PhD
Sponsor Representative
Appendix 1  (continued)
Protocol, Amendments and Deviations

PROTOCOL AMENDMENT NO. 5

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits
with a 4 Week Recovery Period

Test Facility Study No. 520419
Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.
1. Section. 27. Attachment

Justification(s):
To include the Responsible Scientists Standard Operating Procedure for the Anti-MenPF1 Rabbit
Immunoglobulin ELISA.
Appendix 1  (continued)
Protocol, Amendments and Deviations

National Institute for Biological Standards and Control

Division of Bacteriology

Standard Operating Procedure

Title: Anti-MenPF-1 Rabbit Immunoglobulin ELISA

(Relevant to Study Number: 520419)

Changes to previous version are tracked in red with deletions shown as ^
Appendix 1 (continued)
Protocol, Amendments and Deviations

National Institute for Biological Standards and Control

1. INTRODUCTION

MenPF-1 is a developmental vaccine against disease caused by Neisseria meningitidis (the meningococcus). The major antigens in this vaccine are the outer membrane proteins PorA and FexA. The vaccine contains outer membrane vesicles (OMVs) from a meningococcal strain genetically modified to over-express the FexA antigen.

MenPF-1 OMVs, adsorbed to aluminium hydroxide (Al(OH)₃) adjuvant, have been produced by the Norwegian Institute of Public Health (NIPH). A validation batch (Lot number FMOX1102) of the MenPF-1 vaccine will be tested for in vivo toxicity in rabbits. This toxicology study has been contracted to Charles River Laboratories (CRL) by the University of Oxford (Charles River Study Number: 520419). The results of this study will be used to support an application by the University of Oxford for the use of MenPF-1 in a Phase 1 clinical trial in humans.

During the toxicology study, New Zealand White rabbits are given four doses of Al(OH)₃-only control inoculum or Al(OH)₃-adjuvanted MenPF-1 OMVs. Rabbits receiving MenPF-1 OMVs are given either a single human dose (25µg total protein) or double human dose (50µg total protein). Doses are given on days 1, 22, 43 and 64. Blood samples are collected from each rabbit pre-immunisations, before dosing on day 22, before dosing on day 64 and on day 92. Blood samples are processed at Charles River Laboratories, and extracted serum samples are stored at -20°C.

The immunological testing of serum samples has been sub-contracted to NIBSC by CRL. An in vitro Enzyme Linked Immunosorbent Assay (ELISA) is used at NIBSC to determine seroconversion of rabbits in the study. Seroconversion is defined as the development of detectable specific antibodies raised against the vaccine in response to immunisation. The ELISA will be used to demonstrate seroconversion in the rabbits which should switch from MenPF-1 seronegative to MenPF-1 seropositive if successfully immunised. The binding of antibodies in pre- and post-vaccination sera to MenPF-1 OMVs will be assessed using a validated assay of suitable sensitivity and specificity.

2. SAMPLE RECEIPT AND DOCUMENTATION

Serum samples are shipped by CRL to NIBSC on dry ice and delivered to the responsible operator in Bacteriology. On receipt within the division of Bacteriology, receipt will be recorded according to BACT/REC (Document S/N 369). All documents relevant to this study will be labelled with the Charles River Study Number: 520419. Scanned copies of all documents will be stored in the bact/MenPFtox drive. Samples are stored at -20°C in freezer BT077.

3. FORMS USED IN CONNECTION WITH THIS SOP

Buffer and reagent forms:

- SOP: BACT/BUF (Document S/N 388)
- BACT/MEDIA (Document S/N 498)
- BACT/MEDIA10xPBS (Document S/N 2965)
- BACT/MEDIA50mMPBS (Document S/N 2964)
- BACT/MEDIA IMSA (Document S/N 2966)

Other forms:

- MenPF-1 Rabbit ELISA test record form (Document S/N 6116)
- BACT/REC (Document S/N 369)
- SOP: TDI/SOP/RANDOM (Document S/N 4628)
Appendix 1  (continued)
Protocol, Amendments and Deviations

4. MATERIALS

Unless otherwise stated in the SOP, there is no requirement to use volumetric glassware in traceable calibration for the preparation of reagents, solutions or dilutions. Semi-automated pipettes, disposable plastic graduated pipettes, and measuring cylinders, appropriate to the volume being used, are adequate for this purpose. Volumes of less than 1.0 ml are dispensed using suitable pipettes in calibration. All salts used in the preparation of buffers should be of minimum General Purpose Reagent grade, unless otherwise stated.

5. EQUIPMENT

*TREND-monitored -4°C refrigerator BT076.
*TREND-monitored -20°C freezer BT077.
*Suitable pipettes in calibration.
*Multichannel pipette in calibration.
*Bibbyjet pipettor.
*Lab Timer.
*Plate washer.
*Microplate reader.
*Vortex mixer.
96 well microtitre plates (Nunc Maxisorb). Measuring cylinders.
Marker pen.
Racks for tubes and universals.
Buffer reservoirs for multichannel pipettes.
Disposable, sterile serological pipettes.
Container for incubating plates.
Paper towels.
Plastic microtubes.
Plastic universals.
Plastic bijoux.
Glass beakers.

*Equipment records available.

6. RISK ASSESSMENT

Safety glasses and gloves must be worn when handling material of animal origin. A risk assessment for this procedure can be found on the NIBSC Safety Organiser database.

7. CRITICAL REAGENTS

Coating Antigen: Unadsorbed MenPF-1 OMVs (Validation batch 1), sterile, in 3% Sucrose/0.01% Thimerosal with a total protein concentration of 0.45mg/ml. OMVs were produced at NIPH and shipped to NIBSC on 10/06/2011. The OMVs are assigned an expiry date of 6 months from receipt. Upon receipt, OMVs are stored at +4°C in a suitable container to protect from light. The container is labelled with content details and dates of receipt and expiry.

Positive control: Anti-MenPF-1 Rabbit serum (NIBSC 11/1475). A pool of serum from four rabbits is used as the positive control. Aliquots of the positive serum are stored at -20°C, and allowed to thaw at room temperature before use. Aliquots are marked each time they are thawed, and discarded after three freeze/thaw cycles. Serum is assigned a shelf life of one year when stored at -20°C.
Negative control: Normal Rabbit serum (Sigma #R9133, Lot number 089K6004). Stored at -20°C in suitable aliquots. Aliquots are marked each time they are thawed, and discarded after three freeze/thaw cycles. An expiry date of 1 year from receipt is assigned to this reagent.

8. OTHER REAGENTS

All buffers should be prepared when required according to SOP: BACT/BUF (Document S/N 388). Reagents marked with ** can be obtained from Scientific Support Services (SSS), or can be made according to the SOPs.

**Coating Buffer:** Prepared following instructions on form BACT/MEDIAACB (Document S/N 498).

**Phosphate Buffered Saline (PBS) (x 10 concentrate):** Prepared following instructions on form BACT/MEDIA10xPBS (Document S/N 2965).

**Phosphate Buffered Saline (PBS) (x 1):** Prepared following instructions on form BACT/MEDIA50mMPBS (Document S/N 2964).

Wash Buffer: 1 x PBS containing 0.01% polycethylene sorbitan monolaurate (Tween 20, supplied by Sigma Aldrich, #P1379) (PBST). Prepared on the day of the assay by diluting 10 x PBS 1:10 in purified water containing 0.01% (v/v) Tween 20.

Newborn Bovine or Foetal Calf Serum: Supplied by SSS. Each new batch needs to be validated by testing 3x in parallel using the previously validated batch three months prior to replacing the batch being used. Stored at -20°C.

Dilution Buffer: PBS containing 5% (v/v) Foetal calf serum; prepared on the day of the assay.

Goat Anti-rabbit HRP conjugate (Sigma #A6154 or equivalent): Stored at -20°C in suitable aliquots. Aliquots are marked each time they are thawed, and discarded after three freeze/thaw cycles. An expiry date of 1 year from receipt is assigned to this reagent.

TMBBlue Substrate: Supplied by Universal Biologicals Ltd #T118. Stored at +4°C. An expiry date of 1 year from receipt is assigned to this reagent.

**1M Sulphuric acid:** Prepared following instructions on form BACT/MEDIA 1M SA (Document S/N 2966).
9. PROCEDURE – Enzyme Linked Immunosorbent Assay

Record all details of the test, including samples tested, dilutions made, critical timings, pipette serial numbers, and buffers and reagents used on the MenPF-1 Rabbit ELISA test record form (Document S/N 6116).

a). Prepare a solution of 2µg/ml MenPF-1 OMV in coating buffer according to the following table:

| Number of Plates | Total Solution Volume (ml) | Volume Coating Buffer (ml) | Volume OMV stock (µl) |
|------------------|---------------------------|---------------------------|----------------------|
| 1                | 12                        | 11.947                    | 53                   |
| 2                | 24                        | 23.893                    | 107                  |
| 3                | 34                        | 33.849                    | 151                  |
| 4                | 45                        | 44.800                    | 200                  |
| 5                | 55                        | 54.756                    | 244                  |
| 6                | 65                        | 64.711                    | 289                  |

Coat the appropriate wells of microtitre plates with 100µl of solution. Cover and incubate the plates at +4°C for a minimum of 16 hours in a sealed container which has been labelled to be identifiable to the test operator.

b). Wash the ELISA plates with Wash Buffer using the Skatran Plate washer. If the machine has been switched off or the connected buffer has been changed from that required by this assay, a blank plate must first be used to Rinse the machine with pure water, and then Prime the machine with the required Wash Buffer. All buffer changes should be recorded on the test record form.

c). Block plates with 100µl per well of Dilution Buffer. Cover the plates and incubate for a minimum of 1 hour (+10 minutes) at room temperature in a sealed container.

d). Wash the ELISA plates as in step b).

e). Prepare dilutions of the sera to be tested and the positive control by diluting in Dilution Buffer as follows:

- For positive control sera, dilute 1:500 (1:10 followed by 1:50);
- For negative control sera, dilute 1:100 (1:10 followed by 1:10);
- For test sera taken on day 0 (Test Sample 1), dilute 1:100 (1:10 followed by 1:10);
- For test sera taken on day 22 (Test Sample 2), dilute 1:300 (1:10 followed by 1:30);
- For test sera taken on day 64 (Test Sample 3), dilute 1:900 (1:10 followed by 1:90);
- For test sera taken on day 92 (Test Sample 4), dilute 1:900 (1:10 followed by 1:90).

f). Prepare ELISA plates. One 96-well plates is required to test all serum samples extracted from each rabbit and the positive control serum at a maximum of 8 dilutions for each serum sample. All samples (except Blank and negative controls) are tested in duplicate columns (see example plate layout in Figure 1). Samples are assigned randomly to columns following the method described in SOP: TDESOP/RANDOM (Document S/N 4628). For a standard assay, random plate layouts have been generated and can be found on the MenPF-1 Rabbit ELISA test record form (Document S/N 6116). For rabbits for which less than four serum samples are available, columns listed as “Test Sample 4” are left Blank. Record which template is being used in the assay on the MenPF-1 Rabbit ELISA test record form (Document S/N 6116). Use a different template for each assay and rotate in the order 1 through to 8.
Appendix 1 (continued)
Protocol, Amendments and Deviations

National Institute for Biological Standards and Control

Figure 1: Example plate layout (all samples and controls are randomised across the plate). See appendix for detailed plate layout templates.

|   | TS2 | ±ve | ±ve | ±ve | TS1 | TS3 | TS4 | ±ve | TS2 | TS4 | TS3 | TS1 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*| 1/1*|
| B | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 | 1/3 |
| C | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 |
| D | 1/27| 1/27| 1/27| 1/27| 1/27| 1/27| 1/27| 1/27| 1/27| 1/27| 1/27| 1/27|
| E | etc.| etc.| etc.| etc.| etc.| etc.| etc.| etc.| etc.| etc.| etc.| etc.|
| F |     |     |     |     |     |     |     |     |     |     |     |     |     |
| G |     |     |     |     |     |     |     |     |     |     |     |     |     |
| H |     |     |     |     |     |     |     |     |     |     |     |     |     |

*Pre-diluted test sample or positive control.
TS = Test serum
±ve = Positive control serum
±ve = Negative control serum

h). Fill wells in rows B-H with 100μl of Dilution Buffer, leaving row A empty.
i). 150μl of each diluted preparation is added to wells in row A. 50μl of each sample is then removed and transferred to the appropriate wells in row B. Following mixing for a minimum of 5 times, 50μl volumes are transferred to the next row (C). This procedure is repeated down the plate. Following mixing of row H 50μl of sample is discarded from each well. Each well in rows A through to H should now contain 100μl volumes. Plates are then covered and incubated at room temperature in the sealed container for a minimum of 1 hours (+10 minutes).

j). Wash the ELISA plates as in step b).
k). Dilute goat anti-rabbit HRP conjugate 1:2000 in Dilution Buffer. Add 100μl to all wells. Cover and incubate plates for a minimum of 1 hour (+10 minutes) in a sealed container at room temperature.

l). Wash the ELISA plates as in step b).
m). Add 100μl TMBBlue substrate to all wells. Incubate at room temperature for up to ten minutes. Following colour development, add 100μl 1M sulphuric acid to all wells to stop the reaction. The plates are read at 450 nm using a microplate reader. Logon to the computer linked to the plate reader and read the plates using Genesis (or equivalent) plate reader software. Each plate that is read is automatically assigned (by the software) a file name with the date and a sequential number, e.g. 20JUN08W.001. Operator's initials should be added to the beginning of this file name, e.g. HS. 20JUN08W.001. Record the file name(s) and software protocol used to read the plate(s) on the test record form. Data generated by the plate reader is saved by default to the C:\ drive on the computer linked to the plate reader and can be found in C:\genesis\protocol*, where * is the protocol used for reading the plates. Copy the data from C:\ to the "Raw Data" file in the bactMenPFlox drive.
n). Raw data should be printed out immediately, signed and dated.

10. DATA ANALYSIS, VALIDITY AND DETERMINATION OF SEROCONVERSION

Absorbance levels across the dilution series from each test sample are used to directly compare the levels of IgG binding following immunisation of each rabbit to pre-trial sera, in order to determine whether each rabbit was seroconverted. All calculations will be recorded on the print-out of the raw data and reviewed by the Study Director. No computer software is required for data analysis.

10.1. DATA ANALYSIS

a) Referring to the dilution series listed below, for each test sample determine the highest dilution factor at which the absorbance at 450nm is higher than 0.70 (where at least two consecutive dilutions are higher than the threshold, except where only a 1/100 dilution of a sample has an absorbance higher than 0.70). The dilution factor is recorded as “IG”. If a sample does not result in absorbance higher than 0.70 at a dilution of 1:100, IG is recorded as 100. If higher or lower dilutions (to a minimum of 1/100) are required to determine IG, the test sample must be repeated with appropriate dilutions.

For duplicates of a single sample, if IG values are one dilution apart, a mean value is taken as the IG for that sample. If IG values for duplicates of a single sample are greater than one dilution apart, that sample must be repeated.

| Row | Positive | Test Sample 1 (Negative) | Test Sample 2 | Test Sample 3/4 |
|-----|----------|--------------------------|---------------|-----------------|
| A   | 500      | 100                      | 300           | 900             |
| B   | 1500     | 300                      | 900           | 2700            |
| C   | 4500     | 900                      | 2700          | 8100            |
| D   | 13500    | 2700                     | 8100          | 24300           |
| E   | 40500    | 8100                     | 24300         | 72900           |
| F   | 121500   | 24300                    | 72900         | 218700          |
| G   | 364500   | 72900                    | 218700        | 656100          |
| H   | 1095500  | 218700                   | 656100        | 1968300         |

b) For each test sample 2, 3 and 4, calculate the increase in binding following vaccination as follows:

\[
\Delta_{IG} = \frac{IG(\text{Test Sample } n)}{IG(\text{Test Sample 1})}
\]

Where “n” = 2, 3 or 4.

For the positive control serum, \(\Delta_{IG}\) is calculated as follows:

\[
\Delta_{IG} = \frac{IG(\text{Positive control serum})}{IG(\text{Negative control serum})}
\]

c) Record values for \(\Delta_{IG}\) on the test record form.

10.2. VALIDITY REQUIREMENTS

In order for the test to be valid:
Appendix 1  (continued)
Protocol, Amendments and Deviations

National Institute for Biological Standards and Control

i). The maximum absorbance at 450nm for the positive control serum must be greater than 3.0 for both repeats.

ii). The minimum absorbance at 450nm for the positive control serum must be less than 0.7 for both repeats.

iii). The maximum absorbance at 450nm for the negative control serum must be greater than 0.7.

iv). The ΔIG value calculated for the positive control serum must be between 90 and 810.

Validity of the assay is recorded on the test record form.

A test is repeated if it does not meet the validity requirements, if IG values are greater than one dilution apart for duplicates of any test sample, or if alternative dilutions are required to determine IG values for any test sample.

10.3. DETERMINATION OF SEROCONVERSION

When analysis of serum samples from all animals is complete, seroconversion is determined for each time point after initiation of the trial (Day 22, Day 64 and Day 92). For each post-vaccination serum sample, when ΔIG ≥ 4 seroconversion is determined to have occurred.

11. RECORDING OF RESULTS

Copies of all raw and analysed data, as well as scanned copies of all test record forms, will be stored in the bact:MenPFlex drive. Hard copies of all test record forms and raw data will be stored in B38. When analysis of all serum samples is complete all printed and electronic data will be sent to Charles River Laboratories for review and incorporation into their test report.

12. INTERNAL DATA MONITORING

IG values obtained for positive and negative control sera will be recorded in the file “Data Monitoring” in bact:MenPFlex:Raw Data:Data Monitoring. A table of the results can be viewed at any time.

13. COMPETENCY

This test has been developed for a single use over a time period of less than six months. Initial competency has been determined during assay development and will remain valid throughout the time period required for test completion. If the use of this test is delayed, or if it becomes necessary to repeat the test at a later date, competency for this test may be obtained through completion of similar assays. If no similar assay has been completed by the operator within 12 months prior to the start of the test, the competency of that operator must be re-evaluated before testing can begin.

14. UNCERTAINTY OF MEASUREMENT

Uncertainty in the procedure covered by this SOP may result from a number of general factors, such as:

- Variability in assay system
- Human factors
- Homogeneity of the sample
Appendix 1  (continued)
Protocol, Amendments and Deviations

National Institute for Biological Standards and Control

- Environmental factors—temperature of laboratory
- Dilutions of test samples and references preparations
- Instrumental factors

Instrumental factors are listed in the Table below:

| Measurement               | Equipment            | Estimated Uncertainty | Documentation                                           |
|---------------------------|----------------------|-----------------------|---------------------------------------------------------|
| Volumes                   | Gilson pipettes      | <2.5%                 | Certification of calibration of pipettes, SOP: BACT/PIP and associated log books |
| Volumes                   | Disposable plastic pipettes | <1%                   | Manufacturers specifications                             |
| Volumes                   | Measuring cylinder   | 1%                    | Manufacturers specifications                             |
| pH (of buffers)           | pH meter             | 0.25%                 | Certificate of calibration of buffers                   |
| Weight (of reagents in buffers) | Balance            | 1%                    | Certificate of calibration of weight                    |

The contributions to the error in the final result made by instrumental factors are small. Furthermore, the result for any test sample in this assay is expressed as a post-vaccination dilution factor relative to a pre-vaccination sample included in the same assay, and so any sources of error due to environmental factors will cancel out. The remaining sources of error are random human operational error and variability in the assay system.

The uncertainty of measurement for this ELISA test has been considered. The contributions of instrumental sources or error to the final result of the test are small and can be considered negligible.
Appendix 1 (continued)
Protocol, Amendments and Deviations

National Institute for Biological Standards and Control

Appendix

ELISA PLATE LAYOUT TEMPLATES

Random plate layouts for Anti-MenPF-1 Rabbit Immunoglobulin ELISA

Test sample, reference, and positive control added to row A of 96 well plates as indicated below:

Key:

+: Positive serum
B: Blank wells
TS: Test sample

| Template 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|------------|---|---|---|---|---|---|---|---|---|----|----|----|
| T52        | + | B | B | T51 | T53 | T54 | + | T52 | T54 | T53 | T51 |
| Template 2 | B | T53 | T54 | + | B | T53 | T51 | T54 | + | T51 | T52 | T52 |
| Template 3 | T53 | T52 | T51 | B | T54 | T52 | T51 | T53 | + | T54 | B | + |
| Template 4 | T51 | T53 | B | T54 | + | T52 | + | T51 | T52 | T54 | B | T53 |
| Template 5 | B | T51 | T54 | T52 | B | + | T53 | T52 | T54 | T51 | T53 | + |
| Template 6 | B | T52 | T54 | T52 | T51 | T53 | + | T51 | T53 | B | T54 | + |
| Template 7 | T54 | + | T54 | B | T52 | T52 | + | T53 | B | T51 | T53 | T51 |
| Template 8 | T53 | T53 | T54 | T51 | + | T51 | T52 | + | T54 | B | B | T52 |
Appendix 1  (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 5

Amendment Approval:

Bruce Robertson, BSc
Study Director

Date: 18 Nov 2011

Andrew J Pollard, FRCPCH PhD
Sponsor Representative

Date: 8/2/12
Appendix 1  (continued)
Protocol, Amendments and Deviations

PROTOCOL AMENDMENT NO. 6

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits
with a 4 Week Recovery Period

Test Facility Study No. 520419
Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.

1. Section 7. Responsible Personnel

Test Facility-designated Individual Scientists (IS)
Pathologist  TBC Lise Bertrand, DVM, MSc, DESV, DiplECVP
Charles River Laboratories
Address as cited for Test Facility
Tel: +44 (0)1875-618512
Fax: +44 (0)1875 614555
E-mail: name.lise.bertrand@crl.com

Each IS is required to report any deviations or other circumstances that could affect the quality or
integrity of the study to the Study Director in a timely manner. Each IS will provide a report
addressing their assigned phase of the study, which will be included as an appendix to the Final
Report.

Justification(s):
To include details of the study pathologist following confirmation, for completeness.
Appendix 1  (continued)
Protocol, Amendments and Deviations

Protocol Amendment No. 6

Amendment Approval:

Bruce Robertson, BSc
Study Director

Date: 25 Aug 2011

Andrew J Pollard, FRCP EH PhD
Sponsor Representative

Date: Day / Mon / Year
Appendix 1 (continued)
Protocol, Amendments and Deviations

Protocol Deviations

Protocol section 11 Test System

The target age and weight for the animals at the start of dosing was 12 weeks and 2.5 kg, respectively. At the initiation of dosing animals were approximately 13-14 weeks of age and weighed 2.6-2.8 kg for males and 2.7-3.1 kg for females. The difference between the actual age and weight and the target was considered to be small and rabbits at 13-14 weeks old are still considered to be young adults. This deviation was considered not to have impacted on the outcome or integrity of the study.

Protocol section 11.3 Environmental Acclimatisation

The protocol stated animals would be acclimatised for a period of up to 2 weeks before the first administration. Animals were acclimatised for 15 days before administration of MOX control or MenPF-1 vaccine. This additional day before the start of dosing had no observable effect on the animals and was considered not to have impacted on the outcome or integrity of the study.

Protocol section 12.2 Environmental Conditions

On several occasions the humidity and temperature in the animal room was outside the target range of 16-20°C for temperature and 40-85% humidity. The actual temperature range was 14-22°C, while the humidity range was 29-71%. The environmental deviations did not cause any overt effect in any animal, consequently it was considered that the study outcome was unaffected.

Protocol section 15.2 Antibody Sample Collection, Processing and Analysis

A discrepancy between the protocol and the Standard Operating Procedure (SOP) provided by NIBSC and authorised in Amendment 5 (dated 18 November 2011) was noted in the storage temperatures of antibody samples at NIBSC. The protocol stated on receipt of antibody samples at NIBSC, samples would be stored at \( \leq -20^\circ\text{C} \), whereas the SOP stated samples would be stored at \(-20^\circ\text{C}\). A review of the trend data monitoring the freezers at NIBSC indicated the samples were stored in a freezer which was running at \( \text{ca} -26^\circ\text{C} \). Although this was consistent with the Protocol, this was a deviation to the SOP which NIBSC were using for antibody sample analysis. As samples were stored at Charles River in a freezer set to maintain -80°C, and as the samples were held at NIBSC only slightly cooler than the SOP, samples were still held frozen and within ranges which samples were held at Charles River and NIBSC. As a result this deviation was considered not to have impacted on the outcome or integrity of the study or conclusions drawn.
## Appendix 2
Certificates of Analysis for Test and Control Items

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### CERTIFICATE OF ANALYSIS
CoA

- **Product:** Men PF-1
- **Storage:** 2-8°C
- **Batch number:** FMOX1102
- **Expiry date:** N.a

| Test                  | Specification: 07SPE-MOX-003 Version: 2.0 | Test result          | Journal number    |
|-----------------------|-------------------------------------------|----------------------|-------------------|
| Aluminium             | < 1.25 mg/ml                              | 1.0 mg/ml            | J17-11/005        |
| Endotoxin             | < 1 x 10^5 IU/ml                          | < 1 x 10^5 IU/ml     | J44-11/037        |
| Identity              | 70 kDa (Pf A P3-3): Detected Class 1, (P1.16): Detected Class 2 (P3.15): Detected | Detected Detected Detected | J3-11/017        |
| pH                    | To be reported                            | 6.1                  | J13-11/023        |
| Potency               | To be reported                            | See comments         | N.a               |
| Pyrogenicity          | Pass                                      | Pass                 | J41-11/007        |
| Sterility             | Pass                                      | Pass                 | J40-11/038        |
| Appearance            | Opaque, even, milky suspension; easily redispersed | Opaque, even, milky suspension; easily redispersed | J6-11/019        |
| Extractable volume    | ≥ 0.50 ml                                 | ≥ 0.50 ml            | J7-11/009         |
| Degree of adsorption  | To be reported                            | > 93%                | J16-11/006        |
Appendix 2 (continued)
Certificates of Analysis for Test and Control Items

CERTIFICATE OF ANALYSIS

CoA

Product: Men PF-1

Tested in accordance with GMP

Storage: 2-8°C

Batch number: FMOX1102

Expiry date: N.a

Deviations from SOP, manufacturing formula, specifications. Deviation No:

| Date | Sign. |
|------|-------|
| 06/07/2011 | VB |
| 01/11/2011 | NMJ |

The values are correctly transferred from specification and primary data

The test results fulfil the specifications for the product: Yes / No— 06/07/2011 Vegard Bråthen Head of QC

Comments:

Test Abnormal toxicity: Pass (J26-11/001)

A11/098: One cassette had too high Al-content. This cassette is discarded.

Potency test: The test is not established yet and will be performed in August 2011.

The quality control of the product is: Approved / Not-Approved

06/07/2011

Vegard Bråthen
Head of Quality Control
### CERTIFICATE OF ANALYSIS

**Product:** Men PF-1, bulk  
**Storage:** 2-8°C  
**Batch number:** MOX1101  
**Expiry date:** N.a

| Test                          | Specification: | 07SPE-MOX-002 | Test result | Journal number |
|------------------------------|----------------|---------------|-------------|----------------|
| **Inactivation control**     | Inactivated material |                | Pass        |                |
| **Total protein, pre formulation** | To be reported | 0.74 mg/ml   |             | J9-11/018      |
| **Bioburden, pre sterile filtration** | TAMC < 10⁷/ml  | TAMC < 10⁷/ml  |             | J46-11/019     |
|                              | TYMC < 10⁷/ml  | TYMC < 10⁷/ml  |             |                |
|                              | Total < 10⁷/ml | Total < 10⁷/ml |             |                |
| **Antigen pattern**          |                 |               |             |                |
| 70kD Fet A (F 3-3)           |                 |               |             |                |
| Class 1 P1.16                |                 |               |             | J3-11/017      |
| Class 3 P 3.15               |                 |               |             |                |
| **Antigen pattern**          |                 |               |             |                |
| 70kD Fet A F 3-3             |                 |               |             |                |
| Class 1 (P1.16)              |                 |               |             | J2-11/005      |
| Class 3 (P 3.15)             |                 |               |             |                |
| LPS 3,7,9                    | Detected        | Detected      |             |                |
| **Deoxycholate**             | < 0.4 µg/µg protein | 0.26 µg/µg protein |             | J14-11/007     |
| **LPS (Lipopoly saccharide)** | Total: < 0.12   | 0.044 µg/µg protein |             | J5-11/006      |
|                              | LPS 3,7,9: to be reported | 0.043 µg/µg protein |             |                |
| **Total protein**            | 0.45 - 1.24 mg/ml | 0.60 mg/ml |             | J9-11/019      |
| **Appearance**               | Turbid, white to yellow, even suspension, easily redispersed | Turbid, white to yellow, even suspension, easily redispersed | | J6-11/020 |
| **pH**                       | To be reported  | 7.3           |             | J13-11/024     |
Appendix 2  (continued)
Certificates of Analysis for Test and Control Items

Norwegian Institute of Public Health

CERTIFICATE OF ANALYSIS

CoA
Tested in accordance with GMP

Product: Men PF-1, bulk
Storage: 2-8°C
Batch number: MOX1101
Expiry date: N.a

Deviations from SOP, manufacturing formula, specifications. Deviation No:

| Date   | Sign          |
|--------|---------------|
| 01/07/2011 | VB           |
| 01/07/2011 | S.O.         |

The values are correctly transferred from specification and primary data

The test results fulfil the specifications for the product: Yes / No

| Date   | Sign                  |
|--------|-----------------------|
| 01/07/2011 | Vegard Bråthen   |

Head of QC

Comments:

The quality control of the product is: Approved / Not Approved

01/07/2011
Date

Vegard Bråthen
Head of Quality Control
Appendix 2  (continued)
Certificates of Analysis for Test and Control Items

CERTIFICATE OF ANALYSIS
CoA

Product: MOX Control
Storage: 2-8°C
Batch number: FMOX1103

| Test                      | Specification: 07SPE-MOX-004 Version: 2.0 | Test result     | Journal number |
|---------------------------|---------------------------------------------|-----------------|----------------|
| Extractable volume        | ≥ 0.5 ml                                    | ≥ 0.5 ml        | J7-11/008      |
| Aluminium                 | < 1.25 mg/ml                                | 1.1 mg/ml       | J17-11/006     |
| Test for sterility        | Pass                                        | Pass            | J40-11/042     |
| pH                        | To be reported                              | pH 6.0          | J13-11/028     |
| Endotoxin                 | ≤ 5 IU/ml                                   | ≤ 5 IU/ml       | J44-11/035     |
| Appearance                | Opaque, even, milky suspension; easily dispersed | Opaque, even, milky suspension; easily dispersed | J6-11/024     |

Deviations from SOP, manufacturing formula, specifications. Deviation No:

| Deviation No |  |  |
|--------------|---|---|
Appendix 2 (continued)
Certificates of Analysis for Test and Control Items

CERTIFICATE OF ANALYSIS
CoA

Product: MOX Control
Batch number: FMOX1103

Tested in accordance with GMP
Storage: 2-8°C
Expiry date: N.a

| Date     | Sign. |
|----------|-------|
| 06/07/2011 | VB   |
| 06/07/2011 | WREJ |
| 06/07/2011 | Vargad Bråthen |

The values are correctly transferred from specification and primary data

The test results fulfil the specifications for the product:
Yes / -No-

Comments:
N.a

The quality control of the product is: Approved / Not Approved

06/07/2011  Vargad Bråthen
Date  Head of Quality Control
Appendix 3
Individual Clinical Observations: Treatment Period

Key to Appendix 3

Pre = Predose
IPD = Immediate post dose
+3h = 3 hour post dose

The in-life data capture system records the first day of treatment as Day 0.
### Appendix 3 (continued)

### Individual Clinical Observations: Treatment Period

| Group | Test Item | Dosage (µg/dose) | Observation                                                                 | Days          |
|-------|-----------|------------------|-----------------------------------------------------------------------------|---------------|
| M     | Control   | 0                | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | MenPF-1   | 25               | No dosing abnormalities.                                                    | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | MenPF-1   | 50               | No dosing abnormalities.                                                    | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 1         | 1                | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 1         | 2                | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 1         | 3                | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 1         | 19               | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 1         | 20               | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 1         | 21               | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 2         | 4                | Few area(s) of sparse hair                                                  | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 2         | 5                | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42,49,56,63 0 (Pre)-20,21 (Pre)-41,42 (Pre)-62,63 (Pre)-65 65 |
| M     | 2         | 6                | General Observation, No Abnormality Detected.                               | -7-0,7,14,21,28,35,42 |
### Appendix 3  (continued)

**Individual Clinical Observations: Treatment Period**

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
| 1     | Control   | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

| Sex  | Group | Animal | Observation                                                                 | Days                        |
|------|-------|--------|-----------------------------------------------------------------------------|-----------------------------|
| M    | 2     | 6      | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
|      |       |        | discoloured skin on, limb(s), dose site 1                                   | 56, 63                      |
|      |       |        | terminal kill.                                                               |                             |
| M    | 2     | 22     | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
| M    | 2     | 23     | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
| M    | 2     | 24     | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
| M    | 3     | 7      | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
|      |       |        | terminal kill.                                                               |                             |
| M    | 3     | 8      | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
|      |       |        | terminal kill.                                                               |                             |
| M    | 3     | 9      | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
|      |       |        | terminal kill.                                                               |                             |
| M    | 3     | 25     | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
|      |       |        | No dosing abnormalities.                                                     | 0 (Pre)-20, 21 (Pre)-41, 42 (Pre)-62, 63 (Pre)-65 |
|      |       |        | terminal kill.                                                               |                             |
| M    | 3     | 26     | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63 |
### Individual Clinical Observations: Treatment Period

| Sex | Group | Animal | Observation | Days |
|-----|-------|--------|-------------|------|
| M   | 3     | 26     | No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
| M   | 3     | 27     | General Observation, No Abnormality Detected. No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
|     |       |        | discoloured skin on, limb(s), left hind limb One lesion(s) on, limb(s), left hind limb | 24-25, 28 |
| F   | 1     | 10     | General Observation, No Abnormality Detected. No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
|     |       |        | terminal kill. | 65 |
| F   | 1     | 11     | General Observation, No Abnormality Detected. No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
|     |       |        | terminal kill. | 65 |
| F   | 1     | 12     | General Observation, No Abnormality Detected. No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
|     |       |        | terminal kill. | 65 |
| F   | 1     | 28     | General Observation, No Abnormality Detected. No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
| F   | 1     | 29     | General Observation, No Abnormality Detected. No dosing abnormalities. | 14, 21, 28, 35, 42, 49, 56, 63 |
|     |       |        | One area(s) of sparse hair | 7-0, 7 |
| F   | 1     | 30     | General Observation, No Abnormality Detected. No dosing abnormalities. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
| F   | 2     | 13     | General Observation, No Abnormality Detected. | 0(Pre)-20, 21(Pre) -41, 42(Pre) -62, 63(Pre) -65 |
### Appendix 3 (continued)

**Individual Clinical Observations: Treatment Period**

| Group | Test Item | Dosage (µg/dose) | Observation                                                                 | Days          |
|-------|-----------|------------------|-----------------------------------------------------------------------------|---------------|
|       |           |                  | Muzzle, swollen                                                             | 40, 42        |
|       | Control   | 0                | No dosing abnormalities.                                                   | 0 (Pre) -20, 21 (Pre) -41, 42 (Pre) -62, 63 (Pre) -65 |
|       |           |                  | One scab(s) on, muzzle terminal kill.                                       | 38, 40, 65    |
|       | MenPF-1   | 25               | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63                  |
|       |           |                  | No dosing abnormalities.                                                   | 0 (Pre) -20, 21 (Pre) -41, 42 (Pre) -62, 63 (Pre) -65 |
|       | MenPF-1   | 50               | terminal kill.                                                              | 65            |
|       |           |                  | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63                  |
|       |           |                  | No dosing abnormalities.                                                   | 0 (Pre) -20, 21 (Pre) -41, 42 (Pre) -62, 63 (Pre) -65 |
|       |           |                  | terminal kill.                                                              | 65            |
|       |           |                  | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63                  |
|       |           |                  | No dosing abnormalities.                                                   | 0 (Pre) -20, 21 (Pre) -41, 42 (Pre) -62, 63 (Pre) -65 |
|       |           |                  | Skin, scab at dose site 1                                                   | 49-62, 63 (Pre) -65                                   |
|       |           |                  | Skin, discoloured dose site 1                                               | 22-27         |
|       |           |                  | General Observation, No Abnormality Detected.                               | 28-41, 42 (Pre) -48                                   |
|       |           |                  | No dosing abnormalities.                                                   |               |
|       |           |                  | terminal kill.                                                              |               |
|       |           |                  | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63                  |
|       |           |                  | No dosing abnormalities.                                                   | 0 (Pre) -20, 21 (Pre) -41, 42 (Pre) -62, 63 (Pre) -65 |
|       |           |                  | terminal kill.                                                              | 65            |
|       |           |                  | General Observation, No Abnormality Detected.                               | -7-0, 7, 14, 21, 28, 35, 42, 49, 56, 63                  |
|       |           |                  | No dosing abnormalities.                                                   | 0 (Pre) -20, 21 (Pre) -41, 42 (Pre) -62, 63 (Pre) -65 |
|       |           |                  |                                                                           |               |
### Appendix 3  (continued)
#### Individual Clinical Observations: Treatment Period

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
| 1     | Control   | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

| Sex  | Group | Animal | Observation                                                                 | Days          |
|------|-------|--------|-----------------------------------------------------------------------------|---------------|
| F    | 3     | 17     | terminal kill.                                                              | 65            |
| F    | 3     | 18     | General Observation, No Abnormality Detected.                              | 65            |
|      |       |        | No dosing abnormalities.                                                    |               |
| F    | 3     | 34     | terminal kill.                                                              | 65            |
|      |       |        | General Observation, No Abnormality Detected.                              |               |
|      |       |        | No dosing abnormalities.                                                    |               |
| F    | 3     | 35     | General Observation, No Abnormality Detected.                              |               |
|      |       |        | No dosing abnormalities.                                                    |               |
| F    | 3     | 36     | General Observation, No Abnormality Detected.                              |               |
|      |       |        | No dosing abnormalities.                                                    |               |

Test Facility Study No. 520419
Appendix 4
Individual Clinical Observations: Recovery Period

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

Key to Appendix 4

Pre = Predose

The in-life data capture system records the first day of treatment as Day 0.
### Individual Clinical Observations: Recovery Period

| Group | Test Item | Dosage (µg/dose) | Observation                                                                 | Days          |
|-------|-----------|------------------|-----------------------------------------------------------------------------|---------------|
| 1     | Control   | 0                | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|       |           |                  |                                                                             | 63(Pre)-91    |
| 2     | MenPF-1   | 25               | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|       |           |                  |                                                                             | 63(Pre)-91    |
| 3     | MenPF-1   | 50               | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|       |           |                  |                                                                             | 63(Pre)-91    |

#### Individual Clinical Observations

| Sex  | Group | Animal | Observation                                                                 | Days          |
|------|-------|--------|-----------------------------------------------------------------------------|---------------|
| M    | 1     | 19     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 1     | 20     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 1     | 21     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 2     | 22     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 2     | 23     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 2     | 24     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 3     | 25     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 3     | 26     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| M    | 3     | 27     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| F    | 1     | 28     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
|      |       |        |                                                                             | 63(Pre)-91    |
| F    | 1     | 29     | General Observation, No Abnormality Detected.                                | 63,70,77,84,91|
## Individual Clinical Observations: Recovery Period

| Group | Test Item | Dosage (µg/dose) | Observation | Days       |
|-------|-----------|------------------|-------------|------------|
|       | 1         | Control          | No dosing abnormalities. recovery kill. | 63(Pre)-91 |
|       | 2         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63,70,77,84,91 |
|       | 2         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63(Pre)-91 |
|       | 2         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63,70,77,84,91 |
|       | 2         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63(Pre)-91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63(Pre)-91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63,70,77,84,91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63(Pre)-91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63,70,77,84,91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63(Pre)-91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63,70,77,84,91 |
|       | 3         | MenPF-1          | General Observation, No Abnormality Detected. No dosing abnormalities. recovery kill. | 63(Pre)-91 |
### Appendix 5

**Injection Site Reaction Scores: Individual Findings**

| Group / Test Item | Dosage (µg/dose) | 1   | 2   | 3   |
|-------------------|------------------|-----|-----|-----|
| Control           | 0                |     |     |     |
| MenPF-1           | 25               |     |     |     |
| MenPF-1           | 50               |     |     |     |

| Group / sex | Animal No. | Day 1 | Day 22 | Day 43 | Day 64 |
|-------------|------------|-------|--------|--------|--------|
|             |            | + 0 h | + 24 h | + 48 h | + 0 h | + 24 h | + 48 h | + 0 h | + 24 h | + 48 h |
| 1M          | 1          | E     | O      | E      | O      | E      | O      | E      | O      | E      | O      |
|             | 2          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 3          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 19         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 20         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 21         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| 2M          | 4          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 5          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 6          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1      | 0      |
|             | 22         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 23         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 1      |
|             | 24         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| 3M          | 7          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 8          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 9          | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 25         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 26         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
|             | 27         | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |

*E = Erythema (0 = None, 1 = very slight, barely perceptible); O = Oedema (0 = none)*
### Appendix 5 (continued)

**Injection Site Reaction Scores: Individual Findings**

| Group / sex | Animal No. | Day 1 | Day 22 | Day 43 | Day 64 |
|-------------|------------|-------|--------|--------|--------|
|             |            | + 0 h | + 24 h | + 48 h | + 0 h  | + 24 h | + 48 h | + 0 h  | + 24 h | + 48 h |
|             |            | E     | O      | E      | O      | E      | O      | E      | O      | E      |

1F 10 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
11 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
12 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
28 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
29 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
30 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

2F 13 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
14 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
15 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
31 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
32 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
33 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

3F 16 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
17 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
18 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
34 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
35 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
36 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

E = Erythema (0 = None, 1 = very slight, barely perceptible); O = Oedema (0 = None, 1 = very slight, barely perceptible)

*Animal 32 – Erythema recorded up to 120 hours following dose on Day 22; results maintained in the study data*

*Animal 18 – Erythema recorded up to 72 hours following dose on Day 22; results maintained in the study data*
## Appendix 6

### Body Weights with Change (kg): Individual Values: Treatment Period

| Group / sex | Animal No. | Day | 1     | 2     | 3     |
|-------------|------------|-----|-------|-------|-------|
| 1M          |            |     |       |       |       |
| 1           | 2.6        | 2.7 | 2.8   | 2.8   | 2.8   |
| 2           | 2.5        | 2.7 | 2.7   | 2.8   | 2.8   |
| 3           | 2.6        | 2.8 | 2.8   | 2.9   | 2.9   |
| 19          | 2.7        | 2.8 | 2.9   | 3.0   | 3.1   |
| 20          | 2.6        | 2.7 | 2.8   | 2.9   | 3.0   |
| 21          | 2.7        | 2.8 | 2.8   | 2.9   | 3.0   |
| 2M          |            |     |       |       |       |
| 4           | 2.6        | 2.8 | 2.8   | 3.0   | 3.1   |
| 5           | 2.8        | 2.8 | 2.9   | 3.0   | 3.0   |
| 6           | 2.6        | 2.7 | 2.8   | 2.9   | 3.0   |
| 22          | 2.6        | 2.8 | 2.9   | 3.1   | 3.2   |
| 23          | 2.6        | 2.7 | 2.8   | 2.9   | 3.0   |
| 24          | 2.6        | 2.7 | 2.8   | 2.9   | 3.0   |
| 3M          |            |     |       |       |       |
| 7           | 2.6        | 2.7 | 2.8   | 2.9   | 3.1   |
| 8           | 2.4        | 2.6 | 2.7   | 2.8   | 2.9   |
| 9           | 2.7        | 2.8 | 2.9   | 3.0   | 3.0   |
| 25          | 2.7        | 2.8 | 2.9   | 3.1   | 3.1   |
| 26          | 2.6        | 2.7 | 2.8   | 2.8   | 2.9   |
| 27          | 2.7        | 2.8 | 2.9   | 3.0   | 3.2   |
### Appendix 6  (continued)

#### Body Weights with Change (kg): Individual Values: Treatment Period

| Group / sex | Animal No. | Day | Change 0 - 63 |
|-------------|------------|-----|---------------|
| 1M          | 1          | 3.0 | 0.4           |
|             | 2          | 3.1 | 0.6           |
|             | 3          | 3.1 | 0.4           |
|             | 19         | 3.4 | 0.7           |
|             | 20         | 3.1 | 0.6           |
|             | 21         | 3.1 | 0.4           |
| 2M          | 4          | 3.5 | 0.7           |
|             | 5          | 3.3 | 0.5           |
|             | 6          | 3.2 | 0.7           |
|             | 22         | 3.4 | 0.8           |
|             | 23         | 3.2 | 0.6           |
|             | 24         | 3.1 | 0.5           |
| 3M          | 7          | 3.4 | 0.9           |
|             | 8          | 3.1 | 0.6           |
|             | 9          | 3.3 | 0.6           |
|             | 25         | 3.2 | 0.4           |
|             | 26         | 3.0 | 0.3           |
|             | 27         | 3.3 | 0.6           |
## Appendix 6 (continued)

### Body Weights with Change (kg): Individual Values: Treatment Period

| Group / sex | Animal No. | 1 | 2 | 3 |
|-------------|------------|---|---|---|
|             | -7 | 0 | 3 | 7 | 10 | 14 | 17 | 21 | 24 | 28 | 31 | 35 | 38 |
| 1F          | 10 | 2.6 | 2.8 | 2.8 | 3.0 | 3.0 | 3.2 | 3.2 | 3.3 | 3.3 | 3.4 | 3.5 | 3.5 | 3.6 | 3.6 |
| 11          | 2.7 | 2.9 | 3.0 | 3.2 | 3.2 | 3.3 | 3.4 | 3.4 | 3.5 | 3.5 | 3.5 | 3.6 | 3.6 | 3.7 | 3.7 |
| 12          | 2.6 | 2.8 | 2.8 | 2.9 | 3.0 | 3.0 | 3.1 | 3.1 | 3.1 | 3.2 | 3.2 | 3.3 | 3.3 | 3.4 | 3.4 |
| 28          | 2.7 | 2.9 | 3.0 | 3.1 | 3.1 | 3.3 | 3.3 | 3.4 | 3.4 | 3.4 | 3.5 | 3.6 | 3.6 | 3.7 | 3.7 |
| 29          | 2.7 | 2.9 | 3.0 | 3.0 | 3.1 | 3.2 | 3.2 | 3.3 | 3.3 | 3.4 | 3.5 | 3.5 | 3.6 | 3.7 | 3.7 |
| 30          | 2.9 | 3.1 | 3.2 | 3.4 | 3.5 | 3.6 | 3.8 | 3.9 | 3.9 | 4.0 | 4.0 | 4.1 | 4.1 | 4.1 | 4.1 |
| 2F          | 13 | 2.7 | 2.8 | 2.9 | 3.0 | 3.0 | 3.1 | 3.2 | 3.2 | 3.2 | 3.3 | 3.4 | 3.4 | 3.4 | 3.5 |
| 14          | 2.6 | 2.7 | 2.8 | 2.9 | 3.0 | 3.1 | 3.2 | 3.2 | 3.3 | 3.3 | 3.3 | 3.4 | 3.5 | 3.5 | 3.5 |
| 15          | 2.8 | 3.0 | 3.2 | 3.3 | 3.4 | 3.5 | 3.6 | 3.6 | 3.7 | 3.7 | 3.8 | 3.9 | 3.9 | 3.9 | 3.9 |
| 31          | 2.5 | 2.7 | 2.8 | 3.0 | 2.9 | 3.1 | 3.1 | 3.2 | 3.3 | 3.3 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 |
| 32          | 2.5 | 2.7 | 2.8 | 2.9 | 3.0 | 3.1 | 3.2 | 3.2 | 3.3 | 3.3 | 3.4 | 3.4 | 3.4 | 3.5 | 3.5 |
| 33          | 2.6 | 2.8 | 2.8 | 3.0 | 3.0 | 3.1 | 3.1 | 3.2 | 3.2 | 3.3 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 |
| 3F          | 16 | 2.6 | 2.7 | 2.8 | 2.8 | 2.9 | 2.9 | 3.0 | 3.0 | 3.0 | 3.0 | 3.1 | 3.1 | 3.1 | 3.2 |
| 17          | 2.9 | 3.0 | 3.0 | 3.2 | 3.3 | 3.4 | 3.4 | 3.5 | 3.5 | 3.5 | 3.7 | 3.6 | 3.8 | 3.8 | 3.8 |
| 18          | 2.4 | 2.7 | 2.8 | 3.0 | 3.1 | 3.3 | 3.4 | 3.4 | 3.5 | 3.5 | 3.6 | 3.7 | 3.8 | 3.8 | 3.9 |
| 34          | 2.8 | 2.9 | 3.0 | 3.1 | 3.2 | 3.3 | 3.4 | 3.4 | 3.5 | 3.5 | 3.6 | 3.6 | 3.7 | 3.7 | 3.7 |
| 35          | 2.7 | 3.0 | 3.1 | 3.3 | 3.5 | 3.5 | 3.5 | 3.6 | 3.7 | 3.8 | 3.8 | 3.9 | 3.9 | 3.9 | 3.9 |
| 36          | 2.9 | 3.1 | 3.3 | 3.4 | 3.5 | 3.6 | 3.6 | 3.7 | 3.7 | 3.8 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 |
### Appendix 6 (continued)

**Body Weights with Change (kg): Individual Values: Treatment Period**

| Group / sex | Animal No. | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Change 0-63 |
|-------------|------------|-------|-------|-------|-------|-------|-------|-------|-------------|
| 1F          | 10         | 3.7   | 3.6   | 3.7   | 3.8   | 3.9   | 3.9   | 3.9   | 1.1         |
|             | 11         | 3.8   | 3.8   | 3.9   | 3.9   | 4.0   | 4.1   | 4.1   | 1.2         |
|             | 12         | 3.5   | 3.6   | 3.6   | 3.7   | 3.7   | 3.8   | 3.8   | 1.0         |
|             | 28         | 3.6   | 3.7   | 3.7   | 3.8   | 3.9   | 4.0   | 3.9   | 1.0         |
|             | 29         | 3.7   | 3.8   | 3.8   | 3.8   | 3.9   | 4.0   | 4.0   | 1.1         |
|             | 30         | 4.3   | 4.3   | 4.4   | 4.5   | 4.6   | 4.6   | 4.6   | 1.5         |
| 2F          | 13         | 3.5   | 3.5   | 3.4   | 3.5   | 3.6   | 3.6   | 3.6   | 0.8         |
|             | 14         | 3.5   | 3.5   | 3.5   | 3.6   | 3.7   | 3.8   | 3.8   | 1.1         |
|             | 15         | 4.1   | 4.0   | 4.1   | 4.2   | 4.3   | 4.4   | 4.4   | 1.4         |
|             | 31         | 3.5   | 3.6   | 3.6   | 3.6   | 3.7   | 3.7   | 3.7   | 1.0         |
|             | 32         | 3.6   | 3.7   | 3.7   | 3.7   | 3.8   | 3.9   | 3.9   | 1.2         |
|             | 33         | 3.4   | 3.4   | 3.2   | 3.2   | 3.3   | 3.5   | 3.5   | 0.7         |
| 3F          | 16         | 3.1   | 3.2   | 3.2   | 3.2   | 3.2   | 3.2   | 3.2   | 0.5         |
|             | 17         | 3.8   | 3.9   | 3.9   | 3.9   | 3.9   | 4.0   | 4.0   | 1.0         |
|             | 18         | 3.9   | 4.0   | 4.0   | 4.1   | 4.2   | 4.2   | 4.2   | 1.5         |
|             | 34         | 3.7   | 3.8   | 3.9   | 3.9   | 3.9   | 4.0   | 4.0   | 1.1         |
|             | 35         | 4.0   | 4.0   | 4.1   | 4.2   | 4.2   | 4.2   | 4.0   | 1.0         |
|             | 36         | 4.0   | 4.1   | 4.2   | 4.2   | 4.3   | 4.3   | 4.3   | 1.2         |
## Appendix 7

### Body Weights with Change (kg): Individual Values: Recovery Period

| Group / sex | Animal No. | 66 | 70 | 73 | 77 | 80 | 84 | 87 | 91 | Change 66 - 91 |
|-------------|-----------|----|----|----|----|----|----|----|----|---------------|
| 1M          | 19        | 3.6| 3.6| 3.7| 3.7| 3.7| 3.7| 3.8| 0.2|
|            | 20        | 3.2| 3.3| 3.3| 3.3| 3.4| 3.4| 3.5| 0.3|
|            | 21        | 3.3| 3.3| 3.4| 3.4| 3.4| 3.3| 3.5| 0.2|
| 2M          | 22        | 3.6| 3.6| 3.6| 3.7| 3.6| 3.7| 3.7| 0.1|
|            | 23        | 3.3| 3.3| 3.4| 3.3| 3.4| 3.3| 3.4| 0.1|
|            | 24        | 3.3| 3.2| 3.3| 3.3| 3.3| 3.2| 3.3| 0.0|
| 3M          | 25        | 3.2| 3.2| 3.2| 3.3| 3.3| 3.4| 3.4| 0.2|
|            | 26        | 3.0| 2.9| 3.0| 3.0| 3.0| 3.0| 3.0| 0.0|
|            | 27        | 3.4| 3.4| 3.4| 3.3| 3.5| 3.4| 3.5| 0.1|
### Appendix 7 (continued)

**Body Weights with Change (kg): Individual Values: Recovery Period**

| Group / sex | Animal No. | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Change 66 - 91 |
|-------------|------------|-------|-------|-------|-------|-------|-------|----------------|
| 1F          |            |       |       |       |       |       |       |                 |
| 28          | 4.0        | 4.0   | 4.1   | 4.2   | 4.2   | 4.2   | 4.2   | 0.2            |
| 29          | 4.0        | 4.1   | 4.2   | 4.1   | 4.3   | 4.2   | 4.3   | 0.3            |
| 30          | 4.7        | 4.7   | 4.9   | 4.9   | 5.0   | 5.0   | 5.0   | 0.3            |
| 2F          |            |       |       |       |       |       |       |                 |
| 31          | 3.8        | 3.7   | 3.8   | 3.9   | 3.9   | 3.9   | 3.9   | 0.1            |
| 32          | 3.9        | 3.9   | 4.1   | 4.0   | 4.1   | 4.0   | 4.1   | 0.2            |
| 33          | 3.5        | 3.4   | 3.5   | 3.4   | 3.5   | 3.4   | 3.4   | 0.0            |
| 3F          |            |       |       |       |       |       |       |                 |
| 34          | 4.1        | 4.0   | 4.1   | 4.2   | 4.2   | 4.2   | 4.3   | 0.2            |
| 35          | 4.0        | 4.0   | 4.2   | 4.2   | 4.3   | 4.3   | 4.4   | 0.4            |
| 36          | 4.4        | 4.4   | 4.5   | 4.5   | 4.5   | 4.6   | 0.2   |                 |
### Appendix 8

**Food Consumption (g/animal/day): Individual Values: Treatment Period**

| Group | Test Item | Dosage (µg/dose) | Animal No. | Day -4 | 0 | 3 | 7 | 10 | 14 | 17 | 21 | 24 | 28 | 31 | 35 | 38 |
|-------|-----------|------------------|------------|--------|----|----|----|----|----|----|----|----|----|----|----|----|
|       |           |                  |            | 1M     | 2M | 3M |    |    |    |    |    |    |    |    |    |    |
|       |           |                  |            |        |    |    |    |    |    |    |    |    |    |    |    |    |
|       |           |                  |            | 1      | 2  | 3  |    |    |    |    |    |    |    |    |    |    |
| Control | MenPF-1  | MenPF-1         | 0          | 27.0   | 131.7 | 126.5 | 120.9 | 149.6 | 145.7 | 135.8 | 139.3 | 134.0 | 130.2 | 115.6 | 132.2 | 138.8 |
| 25     | 50        |                 | 25         |        |    |    |    |    |    |    |    |    |    |    |    |    |

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### Appendix 8 (continued)

**Food Consumption (g/animal/day): Individual Values: Treatment Period**

| Group / sex | Animal No. | Day | 1 | 2 | 3 |
|-------------|------------|-----|---|---|---|
| 1M          | 42         | 52  | 49 | 52 | 59 | 63 |
| 1           | 110.0      | 98.8| 100.5| 119.7| 118.3| 107.3| 116.5|
| 2           | 126.3      | 132.4| 135.5| 140.3| 133.8| 130.3| 133.0|
| 3           | 137.0      | 128.5| 130.3| 143.7| 117.0| 134.0| 139.3|
| 19          | 161.7      | 160.5| 164.8| 175.7| 165.3| 157.0| 166.0|
| 20          | 125.9      | 119.1| 136.0| 125.0| 135.3| 127.0| 137.5|
| 21          | 129.6      | 132.6| 147.8| 146.0| 140.3| 133.7| 153.3|
| 2M          | 4          | 52  | 49 | 52 | 59 | 63 |
| 4           | 144.7      | 141.0| 143.3| 141.0| 130.5| 138.0| 136.5|
| 5           | 163.4      | 126.4| 139.0| 155.3| 149.0| 150.7| 151.3|
| 6           | 124.1      | 138.1| 128.0| 158.3| 145.8| 169.7| 156.0|
| 22          | 158.2      | 158.9| 157.3| 165.3| 160.3| 161.3| 152.8|
| 23          | 123.0      | 93.0| 112.3| 98.7| 113.5| 122.7| 110.8|
| 24          | 112.6      | 93.7| 108.0| 106.3| 118.5| 132.3| 127.3|
| 3M          | 7          | 52  | 49 | 52 | 59 | 63 |
| 7           | 151.3      | 136.5| 145.3| 144.7| 154.8| 144.3| 150.8|
| 8           | 133.4      | 134.1| 128.8| 140.3| 137.0| 148.7| 130.3|
| 9           | 142.3      | 130.7| 131.3| 131.3| 139.8| 139.0| 118.5|
| 25          | 129.0      | 115.2| 117.5| 127.7| 128.5| 124.7| 111.3|
| 26          | 93.4       | 99.8| 112.0| 111.3| 100.3| 102.7| 103.5|
| 27          | 137.4      | 123.1| 139.3| 144.0| 119.3| 121.7| 117.8|
## Appendix 8 (continued)

### Food Consumption (g/animal/day): Individual Values: Treatment Period

| Group / sex | Animal No. | Day -4 | 0 | 3 | 7 | 10 | 14 | 17 | 21 | 24 | 28 | 31 | 35 | 38 |
|-------------|------------|-------|---|---|---|----|----|----|----|----|----|----|----|----|
| 1F          |            | 1     | 2 | 3 | 3 | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
| 1           | 10         | 149.3 | 161.0 | 117.1 | 174.7 | 142.7 | 163.8 | 162.7 | 180.8 | 152.7 | 170.0 | 171.7 | 140.8 | 153.0 |
| 11          | 151.0      | 154.8 | 142.7 | 171.5 | 142.7 | 168.0 | 157.0 | 173.5 | 157.0 | 165.3 | 160.7 | 171.0 | 158.3 |       |
| 12          | 121.0      | 126.8 | 123.5 | 110.1 | 121.7 | 133.0 | 108.0 | 133.3 | 122.0 | 119.8 | 121.3 | 142.0 | 123.3 |       |
| 28          | 150.0      | 150.3 | 130.6 | 152.6 | 129.3 | 155.5 | 142.0 | 151.3 | 141.3 | 140.0 | 140.0 | 138.3 | 147.3 |       |
| 29          | 158.3      | 154.0 | 143.0 | 155.5 | 130.3 | 166.8 | 157.7 | 158.5 | 144.3 | 167.0 | 157.3 | 177.0 | 169.3 |       |
| 30          | 165.3      | 180.3 | 177.2 | 195.4 | 198.0 | 209.8 | 195.0 | 212.5 | 198.3 | 205.3 | 183.3 | 198.8 | 205.7 |       |
| 2F          |            | 1     | 2 | 3 | 3 | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
| 13          | 120.0      | 155.3 | 136.9 | 145.4 | 125.7 | 153.8 | 149.0 | 160.0 | 122.0 | 140.3 | 150.7 | 143.3 | 131.7 |       |
| 14          | 147.0      | 156.8 | 134.7 | 162.2 | 140.7 | 162.5 | 147.7 | 159.8 | 134.3 | 158.8 | 160.7 | 177.0 | 156.0 |       |
| 15          | 129.7      | 159.0 | 159.3 | 169.0 | 172.7 | 189.3 | 164.0 | 171.5 | 171.3 | 171.8 | 186.0 | 179.3 | 183.7 |       |
| 31          | 126.7      | 136.5 | 122.9 | 145.1 | 144.3 | 151.0 | 120.0 | 149.3 | 133.7 | 129.3 | 113.0 | 156.3 | 126.3 |       |
| 32          | 120.3      | 137.0 | 132.2 | 147.9 | 136.3 | 162.8 | 151.7 | 184.0 | 148.0 | 144.3 | 143.3 | 169.3 | 159.0 |       |
| 33          | 127.3      | 150.0 | 130.3 | 140.4 | 148.3 | 146.0 | 132.3 | 153.0 | 133.3 | 143.0 | 129.3 | 155.0 | 134.0 |       |
| 3F          |            | 1     | 2 | 3 | 3 | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
| 16          | 129.3      | 117.5 | 113.3 | 122.6 | 119.3 | 110.5 | 123.0 | 116.0 | 105.7 | 110.3 | 116.7 | 115.5 | 115.0 |       |
| 17          | 159.7      | 174.5 | 148.2 | 163.1 | 161.0 | 176.3 | 156.3 | 178.0 | 157.3 | 172.5 | 151.7 | 182.8 | 166.0 |       |
| 18          | 135.3      | 155.3 | 180.9 | 205.1 | 183.0 | 247.0 | 201.3 | 213.5 | 200.7 | 211.5 | 222.3 | 222.8 | 212.0 |       |
| 34          | 164.3      | 168.5 | 150.4 | 158.3 | 148.0 | 172.5 | 162.7 | 165.3 | 162.3 | 168.3 | 157.0 | 161.0 | 162.7 |       |
| 35          | 184.7      | 187.5 | 176.4 | 203.7 | 188.0 | 202.3 | 181.3 | 213.8 | 187.0 | 202.5 | 204.0 | 206.3 | 206.3 |       |
| 36          | 182.3      | 184.8 | 172.7 | 205.4 | 174.3 | 199.3 | 185.3 | 198.3 | 169.3 | 186.8 | 185.3 | 194.8 | 185.3 |       |
### Appendix 8 (continued)
**Food Consumption (g/animal/day): Individual Values: Treatment Period**

| Group / sex | Animal No. | 1 | 2 | 3 |
|-------------|------------|---|---|---|
| Test Item   |            | 0 | 25 | 50 |
| Dosage (µg/dose) |         |   |    |    |

| Group / sex | Animal No. | Day |   |
|-------------|------------|-----|---|
| 1F          |            | 42  |   |
|             |            | 45  | 49 |
|             |            | 52  | 56 |
|             |            | 59  | 63 |
| 2F          |            | 42  |   |
|             |            | 45  | 49 |
|             |            | 52  | 56 |
|             |            | 59  | 63 |
| 3F          |            | 42  |   |
|             |            | 45  | 49 |
|             |            | 52  | 56 |
|             |            | 59  | 63 |

| Group / sex | Animal No. | Day |   |
|-------------|------------|-----|---|
| 1F          |            | 0   | 25 |
|             |            | 25  | 50 |
| 2F          |            | 0   | 25 |
|             |            | 25  | 50 |
| 3F          |            | 0   | 25 |
|             |            | 25  | 50 |

**Test Facility Study No. 520419**
## Appendix 9

### Food Consumption (g/animal/day): Individual Values: Recovery Period

| Group / sex | Animal No. | Day | 1 (Control) | 2 (MenPF-1) | 3 (MenPF-1) |
|-------------|------------|-----|-------------|-------------|-------------|
| 1M          | 19         | 77  | 179.7       | 163.5       | 152.5       |
|             | 20         | 80  | 123.0       | 122.3       | 123.3       |
|             | 21         | 91  | 143.0       | 92.3        | 104.7       |
| 2M          | 22         | 73  | 134.0       | 122.3       | 118.3       |
|             | 23         | 84  | 121.0       | 122.3       | 112.3       |
|             | 24         | 87  | 125.7       | 119.0       | 111.7       |
| 3M          | 25         | 70  | 102.0       | 126.3       | 126.3       |
|             | 26         | 80  | 104.7       | 99.3        | 129.5       |
|             | 27         | 87  | 118.3       | 123.8       | 121.0       |
## Appendix 9 (continued)

### Food Consumption (g/animal/day): Individual Values: Recovery Period

| Group / sex | Test Item | Dosage (µg/dose) | Animal No. | Day | 1 | 2 | 3 |
|-------------|-----------|------------------|------------|-----|---|---|---|
| 1F          | Control   | 0                | 66         | 70  | 73 | 77 | 80 | 84 | 87 | 91 |
|             | MenPF-1   | 25               | 29         | 146.7 | 141.3 | 151.7 | 158.3 | 152.0 | 150.0 | 147.0 | 156.0 |
|             |           | 50               | 30         | 198.0 | 209.0 | 201.7 | 207.0 | 198.3 | 198.8 | 198.0 | 194.0 |
| 2F          | Control   | 0                | 31         | 110.7 | 133.5 | 114.3 | 149.0 | 121.3 | 132.8 | 120.3 | 127.0 |
|             | MenPF-1   | 25               | 32         | 146.3 | 151.0 | 133.0 | 172.3 | 127.0 | 152.0 | 124.7 | 143.3 |
|             |           | 50               | 33         | 96.3  | 108.5 | 88.3  | 114.8 | 88.0  | 85.5  | 87.3  | 108.8 |
| 3F          | Control   | 0                | 34         | 160.7 | 145.3 | 158.3 | 164.0 | 147.0 | 156.3 | 141.0 | 149.3 |
|             | MenPF-1   | 25               | 35         | 104.3 | 144.8 | 175.3 | 202.0 | 147.3 | 176.0 | 172.3 | 182.5 |
|             |           | 50               | 36         | 174.0 | 172.5 | 164.3 | 185.5 | 161.7 | 184.8 | 167.0 | 171.5 |
## Appendix 10
### Individual Ophthalmoscopy Findings

| Group | Test Item | Dosage (µg/dose) | Animal No./Sex | Finding | Timepoint |
|-------|-----------|----------------|---------------|---------|-----------|
|       | 1         | Control        | 1M             | Right eye: Persistent pupillary membranes in the iris. | Pretrial |
|       |           |                |                | Left eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Right eye: NAD | Week 10 |
|       | 2         | MenPF-1        |                | Right eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Left eye: Focal opacity, posterior in the cortex of the lens. | Pretrial, Week 10 |
|       | 3         | MenPF-1        | 2M             | Right eye: Persistent pupillary membranes in the iris. | Pretrial |
|       |           |                |                | Left eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Right eye: Retinal dysplasia in the fundus. | Pretrial, Week 10 |
|       |           |                |                | Left eye: Retinal dysplasia in the fundus. | Pretrial, Week 10 |
|       | 4         | MenPF-1        | 21M            | Right eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Left eye: Focal opacity, posterior in the cortex of the lens. | Pretrial, Week 10 |
|       | 5         | MenPF-1        | 4M             | Right eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Left eye: Focal opacity, posterior in the cortex of the lens. | Pretrial, Week 10 |
|       | 6         | MenPF-1        | 22M            | Right eye: Persistent pupillary membranes in the iris. | Pretrial |
|       |           |                |                | Left eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Right eye: Retinal dysplasia in the fundus. | Pretrial, Week 10 |
|       |           |                |                | Left eye: Retinal dysplasia in the fundus. | Pretrial, Week 10 |
|       | 7         | MenPF-1        | 7M             | Right eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Left eye: Focal opacity, posterior in the cortex of the lens. | Pretrial, Week 10 |
|       | 8         | MenPF-1        | 26M            | Right eye: NAD | Pretrial, Week 10 |
|       |           |                |                | Left eye: Focal opacity, posterior in the cortex of the lens. | Pretrial, Week 10 |

NAD – No abnormalities detected
Animals not reported were normal
### Appendix 10 (continued)
**Individual Ophthalmoscopy Findings**

| Group | Test Item | Dosage (µg/dose) | Finding                                                                 | Timepoint       |
|-------|-----------|------------------|-------------------------------------------------------------------------|-----------------|
| 1     | Control   | 0                | Persistent pupillary membranes in the iris                             | Pretrial, Week 10|
|       | MenPF-1   | 25               | NAD                                                                     |                 |
| 2     | MenPF-1   | 50               | Focal opacity, anterior in the cortex of the lens.                       |                 |
| 3     | MenPF-1   | 0                | NAD                                                                     |                 |

| Group | Animal No./Sex | Finding                                                                 | Timepoint       |
|-------|----------------|-------------------------------------------------------------------------|-----------------|
| 1     | 11F            | Persistent pupillary membranes in the iris                             | Pretrial, Week 10|
|       | Left eye: NAD  |                                                                         |                 |
| 30F   | Right eye: NAD |                                                                         |                  |
|       | Left eye: Focal opacity, anterior in the cortex of the lens.            | Pretrial, Week 10|
| 2     | 14F            | Persistent pupillary membranes in the iris                             | Pretrial, Week 10|
|       | Both eyes: NAD |                                                                         |                 |
| 32F   | Right eye: Multi-focal opacity, anterior in the cortex of the lens       | Pretrial, Week 10|
|       | Left eye: Focal opacity, anterior in the cortex of the lens.            |                  |
|       | Right eye: NAD    |                                                                         |                 |
| 33F   | Both eyes: NAD |                                                                         |                 |
| 3     | 18F            | Retinal dysplasia in the Fundus.                                        | Pretrial, Week 10|
| 34F   | Right eye: NAD  |                                                                         | Pretrial         |
|       | Left eye: Focal opacity, anterior in the cortex of the lens.            |                  |
|       | Left eye: Diffuse opacity, posterior in the cortex of the lens.         | Week 10          |
|       | Left eye: NAD |                                                                         |                 |
| 36F   | Right eye: NAD  |                                                                         | Pretrial, Week 10|

NAD – No abnormalities detected
Animals not reported were normal
### Appendix 11

**Body Temperatures (°C): Individual Recordings**

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|-----------------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| Group / sex | Animal No. | Pretrial | Day 1 | Day 22 | Day 43 |
|-------------|------------|----------|-------|-------|-------|
| 1M          | 1          | 38.7     | 39.4  | 39.1  | 38.6  | 35.2  | 39.5  | 40.6  | 37.6  | 37.7  | 39.5  | 37.9  | 39.5  | 38.5  | 38.8  | 37.0  | 35.8  |
|             | 2          | 37.3     | 38.6  | 38.6  | 36.6  | 36.6  | 40.1  | 39.4  | 37.6  | 37.6  | 39.7  | 37.1  | 38.4  | 36.8  | 38.5  | 36.9  | 37.5  |
|             | 3          | 35.3     | 36.3  | 38.2  | 36.8  | 37.9  | 37.3  | 39.6  | 38.0  | 37.7  | 35.2  | 36.9  | 39.6  | 36.5  | 35.8  | 35.8  | 37.6  |
|             | 19         | 37.0     | 39.1  | 38.8  | 38.5  | 40.3  | 39.9  | 40.5  | 36.9  | 37.8  | 39.6  | 38.8  | 39.9  | 38.2  | 39.2  | 39.4  | 37.5  |
|             | 20         | 36.6     | 39.5  | 39.2  | 39.1  | 37.1  | 38.1  | 35.4  | 37.9  | 38.6  | 37.9  | 38.6  | 35.6  | 36.6  | 38.2  | 39.1  | 36.9  | 37.5  |
|             | 21         | 37.3     | 39.1  | 38.3  | 39.0  | 39.8  | 39.5  | 39.9  | 38.1  | 39.8  | 38.5  | 38.2  | 40.5  | 38.1  | 38.5  | 38.3  | 38.5  | 37.6  |
| 2M          | 4          | 35.0     | 39.9  | 37.4  | 39.8  | 39.4  | 39.7  | 38.3  | 38.0  | 39.5  | 37.5  | 38.8  | 40.3  | 39.4  | 38.3  | 38.6  | 38.3  |
|             | 5          | 36.0     | 39.5  | 37.9  | 38.5  | 39.1  | 39.1  | 40.0  | 38.4  | 39.2  | 40.3  | 39.2  | 39.9  | 39.4  | 39.6  | 38.0  | 38.8  | 38.9  |
|             | 6          | 36.2     | 40.0  | 38.1  | 40.1  | 39.3  | 37.3  | 38.7  | 38.8  | 36.1  | 39.5  | 38.2  | 39.6  | 37.8  | 37.8  | 39.5  | 39.9  |
|             | 22         | 36.3     | 37.6  | 36.1  | 38.6  | 39.6  | 38.1  | 38.7  | 38.2  | 38.4  | 39.4  | 38.2  | 38.4  | 38.9  | 37.4  | 39.1  | 39.7  |
|             | 23         | 36.8     | 36.1  | 37.9  | 37.1  | 39.3  | 38.8  | 38.0  | 37.5  | 37.2  | 36.7  | 36.2  | 39.4  | 38.3  | 37.6  | 39.2  | 38.5  |
|             | 24         | 34.9     | 37.9  | 38.4  | 38.6  | 39.5  | 38.5  | 38.2  | 36.2  | 39.5  | 39.5  | 39.5  | 38.0  | 38.4  | 37.7  | 39.3  | 39.8  |
| 3M          | 7          | 35.1     | 37.2  | 38.2  | 36.4  | 40.8  | 39.1  | 39.6  | 37.5  | 38.2  | 39.0  | 38.7  | 39.5  | 38.9  | 39.6  | 39.0  | 39.7  |
|             | 8          | 34.8     | 37.2  | 39.0  | 37.0  | 38.5  | 39.2  | 39.9  | 38.0  | 39.3  | 39.0  | 39.2  | 39.6  | 39.0  | 38.7  | 39.0  | 38.4  |
|             | 9          | 34.8     | 39.4  | 38.2  | 36.9  | 38.5  | 39.4  | 40.2  | 38.5  | 38.4  | 38.6  | 37.1  | 38.9  | 38.6  | 38.9  | 39.1  | 39.3  |
|             | 25         | 35.7     | 37.4  | 38.1  | 34.8  | 38.7  | 39.7  | 39.7  | 38.4  | 38.1  | 38.9  | 39.3  | 38.5  | 38.1  | 38.7  | 38.5  | 39.4  |
|             | 26         | 35.4     | 35.7  | 38.4  | 37.5  | 37.6  | 37.5  | 37.4  | 37.9  | 39.8  | 36.2  | 38.6  | 39.5  | 37.9  | 38.8  | 39.4  | 39.5  |
|             | 27         | 34.1     | 37.1  | 38.1  | 36.1  | 38.1  | 37.5  | 39.4  | 37.2  | 38.4  | 39.1  | 38.9  | 38.3  | 36.6  | 39.0  | 39.4  | 39.2  |

*IBD = immediately before dose*
### Appendix 11  (continued)

**Body Temperatures (°C): Individual Recordings**

| Group / Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------------------|------------------|---|---|---|
| Control           | Control          | 0 | 25| 50|
| MenPF-1           | MenPF-1          | 25| 50| 75|

| Group / sex / Animal No. | Day 64 |
|--------------------------|--------|
|                          | IBD + 1 h | + 3 h | + 24 h | + 48 h |
| 1M 1                     | 38.8    | 37.7 | 39.5 | 39.0 | 37.4 |
|                          | 39.6    | 36.2 | 38.7 | 39.1 | 37.9 |
|                          | 39.9    | 36.7 | 38.6 | 39.1 | 37.2 |
|                          | 38.1    | 37.2 | 38.0 | 39.4 | 37.3 |
|                          | 36.8    | 37.0 | 38.2 | 38.1 | 37.0 |
|                          | 39.5    | 36.3 | 38.3 | 39.8 | 38.0 |
| 2M 4                     | 39.1    | 37.4 | 38.6 | 39.3 | 38.0 |
|                          | 38.1    | 37.3 | 37.3 | 38.2 | 38.3 |
|                          | 39.3    | 37.4 | 38.8 | 37.4 | 39.6 |
|                          | 38.6    | 37.9 | 39.3 | 37.4 | 39.5 |
|                          | 38.4    | 37.1 | 38.0 | 37.3 | 38.1 |
|                          | 38.6    | 36.3 | 38.2 | 38.4 | 39.3 |
| 3M 7                     | 38.0    | 39.0 | 39.2 | 38.7 | 39.1 |
|                          | 38.0    | 38.4 | 38.2 | 38.6 | 39.0 |
|                          | 38.2    | 38.3 | 38.0 | 38.6 | 38.9 |
|                          | 38.2    | 38.8 | 38.0 | 40.4 | 38.8 |
|                          | 36.6    | 38.5 | 38.1 | 37.4 | 38.9 |
|                          | 39.0    | 38.0 | 38.0 | 38.6 | 38.8 |

*IBD = immediately before dose*
### Appendix 11 (continued)

**Body Temperatures (°C): Individual Recordings**

| Group / sex | Animal No. | Pretrial | Day 1 | Day 22 | Day 43 |
|-------------|------------|----------|-------|--------|--------|
|             |            | IBD + 1 h | + 3 h | + 24 h | + 48 h | IBD + 1 h | + 3 h | + 24 h | + 48 h | IBD + 1 h | + 3 h | + 24 h | + 48 h |
| 1F          | 10         | 35.2      | 37.8  | 38.7  | 37.1  | 37.0  | 38.0  | 36.5  | 38.2  | 35.1  | 35.2  | 36.5  | 39.8  | 38.1  | 38.4  | 37.3  | 37.4  |
|             | 11         | 37.8      | 39.5  | 38.8  | 37.6  | 37.4  | 39.9  | 38.5  | 38.8  | 39.5  | 39.0  | 37.5  | 39.8  | 38.3  | 39.0  | 39.2  | 37.7  |
|             | 12         | 35.5      | 37.7  | 38.5  | 36.5  | 36.4  | 37.3  | 37.3  | 37.4  | 39.7  | 36.8  | 34.8  | 40.0  | 38.6  | 37.4  | 37.1  | 38.5  |
|             | 28         | 36.1      | 37.0  | 38.3  | 36.9  | 36.7  | 37.9  | 39.3  | 37.8  | 39.3  | 38.1  | 35.9  | 39.8  | 38.5  | 36.6  | 39.1  | 38.7  |
|             | 29         | 38.6      | 39.0  | 38.1  | 37.0  | 40.1  | 40.0  | 37.7  | 37.0  | 39.4  | 39.6  | 37.9  | 37.5  | 39.4  | 37.9  | 37.4  | 38.7  |
|             | 30         | 37.3      | 38.3  | 37.6  | 38.1  | 38.2  | 37.7  | 38.0  | 37.2  | 39.2  | 38.0  | 36.5  | 38.8  | 38.3  | 37.3  | 37.2  | 38.5  |
| 2F          | 13         | 34.7      | 36.8  | 38.2  | 39.1  | 39.6  | 37.6  | 39.9  | 37.7  | 35.6  | 39.9  | 36.6  | 38.9  | 37.2  | 38.7  | 39.3  | 39.2  |
|             | 14         | 36.7      | 37.0  | 37.9  | 39.0  | 37.5  | 39.2  | 39.7  | 37.3  | 35.8  | 39.1  | 37.7  | 40.0  | 37.5  | 37.9  | 39.1  | 39.2  |
|             | 15         | 37.6      | 37.6  | 38.4  | 39.4  | 38.2  | 39.6  | 37.7  | 38.1  | 36.5  | 39.4  | 38.6  | 39.3  | 38.3  | 37.5  | 37.9  | 38.9  |
|             | 31         | 34.4      | 37.6  | 38.9  | 37.6  | 37.5  | 39.5  | 39.9  | 38.4  | 38.3  | 39.7  | 39.1  | 40.0  | 37.5  | 37.9  | 38.5  | 38.4  |
|             | 32         | 35.2      | 36.1  | 37.2  | 39.7  | 36.0  | 37.6  | 38.7  | 37.5  | 40.2  | 39.6  | 37.0  | 38.7  | 38.6  | 38.0  | 39.7  | 38.5  |
|             | 33         | 36.0      | 37.0  | 37.0  | 38.9  | 39.0  | 39.0  | 38.1  | 37.7  | 38.4  | 38.7  | 39.0  | 38.6  | 36.8  | 37.4  | 38.6  | 39.3  |
| 3F          | 16         | 36.6      | 37.2  | 38.5  | 37.0  | 37.9  | 38.8  | 39.8  | 38.6  | 39.6  | 40.3  | 39.3  | 39.5  | 39.0  | 38.7  | 39.3  | 40.1  |
|             | 17         | 37.0      | 38.8  | 38.5  | 38.5  | 39.8  | 40.1  | 40.3  | 37.7  | 38.8  | 38.5  | 38.9  | 40.2  | 38.9  | 38.7  | 38.9  | 39.6  |
|             | 18         | 35.1      | 38.7  | 38.5  | 38.5  | 38.5  | 37.8  | 39.7  | 39.0  | 38.1  | 38.6  | 38.0  | 39.6  | 37.3  | 38.3  | 38.6  | 39.6  |
|             | 34         | 34.2      | 38.2  | 37.3  | 38.0  | 38.3  | 37.7  | 39.1  | 37.7  | 38.9  | 37.7  | 37.8  | 35.6  | 37.5  | 37.8  | 38.3  | 37.9  |
|             | 35         | 35.1      | 37.4  | 37.8  | 39.3  | 38.8  | 38.1  | 38.0  | 38.5  | 37.1  | 36.6  | 37.2  | 39.0  | 36.1  | 38.5  | 39.3  | 38.9  |
|             | 36         | 35.0      | 36.0  | 38.0  | 39.0  | 38.4  | 37.8  | 38.5  | 38.8  | 39.5  | 38.0  | 38.5  | 38.2  | 37.8  | 37.7  | 37.8  | 37.5  |

*IBD = immediately before dose*
### Appendix 11 (continued)

**Body Temperatures (°C): Individual Recordings**

| Group / sex | Animal No. | IBD | + 1 h | + 3 h | + 24 h | + 48 h |
|-------------|------------|-----|-------|-------|--------|--------|
| 1F          | 10         | 39.7| 36.8  | 38.5  | 37.4   | 37.2   |
|             | 11         | 39.3| 37.2  | 39.4  | 38.8   | 38.9   |
|             | 12         | 38.3| 37.0  | 38.2  | 37.3   | 37.8   |
|             | 28         | 37.9| 36.7  | 37.5  | 37.4   | 37.2   |
|             | 29         | 38.0| 37.7  | 38.2  | 37.2   | 37.1   |
|             | 30         | 38.3| 37.7  | 38.4  | 37.1   | 36.8   |
| 2F          | 13         | 37.7| 38.3  | 37.7  | 37.7   | 38.0   |
|             | 14         | 38.6| 38.2  | 38.7  | 38.6   | 39.7   |
|             | 15         | 39.0| 38.1  | 38.6  | 38.6   | 37.9   |
|             | 31         | 39.1| 38.9  | 38.1  | 39.0   | 38.7   |
|             | 32         | 38.6| 38.9  | 38.1  | 37.0   | 38.8   |
|             | 33         | 38.1| 38.7  | 38.1  | 38.6   | 38.7   |
| 3F          | 16         | 36.7| 38.9  | 39.3  | 38.2   | 39.4   |
|             | 17         | 37.3| 38.7  | 38.0  | 38.5   | 38.8   |
|             | 18         | 40.3| 38.6  | 38.1  | 39.0   | 39.1   |
|             | 34         | 39.1| 37.8  | 37.7  | 39.3   | 39.1   |
|             | 35         | 38.8| 38.8  | 38.8  | 38.5   | 39.0   |
|             | 36         | 37.9| 38.7  | 38.8  | 38.4   | 37.2   |

*IBD = immediately before dose*
Appendix 12
Methods, Units and Abbreviations Used for Laboratory Investigations

| Group | Test Item   | Dosage (µg/dose) |
|-------|-------------|------------------|
|       | Control     | 0                |
|       | MenPF-1     | 25               |
|       | MenPF-1     | 50               |

**Haematology**

| Parameters              | Methods                                                                 | Units   |
|-------------------------|------------------------------------------------------------------------|---------|
| Red Blood Cell Count:   | Siemens, ADVIA 120 haematology analyser developed from Tycko et al 1985, Applied Optics 24(9):1355-1365. | x10^{12}/L |
| (RBC)                   |                                                                        |         |
| Haemoglobin:            | Siemens, ADVIA 120 haematology analyser obtained from the direct measurements of red cell volume and haemoglobin concentration using the RBC/pH method. | g/dL    |
| (Hb)                    |                                                                        |         |
| Haematocrit:            | Siemens, ADVIA 120 haematology analyser derived from the measured red cell volume (MCV) and the red cell count (RBC). | L/L     |
| (Hct)                   |                                                                        |         |
| Mean Cell Volume:       | Siemens, ADVIA 120 haematology analyser derived from mean of RBC volume histogram. | fL      |
| (MCV)                   |                                                                        |         |
| Mean Cell Haemoglobin Concentration: | Siemens, ADVIA 120 haematology analyser. Calculated parameter from haemoglobin concentration, red blood cell count and mean cell volume. | g/dL    |
| (MCHC)                  |                                                                        |         |
| Mean Cell Haemoglobin:  | Siemens, ADVIA 120 haematology analyser. Calculated parameter from haemoglobin concentration and red blood cell count. | pg      |
| (MCH)                   |                                                                        |         |
### Appendix 12  (continued)
#### Methods, Units and Abbreviations Used for Laboratory Investigations

| Group | Test Item  | Dosage (µg/dose) |
|-------|------------|------------------|
|       | Control    | 0                |
|       | MenPF-1    | 25               |
|       | MenPF-1    | 50               |

#### Haematology

| Parameters               | Methods                                                                 | Units  |
|--------------------------|-------------------------------------------------------------------------|--------|
| Reticulocytes: (Reti)    | Siemens, ADVIA 120 haematology analyser measured by light absorption which is proportional to RNA content. | %      |
| Reticulocyte Count: (Ret)| Siemens, ADVIA 120 haematology analyser measured by light absorption which is proportional to RNA content. | x10⁹/L |
| Red Cell Distribution Width: (RDW) | Siemens, ADVIA 120 haematology analyser measured from the amount of variation in size or volume of RBC’s. This is the coefficient of variation of the RBC volume distribution. | %      |
| Platelet Count: (Plat)  | Siemens, ADVIA 120 haematology analyser measured using the MIE theory of light scattering for homogenous spheres. | x10⁹/L |
| White Blood Cell Count: (WBC) | Siemens, ADVIA 120 haematology analyser analysed using two angle laser light signals | x10⁹/L |
| Neutrophils: (Neut)     | Siemens, ADVIA 120 haematology analyser measured quantitatively using both the Peroxidase method and the Basophil/Lobularity method. | x10⁹/L |
## Appendix 12  (continued)
### Methods, Units and Abbreviations Used for Laboratory Investigations

| Group | Test Item     | Dosage (µg/dose) |
|-------|---------------|------------------|
|       | Control       | 0                |
|       | MenPF-1       | 25               |
|       | MenPF-1       | 50               |

### Haematology

| Parameters | Methods | Units |
|------------|---------|-------|
| Lymphocytes: (Lymp) | Siemens, ADVIA 120 haematology analyser measured quantitatively using both the Peroxidase method and the Basophil/Lobularity method. | \(\times 10^9/L\) |
| Monocytes: (Mono) | Siemens, ADVIA 120 haematology analyser measured quantitatively using both the Peroxidase method and the Basophil/Lobularity method. | \(\times 10^9/L\) |
| Eosinophils: (Eos) | Siemens, ADVIA 120 haematology analyser measured quantitatively using both the Peroxidase method and the Basophil/Lobularity method. | \(\times 10^9/L\) |
| Basophils: (Baso) | Siemens, ADVIA 120 haematology analyser measured quantitatively using both the Peroxidase method and the Basophil/Lobularity method. | \(\times 10^9/L\) |
| Large Unclassified Cells: (LUC) | Siemens, ADVIA 120 haematology analyser measured quantitatively using both the Peroxidase method and the Basophil/Lobularity method. | \(\times 10^9/L\) |
### Appendix 12  (continued)
**Methods, Units and Abbreviations Used for Laboratory Investigations**

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
|       | Control   | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

**Coagulation**

| Parameters                  | Methods                                                                                     | Units |
|-----------------------------|---------------------------------------------------------------------------------------------|-------|
| Activated Partial Thromboplastin Time: (APTT) | Instrumentation Laboratory, ACL Advance coagulation analyser. Cephalin with micronized silica for the in vitro determination of APTT in plasma | s     |
| Fibrinogen: (Fib)          | HemosIL PT-Fibrinogen high sensitivity reagent HS PLUS cat# 0008469810 ©. Calcium thromboplastin (rabbit brain) for the simultaneous in vitro determination of PT and fibrinogen in plasma using ACL Advance/Futura coagulation analyser | mg/dL |
| Prothrombin Time: (PT)     | HemosIL PT-Fibrinogen high sensitivity reagent HS PLUS cat# 0008469810 ©. Calcium thromboplastin (rabbit brain) for the simultaneous in vitro determination of PT and fibrinogen in plasma using ACL Advance/Futura coagulation analyser | s     |
Appendix 12  (continued)
Methods, Units and Abbreviations Used for Laboratory Investigations

| Group | Test Item   | Dosage (µg/dose) |
|-------|-------------|-----------------|
|       | Control     | 0               |
| 2     | MenPF-1     | 25              |
| 3     | MenPF-1     | 50              |

Clinical Chemistry

| Parameters | Methods | Units |
|------------|---------|-------|
| Urea:      | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Urease kinetic UV Assay developed from Talke H, Schubert GE. Klin Wschr 1965;43:174-175. | mmol/L |
| Glucose:   | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Hexokinase UV assay, Schmidt F H 1961 Klin Wschr 39:1244 | mmol/L |
| Aspartate Aminotransferase: | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. IFCC Method. | U/L |
| Alanine Aminotransferase: | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. IFCC Method. | U/L |
| Alkaline Phosphatase: | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. IFCC Method. | U/L |
| Creatine Phosphokinase: | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. IFCC Method. Thomas, L ed. Labor und Diagnose, 4th ed. Marburg: Die Medizinische Verlagsgesellschaft. Szasz G et al. Clin Chem 1976;22:650 | U/L |
### Group Test Item Dosage (µg/dose):

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
|       | Control   | 0               |
| 1     | MenPF-1   | 25              |
| 2     | MenPF-1   | 50              |

### Clinical Chemistry

**Parameters** | **Methods** | **Units**
--- | --- | ---
Lactate Dehydrogenase: (LDH) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. IFCC Method. | U/L
Sodium: (Na) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using indirect Ion Selective Electrode. Application of the Nernst equation to an electrode with crown ether membrane type. | mmol/L
Potassium: (K) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using indirect Ion Selective Electrode. Application of the Nernst equation to an electrode with valinomycin liquid membrane type. | mmol/L
Chloride: (Cl) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using indirect Ion Selective Electrode. Application of the Nernst equation to an electrode with quaternary ammonium salt ion exchanger. | mmol/L
Total Protein: (TP) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Biuret colorimetric assay for the formation of protein - biuret reagent complex. | g/L
### Appendix 12  (continued)
Methods, Units and Abbreviations Used for Laboratory Investigations

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
|       | Control   | 0               |
| 2     | MenPF-1   | 25              |
| 3     | MenPF-1   | 50              |

#### Clinical Chemistry

| Parameters          | Methods                                                                 | Units |
|---------------------|-------------------------------------------------------------------------|-------|
| Albumin: (Alb)      | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit Cat. No.11970909 216. Brom cresol green colorimetric assay with endpoint method. Doumas B.T. et al 1971, Clin Chem Acta 31:87. | g/L   |
| Globulin: (Glob)    | Calculated by subtraction of the Albumin concentration from the Total Protein concentration. | g/L   |
| Albumin Globulin Ratio: (AG-R) | Calculated using Clinical Chemistry Plasma/Serum Total Protein and Albumin Concentrations. Calculated Parameter (Albumin/(Total Protein-Albumin)) |       |
| Cholesterol: (Chol) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. CHOD-PAP colorimetric assay for the measurement of cholesterol in serum or plasma. | mmol/L|
| Creatinine: (Crea)  | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Jaffé kinetic colorimetric method. Rate blanked and compensated. Bartels et al 1972, Clin Chem Acta 37:193 | µmol/L|
| Total Bilirubin: (T.Bil) | Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Modified Jendrassik-Grof colorimetric method in strongly acidic conditions. | µmol/L|
Appendix 12  (continued)
Methods, Units and Abbreviations Used for Laboratory Investigations

| Group | Test Item | Dosage (µg/dose) |  |  |
|-------|-----------|-----------------|---|---|
|       | Control   | 0               | 2 | MenPF-1 |
|       | MenPF-1   | 25              | 3 | MenPF-1 |

Clinical Chemistry

Parameters: Methods: Units

Jendrassik L et al. Biochem Z 1938;297:81.

Calcium: (Ca)
Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. O-cresolphthalein complexone coloimetric assay. Gindler E M and King J D 1972, Am. J. Clin. Pathol. 58:376

Phosphate: (Phos)
Roche/Hitachi P Modular 800 Clinical Chemistry Analyser using Roche Test Kit. Molybdate UV method. Henry, R.J. 1974. pg 723 in "Clinical Chemistry" 2nd Edition.

The limit of quantification (LOQ) for the following assays was observed and reported as follows with the LOQ used to calculate means and standard deviations for values below the LOQ:

| Assay            | Limit of Detection | Non-detectable values reported as |
|------------------|--------------------|-----------------------------------|
| Total Bilirubin  | 1.7 µmol/L         | <1.7 µmol/L                       |
### Appendix 13

**Haematology and Coagulation: Individual Values: Pretrial**

| Group / Test Item / Dosage (µg/dose) | 1  | 2  | 3  |
|-------------------------------------|----|----|----|
| Control 0                          |    |    |    |
| MenPF-1 25                         |    |    |    |
| MenPF-1 50                         |    |    |    |

| Group / Animal No. / Hb g/dL / RBC x10¹²/L / Hct L/L / MCH pg / MCV fl / MCHC g/dL / RDW % / Reti % / Ret x10⁵/L / WBC / Neut / Lymph / Mono / Eos / Baso |
|-------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1M                                              | 1      | 13.2   | 6.72   | 0.391  | 19.7   | 58.1   | 33.9   | 11.9   | 2.0    | 136    | 7.50   | 0.69   | 6.05   | 0.09   | 0.24   | 0.42   |
| 2                                                | 2      | 13.1   | 6.32   | 0.381  | 20.7   | 60.3   | 34.3   | 12.3   | 1.3    | 83     | 8.06   | 1.08   | 6.20   | 0.08   | 0.19   | 0.50   |
| 3                                                | 3      | 13.0   | 6.26   | 0.388  | 20.8   | 62.0   | 33.5   | 13.8   | 1.8    | 112    | 6.27   | 1.27   | 4.30   | 0.22   | 0.10   | 0.37   |
| 19                                               | 19     | 12.8   | 5.77   | 0.364  | 22.2   | 63.1   | 35.2   | 13.7   | 5.1    | 294    | 6.18   | 1.51   | 3.79   | 0.19   | 0.16   | 0.53   |
| 20                                               | 20     | 12.4   | 5.98   | 0.356  | 20.7   | 59.5   | 34.7   | 12.8   | 1.9    | 116    | 5.92   | 0.94   | 4.25   | 0.06   | 0.17   | 0.50   |
| 21                                               | 21     | 13.5   | 6.04   | 0.382  | 22.3   | 63.3   | 35.3   | 11.5   | 2.6    | 158    | 5.08   | 1.01   | 3.40   | 0.07   | 0.15   | 0.44   |
| 2M                                               | 4      | 12.9   | 6.39   | 0.379  | 20.2   | 59.3   | 34.0   | 12.5   | 1.8    | 114    | 5.60   | 0.91   | 4.01   | 0.10   | 0.13   | 0.43   |
| 5                                                | 5      | 13.2   | 6.12   | 0.373  | 21.5   | 61.0   | 35.3   | 12.3   | 2.2    | 133    | 5.43   | 1.09   | 3.69   | 0.00   | 0.11   | 0.54   |
| 6                                                | 6      | 12.7   | 6.51   | 0.373  | 19.4   | 57.3   | 33.9   | 12.5   | 1.4    | 91     | 7.65   | 2.44   | 4.31   | 0.12   | 0.20   | 0.55   |
| 22                                               | 22     | 12.7   | 5.73   | 0.360  | 22.2   | 62.8   | 35.3   | 12.2   | 1.9    | 111    | 7.43   | 3.48   | 3.15   | 0.10   | 0.14   | 0.55   |
| 23                                               | 23     | 13.0   | 6.42   | 0.375  | 20.2   | 58.4   | 34.7   | 12.7   | 1.9    | 119    | 6.25   | 1.19   | 4.17   | 0.10   | 0.21   | 0.58   |
| 24                                               | 24     | 12.1   | 5.73   | 0.356  | 21.2   | 62.2   | 34.0   | 12.7   | 1.9    | 107    | 5.73   | 0.92   | 4.20   | 0.14   | 0.08   | 0.38   |
| 3M                                               | 7      | 12.3   | 6.08   | 0.367  | 20.2   | 60.4   | 33.5   | 11.6   | 2.1    | 126    | 4.28   | 0.65   | 3.17   | 0.07   | 0.07   | 0.31   |
| 8                                                | 8      | 12.3   | 6.20   | 0.364  | 19.8   | 58.8   | 33.7   | 11.3   | 1.5    | 93     | 6.36   | 1.46   | 4.25   | 0.08   | 0.09   | 0.47   |
| 9                                                | 9      | 13.1   | 6.19   | 0.375  | 21.1   | 60.7   | 34.8   | 13.3   | 1.8    | 114    | 7.09   | 2.11   | 4.28   | 0.07   | 0.11   | 0.51   |
| 25                                               | 25     | 12.4   | 5.84   | 0.359  | 21.2   | 61.5   | 34.5   | 13.6   | 2.8    | 162    | 7.27   | 1.67   | 4.49   | 0.12   | 0.19   | 0.79   |
| 26                                               | 26     | 13.7   | 6.24   | 0.386  | 21.9   | 61.9   | 35.4   | 12.7   | 2.6    | 162    | 7.41   | 2.17   | 4.42   | 0.09   | 0.18   | 0.53   |
| 27                                               | 27     | 12.8   | 6.29   | 0.375  | 20.4   | 59.5   | 34.3   | 13.1   | 1.7    | 105    | 7.34   | 1.70   | 4.39   | 0.15   | 0.27   | 0.81   |

*Animal 19 - Initial haematology sample clotted; repeat sample collected*
### Appendix 13 (continued)

**Haematology and Coagulation: Individual Values: Pretrial**

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|------------------|---|---|---|
|       | Control   | 0                |   |   |   |
|       | MenPF-1   | 25               |   |   |   |
|       | MenPF-1   | 50               |   |   |   |

| LUC | Plat ($\times 10^9/L$) | PT (s) | APTT (s) | Fib (mg/dL) |
|-----|------------------------|--------|----------|-------------|
| 1M  | 0.02                   | 367    | 6.7      | 62.9        | 248         |
| 2   | 0.01                   | 357    | 6.3      | 56.8        | 211         |
| 3   | 0.01                   | 471    | 6.0      | 63.4        | 232         |
| 19  | 0.01                   | 502    | 6.3      | 62.1        | 233         |
| 20  | 0.01                   | 421    | 6.3      | 56.7        | 225         |
| 21  | 0.01                   | 333    | 6.1      | 62.5        | 206         |
| 2M  | 0.01                   | 307    | 6.3      | 62.9        | 220         |
| 5   | -                      | 474    | 6.1      | 58.5        | 222         |
| 6   | 0.02                   | 259    | 6.7      | 65.1        | 244         |
| 22  | 0.01                   | 461    | 6.1      | 55.5        | 245         |
| 23  | 0.01                   | 413    | 6.3      | 65.6        | 223         |
| 24  | 0.01                   | 500    | 6.7      | 67.1        | 193         |
| 3M  | 0.01                   | 381    | 6.1      | 54.3        | 232         |
| 8   | 0.01                   | 487    | 6.0      | 50.6        | 265         |
| 9   | 0.01                   | 438    | 6.1      | 60.6        | 231         |
| 25  | 0.02                   | 474    | 5.9      | 60.8        | 209         |
| 26  | 0.02                   | 426    | 6.5      | 67.9        | 231         |
| 27  | 0.02                   | 460    | 6.3      | 59.2        | 210         |

Animal 19 - Initial haematology sample clotted; repeat sample collected
Animal 2 - Initial coagulation sample clotted; repeat sample collected
Animal 5 - Manual differential performed, LUC cancelled
## Appendix 13 (continued)
### Haematology and Coagulation: Individual Values: Pretrial

| Test Item | Dosage (µg/dose) | Group 1 | Group 2 | Group 3 |
|-----------|------------------|---------|---------|---------|
| Control   | 0                | Control | MenPF-1 | MenPF-1 |
| MenPF-1   | 25               | 25      | 50      |         |

| Group / Animal No. | RBC x10^12/L | Hct L/L | MCH pg | MCV fl  | MCHC g/dL | RDW % | Reti % | Ret % x10^5/L | WBC | Neut | Lymph | Mono | Eos | Baso |
|-------------------|--------------|---------|--------|---------|------------|-------|--------|---------------|-----|------|--------|------|-----|------|
| 1F 10             | 12.2         | 5.84    | 0.357  | 20.8    | 61.1       | 34.1  | 12.4   | 2.5           | 148 | 5.03 | 0.77   | 3.53 | 0.04 | 0.13 |
| 11                | 11.1         | 5.02    | 0.328  | 22.0    | 65.4       | 33.7  | 13.3   | 2.7           | 137 | 6.96 | 1.75   | 4.34 | 0.08 | 0.23 |
| 12                | 12.5         | 6.00    | 0.368  | 20.9    | 61.2       | 34.1  | 12.3   | 2.0           | 118 | 8.14 | 3.12   | 4.19 | 0.09 | 0.11 |
| 28                | 12.0         | 5.46    | 0.342  | 21.9    | 62.6       | 35.0  | 12.1   | 3.2           | 177 | 6.73 | 2.96   | 2.56 | 0.13 | 0.20 |
| 29                | 12.2         | 5.91    | 0.365  | 20.6    | 61.8       | 33.4  | 12.0   | 2.2           | 128 | 5.45 | 2.13   | 3.11 | 0.00 | 0.00 |
| 30                | 11.3         | 5.26    | 0.333  | 21.4    | 63.4       | 33.8  | 12.3   | 3.0           | 160 | 6.72 | 1.43   | 4.63 | 0.08 | 0.15 |
| 2F 13             | 12.2         | 5.96    | 0.353  | 20.5    | 59.1       | 34.7  | 13.5   | 3.0           | 180 | 8.65 | 2.82   | 4.94 | 0.10 | 0.12 |
| 14                | 12.5         | 5.70    | 0.365  | 21.9    | 64.0       | 34.2  | 13.4   | 3.5           | 199 | 7.13 | 1.68   | 4.61 | 0.08 | 0.18 |
| 15                | 12.3         | 5.87    | 0.354  | 21.0    | 60.3       | 34.8  | 12.4   | 3.3           | 193 | 6.15 | 1.54   | 3.91 | 0.06 | 0.13 |
| 31                | 12.4         | 5.49    | 0.362  | 22.5    | 65.8       | 34.2  | 12.1   | 2.0           | 112 | 5.09 | 0.82   | 3.58 | 0.08 | 0.10 |
| 32                | 12.5         | 6.01    | 0.362  | 20.7    | 60.2       | 34.4  | 13.4   | 2.9           | 172 | 6.99 | 1.92   | 4.16 | 0.07 | 0.22 |
| 33                | 12.7         | 5.91    | 0.371  | 21.5    | 62.8       | 34.3  | 11.6   | 3.1           | 181 | 7.19 | 2.13   | 4.02 | 0.06 | 0.14 |
| 3F 16             | 12.6         | 5.64    | 0.358  | 22.4    | 63.4       | 35.2  | 11.5   | 2.5           | 141 | 6.46 | 1.62   | 4.09 | 0.06 | 0.17 |
| 17                | 12.0         | 5.61    | 0.347  | 21.3    | 61.8       | 34.5  | 12.8   | 2.8           | 159 | 7.06 | 1.98   | 4.41 | 0.08 | 0.12 |
| 18                | 13.7         | 6.34    | 0.392  | 21.7    | 61.9       | 35.0  | 12.4   | 1.9           | 119 | 5.49 | 2.54   | 2.20 | 0.06 | 0.14 |
| 34                | 11.5         | 5.56    | 0.330  | 20.7    | 59.4       | 34.8  | 12.5   | 2.2           | 122 | 7.80 | 2.44   | 4.47 | 0.17 | 0.22 |
| 35                | 11.8         | 5.69    | 0.357  | 20.8    | 62.8       | 33.1  | 12.8   | 3.0           | 172 | 7.29 | 1.31   | 4.95 | 0.07 | 0.15 |
| 36                | 11.7         | 5.15    | 0.341  | 22.7    | 66.2       | 34.3  | 12.8   | 2.5           | 131 | 6.35 | 1.34   | 4.07 | 0.25 | 0.19 |
### Appendix 13  (continued)
**Haematology and Coagulation : Individual Values: Pretrial**

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|------------------|
|       | Control   | 0                |
|       | MenPF-1   | 25               |
|       | MenPF-1   | 50               |

| Group / Animal No. | LUC | Plat x10^9/L | PT s | APTT s | Fib mg/dL |
|--------------------|-----|-------------|------|--------|-----------|
| 1F                 | 10  | 455         | 6.1  | 53.6   | 156       |
| 11                 | 0.01 | 395        | 6.3  | 59.7   | 162       |
| 12                 | 0.01 | 308        | 6.5  | 54.2   | 149       |
| 28                 | -    | 526         | 5.8  | 53.9   | 186       |
| 29                 | -    | 444         | 6.0  | 55.2   | 189       |
| 30                 | 0.01 | 457         | 6.5  | 56.1   | 165       |
| 2F                 | 13   | 0.03        | 494  | 6.3    | 54.2     | 190       |
| 14                 | 0.02 | 565         | -    | -      | -         |
| 15                 | 0.02 | 483         | 6.5  | 61.7   | 185       |
| 31                 | 0.01 | 466         | 6.6  | 54.6   | 150       |
| 32                 | 0.02 | 350         | 6.3  | 52.1   | 175       |
| 33                 | 0.02 | 417         | 6.3  | 55.0   | 153       |
| 3F                 | 16   | 0.02        | 353  | 6.0    | 53.5     | 198       |
| 17                 | 0.01 | 567         | 6.5  | 51.1   | 157       |
| 18                 | 0.01 | 428         | 6.2  | 60.3   | 191       |
| 34                 | 0.03 | 448         | 6.0  | 42.8   | 184       |
| 35                 | 0.02 | 458         | 6.3  | 43.3   | 175       |
| 36                 | 0.00 | 467         | 6.4  | 57.1   | 168       |

Animals 28 and 29 - Manual differential performed, LUC cancelled
Animals 14, 15 and 31 - Initial coagulation sample clotted, repeat samples collected
Animal 14 - Repeat sample clotted
### Appendix 14

**Haematology and Coagulation: Individual Values: Day 66**

| Group / Animal No. | Hb (g/dL) | RBC x10³/L | Hct L/L | MCH pg | MCV fL | MCHC g/dL | RDW % | Ret % | Ret x10⁶/L | WBC | Neut | Lymph | Mono | Eos | Baso |
|--------------------|-----------|-------------|---------|--------|-------|-----------|-------|-------|-----------|-----|------|-------|------|-----|------|
| **1M**             |           |             |         |        |       |           |       |       |           |     |      |       |      |     |      |
| 1                  | 12.8      | 6.54        | 0.388   | 19.5   | 59.3  | 32.9      | 11.5  | 2.6   | 169       | 8.36| 1.06 | 6.65  | 0.03 | 0.20 | 0.42 |
| 2                  | 12.8      | 6.41        | 0.396   | 19.9   | 61.9  | 32.2      | 11.9  | 2.0   | 131       | 9.41| 1.23 | 7.39  | 0.03 | 0.19 | 0.56 |
| 3                  | 12.9      | 6.28        | 0.392   | 20.6   | 62.5  | 32.9      | 13.1  | 2.2   | 140       | 6.55| 1.15 | 4.90  | 0.04 | 0.08 | 0.38 |
| 19                 | 13.3      | 6.31        | 0.397   | 20.1   | 62.9  | 33.4      | 12.0  | 2.8   | 179       | 8.61| 2.34 | 5.32  | 0.08 | 0.18 | 0.68 |
| 20                 | 13.0      | 6.48        | 0.397   | 20.1   | 61.3  | 32.7      | 11.8  | 2.4   | 154       | 5.91| 1.06 | 4.07  | 0.03 | 0.20 | 0.53 |
| 21                 | 13.8      | 6.48        | 0.410   | 21.3   | 63.2  | 33.7      | 11.4  | 3.1   | 201       | 5.47| 0.88 | 3.77  | 0.06 | 0.23 | 0.52 |
| **2M**             |           |             |         |        |       |           |       |       |           |     |      |       |      |     |      |
| 4                  | 13.2      | 6.70        | 0.402   | 19.8   | 60.0  | 32.9      | 11.7  | 2.1   | 143       | 7.97| 1.98 | 5.31  | 0.07 | 0.15 | 0.44 |
| 5                  | 13.8      | 6.59        | 0.408   | 20.9   | 61.8  | 33.9      | 11.9  | 3.3   | 218       | 5.20| 1.66 | 2.96  | 0.16 | 0.16 | 0.26 |
| 6                  | -         | -           | -       | -      | -     | -         | -     | -     | -         | -   | -    | -     | -    | -   | -    |
| 22                 | 13.5      | 6.18        | 0.401   | 21.9   | 64.9  | 33.7      | 13.0  | 3.4   | 208       | 5.14| 1.17 | 3.02  | 0.29 | 0.14 | 0.52 |
| 23                 | 13.6      | 6.78        | 0.409   | 20.0   | 60.3  | 33.2      | 11.9  | 2.2   | 150       | 8.34| 2.32 | 5.13  | 0.07 | 0.20 | 0.61 |
| 24                 | 12.2      | 5.83        | 0.372   | 20.8   | 63.8  | 32.6      | 12.5  | 2.6   | 152       | 6.57| 1.56 | 4.48  | 0.05 | 0.12 | 0.36 |
| **3M**             |           |             |         |        |       |           |       |       |           |     |      |       |      |     |      |
| 7                  | 12.8      | 6.30        | 0.399   | 20.3   | 63.4  | 32.0      | 12.1  | 2.5   | 156       | 8.92| 2.76 | 5.51  | 0.09 | 0.11 | 0.44 |
| 8                  | 12.3      | 6.31        | 0.382   | 19.4   | 60.6  | 32.1      | 11.4  | 2.2   | 137       | 5.87| 1.58 | 3.69  | 0.07 | 0.13 | 0.38 |
| 9                  | 13.3      | 6.47        | 0.399   | 20.6   | 61.8  | 33.3      | 12.1  | 2.2   | 144       | 8.17| 2.83 | 4.50  | 0.09 | 0.20 | 0.54 |
| 25                 | 12.6      | 6.08        | 0.383   | 20.7   | 63.0  | 32.9      | 12.4  | 2.8   | 167       | 9.40| 2.38 | 6.07  | 0.10 | 0.19 | 0.64 |
| 26                 | 14.1      | 6.65        | 0.419   | 21.2   | 63.0  | 33.7      | 12.3  | 3.6   | 242       | 7.36| 1.59 | 5.05  | 0.07 | 0.16 | 0.48 |
| 27                 | 13.4      | 6.80        | 0.412   | 19.7   | 60.5  | 32.6      | 12.3  | 1.8   | 125       | 10.12| 3.41 | 5.36  | 0.13 | 0.33 | 0.87 |

Animals 6 and 22 - Initial haematology and coagulation samples clotted; repeat samples collected from Animal 22 only.
### Haematology and Coagulation: Individual Values: Day 66

| Group / Animal No. | LUC | Plat x10^9/L | PT s | APTT s | Fib mg/dL |
|--------------------|-----|--------------|------|--------|----------|
| 1M 1               | 0.00| 326          | 6.2  | 57.9   | 251      |
| 2M 2               | 0.01| 373          | 6.4  | 51.3   | 141      |
| 3M 3               | 0.01| 381          | 5.8  | 53.0   | 195      |
| 1M 19              | 0.01| 384          | 6.3  | 57.1   | 195      |
| 2M 20              | 0.01| 408          | 6.0  | 54.0   | 203      |
| 3M 21              | 0.01| 312          | 5.8  | 63.8   | 207      |
| 2M 4               | 0.01| 267          | 5.8  | 59.5   | 282      |
| 2M 5               | -   | 376          | 5.7  | 55.9   | 316      |
| 2M 6               | -   | -            | -    | -      | -        |
| 2M 22              | 0.01| 534          | 5.8  | 60.5   | 249      |
| 2M 23              | 0.00| 413          | 5.8  | 61.7   | 295      |
| 2M 24              | 0.00| 479          | 5.8  | 56.4   | 251      |
| 3M 7               | 0.01| 311          | 5.8  | 50.5   | 290      |
| 3M 8               | 0.00| 357          | 5.8  | 47.6   | 361      |
| 3M 9               | 0.01| 376          | 5.9  | 52.6   | 343      |
| 3M 25              | 0.01| 447          | 5.8  | 52.7   | 315      |
| 3M 26              | 0.01| 428          | 6.0  | 54.5   | 370      |
| 3M 27              | 0.02| 371          | 6.0  | 52.9   | 330      |

Animals 6 and 22 - Initial haematology and coagulation samples clotted; repeat samples collected from Animal 22 only
Animal 21 - Initial coagulation sample clotted; repeat sample collected
Animal 5 - Manual differential performed, LUC cancelled
## Appendix 14 (continued)
### Haematology and Coagulation: Individual Values: Day 66

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|-----------------|---|---|---|
| Control | MenPF-1 | MenPF-1 | | | |

| Group / sex | Animal No. | Hb (g/dL) | RBC x10¹²/L | Hct L/L | MCH pg | MCV fL | MCHC g/dL | RDW % | Reti % | Ret x10⁶/L | WBC x10⁹/L | Neut | Lymph x10⁹/L | Mono | Eos | Baso | |
|-------------|------------|-----------|--------------|---------|--------|--------|-----------|--------|--------|-----------|-------------|------|----------------|------|------|------|--------|
| 1F 10 | 12.6 | 6.21 | 0.381 | 20.2 | 61.3 | 33.0 | 12.4 | 2.9 | 182 | 4.90 | 0.71 | 3.63 | 0.03 | 0.13 | 0.40 |
| 11 | 11.7 | 5.43 | 0.359 | 21.5 | 66.1 | 32.5 | 13.5 | 2.9 | 160 | 6.45 | 1.77 | 3.87 | 0.06 | 0.20 | 0.55 |
| 12 | 12.7 | 6.22 | 0.396 | 20.4 | 63.7 | 32.1 | 12.5 | 2.7 | 171 | 6.58 | 2.37 | 3.42 | 0.00 | 0.13 | 0.66 |
| 28 | 11.0 | 5.23 | 0.337 | 21.1 | 64.6 | 32.7 | 12.8 | 4.0 | 210 | 6.51 | 1.47 | 4.20 | 0.07 | 0.15 | 0.59 |
| 29 | 13.1 | 6.59 | 0.405 | 19.9 | 61.5 | 32.3 | 12.3 | 2.5 | 167 | 16.66 | 1.50 | 13.83 | 0.16 | 0.29 | 0.85 |
| 30 | 11.8 | 5.66 | 0.367 | 20.8 | 64.8 | 32.2 | 12.7 | 3.3 | 184 | 6.33 | 1.22 | 4.53 | 0.06 | 0.18 | 0.33 |
| 2F 13 | 12.9 | 6.31 | 0.381 | 20.5 | 60.4 | 33.9 | 12.3 | 3.5 | 220 | 7.50 | 1.76 | 4.73 | 0.12 | 0.18 | 0.70 |
| 14 | 13.0 | 6.12 | 0.391 | 21.3 | 63.8 | 33.3 | 13.3 | 2.9 | 179 | 7.23 | 1.76 | 4.58 | 0.10 | 0.25 | 0.51 |
| 15 | 12.0 | 6.10 | 0.366 | 19.7 | 60.0 | 32.9 | 12.8 | 3.1 | 187 | 5.97 | 1.30 | 4.03 | 0.05 | 0.10 | 0.49 |
| 31 | 12.6 | 5.72 | 0.378 | 22.1 | 66.1 | 33.4 | 12.2 | 2.6 | 149 | 7.59 | 1.33 | 5.40 | 0.07 | 0.16 | 0.62 |
| 32 | 13.2 | 6.60 | 0.403 | 20.0 | 61.0 | 32.9 | 12.3 | 2.8 | 187 | 6.08 | 1.58 | 3.73 | 0.06 | 0.18 | 0.51 |
| 33 | 13.0 | 6.34 | 0.395 | 20.5 | 62.2 | 33.0 | 11.7 | 2.3 | 143 | 6.95 | 2.85 | 3.54 | 0.07 | 0.14 | 0.35 |
| 3F 16 | 12.2 | 5.83 | 0.361 | 20.9 | 61.9 | 33.7 | 11.0 | 2.9 | 170 | 5.99 | 0.98 | 4.36 | 0.06 | 0.10 | 0.49 |
| 17 | 12.1 | 5.86 | 0.364 | 20.6 | 62.1 | 33.1 | 11.6 | 2.4 | 140 | 8.46 | 2.27 | 5.24 | 0.27 | 0.15 | 0.51 |
| 18 | 12.5 | 6.00 | 0.383 | 20.9 | 63.8 | 32.7 | 12.8 | 2.9 | 174 | 4.72 | 1.32 | 2.75 | 0.06 | 0.11 | 0.47 |
| 34 | 12.2 | 6.22 | 0.377 | 19.7 | 60.6 | 32.5 | 12.5 | 2.5 | 155 | 7.69 | 1.51 | 5.36 | 0.21 | 0.11 | 0.47 |
| 35 | 12.3 | 6.17 | 0.384 | 20.0 | 62.2 | 32.1 | 13.4 | 5.3 | 328 | 7.04 | 2.01 | 4.08 | 0.08 | 0.19 | 0.67 |
| 36 | 11.6 | 5.30 | 0.348 | 21.9 | 65.7 | 33.4 | 12.4 | 2.6 | 139 | 5.73 | 1.64 | 3.29 | 0.08 | 0.24 | 0.47 |

Animal 30 - Insufficient haematology sample to check analysis; repeat samples collected
Animals 35 and 36 - Haematology and coagulation samples clotted; repeat samples taken
### Appendix 14 (continued)

#### Haematology and Coagulation: Individual Values: Day 66

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|------------------|
|       | Control   | 0                |
|       | MenPF-1   | 25               |
|       | MenPF-1   | 50               |

| Group / sex | Animal No. | LUC | Plat x10^9/L | PT s | APTT s | Fib mg/dL |
|-------------|------------|-----|--------------|------|--------|-----------|
| 1F          | 10         | 0.01| 500          | 6.3  | 55.2   | 123       |
|             | 11         | 0.01| 367          | 6.1  | 59.5   | 144       |
|             | 12         |     | 315          | 6.3  | 57.3   | 116       |
|             | 28         | 0.02| 494          | 5.8  | 48.1   | 144       |
|             | 29         | 0.04| 342          | 6.0  | 54.1   | 141       |
|             | 30         | 0.01| 520          | 6.2  | 59.2   | 108       |
| 2F          | 13         | 0.02| 375          | 5.6  | 61.5   | 267       |
|             | 14         | 0.02| 524          | 5.7  | 49.7   | 246       |
|             | 15         | 0.00| 486          | 6.1  | 59.3   | 187       |
|             | 31         | 0.02| 370          | 6.0  | 52.7   | 211       |
|             | 32         | 0.01| 279          | 6.0  | 62.2   | 174       |
|             | 33         |     | 375          | 6.1  | 53.2   | 203       |
| 3F          | 16         | 0.01| 319          | 5.7  | 52.5   | 302       |
|             | 17         | 0.01| 508          | 5.9  | 52.7   | 199       |
|             | 18         | 0.01| 470          | 5.7  | 58.8   | 179       |
|             | 34         | 0.03| 426          | 5.8  | 49.2   | 173       |
|             | 35         | 0.01| 538          | 5.8  | 69.5   | 231       |
|             | 36         | 0.01| 624          | 5.6  | 69.1   | 245       |

Animal 30 - Insufficient haematology sample to check analysis; repeat samples collected.

Animals 35 and 36 - Haematology and coagulation samples clotted; repeat samples taken.

Animals 12 and 33 - Manual differential performed, LUC cancelled.
### Appendix 15

**Haematology and Coagulation: Individual Values: Day 92**

| Group / sex | Animal No. | Hb g/dL | RBC x10^{12}/L | Hct L/L | MCH pg | MCV fl | MCHC g/dL | RDW % | Reti % | Ret x10^{9}/L | WBC | Neut | Lymph | Mono | Eos | Baso |
|-------------|------------|---------|-----------------|---------|--------|--------|-----------|--------|--------|---------------|-----|------|-------|------|-----|------|
| 1M          | 19         | 13.6    | 6.24            | 0.401   | 21.7   | 64.2   | 33.8      | 12.3   | 3.1    | 194           | 7.86| 1.59 | 5.45  | 0.05 | 0.19 | 0.57 |
|             | 20         | 12.9    | 6.26            | 0.394   | 20.6   | 63.0   | 32.7      | 12.4   | 2.4    | 150           | 5.94| 0.96 | 4.22  | 0.02 | 0.23 | 0.49 |
|             | 21         | 14.2    | 6.69            | 0.428   | 21.2   | 64.0   | 33.2      | 11.0   | 2.5    | 166           | 6.13| 0.90 | 4.52  | 0.03 | 0.21 | 0.47 |
| 2M          | 22         | -       | -               | -       | -      | -      | -         | -      | -      | -             | -   | -    | -     | -    | -   | -    |
|             | 23         | 14.3    | 6.95            | 0.431   | 20.6   | 61.9   | 33.3      | 12.0   | 2.3    | 157           | 7.06| 1.75 | 4.44  | 0.05 | 0.19 | 0.62 |
|             | 24         | 12.7    | 5.93            | 0.388   | 21.4   | 65.5   | 32.7      | 11.8   | 1.8    | 105           | 5.00| 0.76 | 3.75  | 0.04 | 0.11 | 0.33 |
| 3M          | 25         | 12.9    | 6.09            | 0.390   | 21.1   | 64.0   | 33.0      | 11.8   | 2.1    | 128           | 6.64| 0.92 | 4.97  | 0.04 | 0.15 | 0.54 |
|             | 26         | 14.3    | 6.64            | 0.417   | 21.5   | 62.8   | 34.2      | 11.4   | 2.3    | 151           | 5.17| 0.75 | 3.80  | 0.03 | 0.17 | 0.41 |
|             | 27         | 13.1    | 6.46            | 0.401   | 20.3   | 62.0   | 32.7      | 13.3   | 2.3    | 148           | 7.63| 1.61 | 5.10  | 0.05 | 0.25 | 0.60 |

Animal 22 - Insufficient haematology sample to check analysis
## Appendix 15  (continued)
### Haematology and Coagulation: Individual Values: Day 92

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| Group / sex | Animal No. | LUC | Plat x10^9/L | PT s | APTT s | Fib mg/dL |
|-------------|------------|-----|--------------|------|--------|-----------|
| 1M          | 19         | 0.02| 341          | 6.4  | 61.1   | 174       |
|             | 20         | 0.01| 445          | 6.0  | 54.1   | 188       |
|             | 21         | 0.01| 277          | 5.8  | 55.6   | 167       |
| 2M          | 22         | -   | -            | -    | -      | -         |
|             | 23         | 0.01| 351          | 5.8  | 57.0   | 211       |
|             | 24         | 0.02| 440          | 6.1  | 55.7   | 166       |
| 3M          | 25         | 0.01| 427          | 5.8  | 54.5   | 194       |
|             | 26         | 0.01| 379          | 6.3  | 57.1   | 195       |
|             | 27         | 0.02| 387          | 6.0  | 57.8   | 150       |

Animal 22 - Insufficient haematology sample to check analysis
## Appendix 15  (continued)

### Haematology and Coagulation : Individual Values: Day 92

| Group | Test Item | Dosage (µg/dose) |
|-------|-----------|-----------------|
|       |           | 1               |
|       |           | 2               |
|       |           | 3               |
|       | Control   | MenPF-1         |
|       | MenPF-1   | MenPF-1         |

| Group | Animal No. | Hb g/dL | RBC x10¹²/L | Hct L/L | MCH pg | MCV fl | MCHC g/dL | RDW % | Reti % | Ret x10⁹/L | WBC       | Neut       | Lymph      | Mono | Eos | Baso |
|-------|-------------|---------|-------------|---------|--------|--------|-----------|-------|--------|------------|-----------|------------|------------|-------|-----|-----|
| 1F    | 28          | 12.4    | 5.82       | 0.376   | 21.4   | 64.6   | 33.1      | 11.5  | 2.4    | 138        | 6.08      | 2.00       | 3.41       | 0.03  | 0.15| 0.47|
|       | 29          | 13.4    | 6.46       | 0.409   | 20.7   | 63.3   | 32.7      | 12.1  | 2.5    | 162        | 6.41      | 0.93       | 4.49       | 0.08  | 0.32| 0.58|
|       | 30          | 12.1    | 5.65       | 0.370   | 21.4   | 65.4   | 32.6      | 11.3  | 2.3    | 130        | 4.85      | 0.84       | 3.50       | 0.05  | 0.14| 0.32|
| 2F    | 31          | 12.7    | 5.58       | 0.380   | 22.8   | 68.2   | 33.4      | 12.4  | 2.3    | 130        | 6.43      | 0.69       | 4.98       | 0.04  | 0.16| 0.54|
|       | 32          | 13.8    | 6.67       | 0.421   | 20.6   | 63.1   | 32.7      | 12.5  | 2.4    | 159        | 6.48      | 1.36       | 4.38       | 0.05  | 0.19| 0.50|
|       | 33          | 12.1    | 5.85       | 0.359   | 20.7   | 61.3   | 33.8      | 10.8  | 1.7    | 97         | 2.22      | 0.28       | 1.65       | 0.01  | 0.07| 0.21|
| 3F    | 34          | 12.3    | 6.00       | 0.375   | 20.5   | 62.5   | 32.9      | 12.6  | 2.0    | 119        | 5.77      | 1.11       | 4.01       | 0.09  | 0.15| 0.39|
|       | 35          | 13.0    | 6.25       | 0.394   | 20.8   | 63.1   | 33.0      | 11.3  | 2.8    | 173        | 6.02      | 1.42       | 3.71       | 0.05  | 0.15| 0.67|
|       | 36          | 12.0    | 5.35       | 0.363   | 22.4   | 68.0   | 33.0      | 12.0  | 2.1    | 113        | 4.28      | 1.03       | 2.73       | 0.04  | 0.13| 0.34|
## Appendix 15  (continued)
### Haematology and Coagulation : Individual Values: Day 92

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|------------------|---|---|---|
| | : | : | Control | MenPF-1 | MenPF-1 |

| Group / sex | Animal No. | LUC | Plat x10^9/L | PT s | APTT s | Fib mg/dL |
|-------------|------------|-----|-------------|------|--------|-----------|
| 1F          | 28         | 0.01 | 467         | 5.8  | 56.3   | 132       |
|             | 29         | 0.01 | 375         | 5.9  | 55.4   | 152       |
|             | 30         | 0.00 | 423         | 5.9  | 57.6   | 114       |
| 2F          | 31         | 0.02 | 436         | 5.8  | 51.4   | 133       |
|             | 32         | 0.01 | 293         | 5.8  | 64.5   | 131       |
|             | 33         | 0.00 | 11          | -    | -      | -         |
| 3F          | 34         | 0.01 | 432         | 5.8  | 49.5   | 129       |
|             | 35         | 0.02 | 445         | 5.7  | 57.2   | 181       |
|             | 36         | 0.01 | 473         | 6.0  | 54.0   | 160       |

Animal 33 - Coagulation sample clotted
### Appendix 16
Clinical Chemistry : Individual Values: Pretrial

| Group | Test Item | Dosage (µg/dose) | 1 | 2 | 3 |
|-------|-----------|------------------|---|---|---|
|       | Control   | 0                |   |   |   |
| MenPF-1 | 25         | 25               |   |   |   |
| MenPF-1 | 50         | 50               |   |   |   |

| Group / sex | Animal No. | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R mmol/L | Na mmol/L | K mmol/L |
|-------------|------------|---------|---------|---------|---------|---------|-------------|------------|--------------|-------------|--------|---------|----------|--------------|-----------|----------|
| 1M          | 1          | 220     | 25      | 12      | 82      | 737     | 7.6         | 7.79       | <1.7         | 0.8         | 51     | 38      | 13       | 2.9          | 140       | 4.5      |
|             | 2          | 139     | 39      | 10      | 56      | 365     | 6.8         | 7.65       | <1.7         | 1.3         | 50     | 40      | 11       | 3.7          | 140       | 4.5      |
|             | 3          | 105     | 20      | 8       | 59      | 399     | 7.2         | 8.20       | <1.7         | 0.9         | 57     | 44      | 13       | 3.3          | 143       | 4.9      |
|             | 19         | 200     | 30      | 13      | 62      | 795     | 7.7         | 8.48       | <1.7         | 0.5         | 56     | 44      | 13       | 3.5          | 145       | 4.3      |
|             | 20         | 139     | 18      | 11      | 60      | 838     | 6.5         | 7.56       | <1.7         | 0.7         | 56     | 43      | 14       | 3.2          | 142       | 4.5      |
|             | 21         | 178     | 23      | 13      | 100     | 665     | 6.5         | 8.39       | <1.7         | 0.6         | 54     | 42      | 12       | 3.4          | 142       | 4.9      |
| 2M          | 4          | 166     | 22      | 12      | 75      | 437     | 8.0         | 8.39       | <1.7         | 0.8         | 60     | 46      | 14       | 3.3          | 140       | 4.8      |
|             | 5          | 141     | 27      | 11      | 55      | 442     | 7.1         | 8.13       | <1.7         | 0.8         | 57     | 43      | 14       | 3.1          | 141       | 4.3      |
|             | 6          | 172     | 43      | 12      | 65      | 557     | 7.3         | 8.08       | <1.7         | 0.5         | 60     | 44      | 16       | 2.7          | 141       | 4.8      |
|             | 22         | 142     | 28      | 12      | 47      | 322     | 6.6         | 8.18       | <1.7         | 0.5         | 58     | 44      | 14       | 3.2          | 141       | 4.0      |
|             | 23         | 148     | 27      | 9       | 59      | 500     | 7.8         | 7.88       | <1.7         | 0.6         | 57     | 43      | 13       | 3.2          | 143       | 4.7      |
|             | 24         | 151     | 28      | 11      | 59      | 717     | 7.7         | 8.24       | <1.7         | 0.8         | 55     | 42      | 13       | 3.2          | 142       | 4.7      |
| 3M          | 7          | 188     | 22      | 12      | 50      | 826     | 7.4         | 8.33       | <1.7         | 1.0         | 58     | 44      | 14       | 3.2          | 141       | 4.5      |
|             | 8          | 163     | 31      | 13      | 93      | 770     | 7.3         | 7.62       | <1.7         | 0.9         | 55     | 41      | 14       | 2.9          | 146       | 4.3      |
|             | 9          | 148     | 29      | 11      | 65      | 436     | 7.1         | 7.73       | <1.7         | 0.5         | 57     | 44      | 13       | 3.4          | 142       | 4.4      |
|             | 25         | 193     | 16      | 9       | 49      | 548     | 7.3         | 8.40       | <1.7         | 1.0         | 59     | 44      | 15       | 2.9          | 144       | 4.5      |
|             | 26         | 234     | 32      | 12      | 62      | 563     | 6.4         | 7.78       | <1.7         | 0.8         | 56     | 43      | 13       | 3.3          | 142       | 4.3      |
|             | 27         | 224     | 19      | 11      | 52      | 442     | 7.3         | 7.99       | <1.7         | 1.0         | 53     | 41      | 13       | 3.2          | 143       | 4.4      |
## Appendix 16  (continued)
### Clinical Chemistry : Individual Values: Pretrial

| Group | Test Item | Dosage (µg/dose) | 1 Cl mmol/L | 1 Phos mmol/L | 1 Ca mmol/L | 1 Crea µmol/L | 2 Cl mmol/L | 2 Phos mmol/L | 2 Ca mmol/L | 2 Crea µmol/L | 3 Cl mmol/L | 3 Phos mmol/L | 3 Ca mmol/L | 3 Crea µmol/L |
|-------|-----------|------------------|-------------|--------------|-------------|---------------|-------------|--------------|-------------|---------------|-------------|--------------|-------------|---------------|
| 2M    | 4 97      | 1.76             | 3.82        | 52           |
| 5 101  | 1.94      | 3.70             | 51           |
| 6 100  | 2.13      | 3.73             | 70           |
| 22 99  | 1.89      | 3.74             | 54           |
| 23 103 | 1.71      | 3.73             | 58           |
| 24 102 | 1.78      | 3.79             | 75           |
| 3M    | 7 101      | 1.97             | 3.76        | 62           |
| 8 105  | 1.63      | 3.60             | 67           |
| 9 99   | 1.90      | 3.75             | 65           |
| 25 99  | 2.14      | 3.78             | 64           |
| 26 102 | 1.95      | 3.68             | 66           |
| 27 103 | 2.07      | 3.57             | 58           |

The table above presents the individual values for various test items and dosages in the context of clinical chemistry. Each row represents an animal, with columns indicating the group, sex, test item, dosage, and measured values for chloride (Cl), phosphorus (Phos), calcium (Ca), and creatinine (Crea) in mmol/L and µmol/L, respectively.
### Appendix 16 (continued)
#### Clinical Chemistry: Individual Values: Pretrial

| Group | Test Item | Dosage (µg/dose) | 1   | 2   | 3   |
|-------|-----------|-----------------|-----|-----|-----|
|       | Control   | 0               | 0   | 0   | 0   |
|       | MenPF-1   | 25              | 25  | 25  | 25  |
|       | MenPF-1   | 50              | 50  | 50  | 50  |

| Group | Animal No. | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R mmol/L | Na mmol/L | K mmol/L |
|-------|------------|---------|---------|---------|---------|---------|-------------|------------|--------------|-------------|--------|---------|----------|--------------|-----------|----------|
| 1F    | 10         | 153     | 13      | 13      | 71      | 2407    | 7.9         | 8.78       | <1.7         | 1.4         | 55     | 42      | 13       | 3.2          | 141       | 4.6      |
|       | 11         | 325     | 18      | 11      | 64      | 465     | 6.4         | 7.29       | <1.7         | 1.1         | 52     | 40      | 12       | 3.4          | 142       | 4.3      |
|       | 12         | 250     | 30      | 12      | 69      | 768     | 6.2         | 7.75       | <1.7         | 1.1         | 55     | 40      | 15       | 2.6          | 143       | 4.2      |
|       | 28         | 208     | 22      | 10      | 73      | 531     | 8.3         | 7.53       | <1.7         | 1.8         | 58     | 44      | 15       | 2.9          | 141       | 4.4      |
|       | 29         | 275     | 28      | 8       | 75      | 868     | 5.4         | 7.67       | <1.7         | 1.4         | 57     | 43      | 14       | 3.1          | 140       | 4.5      |
|       | 30         | 178     | 27      | 11      | 67      | 726     | 7.6         | 7.23       | <1.7         | 1.0         | 55     | 42      | 13       | 3.3          | 143       | 4.3      |
| 2F    | 13         | 181     | 36      | 12      | 52      | 295     | 6.7         | 8.68       | <1.7         | 1.1         | 61     | 46      | 15       | 3.0          | 141       | 4.2      |
|       | 14         | 173     | 22      | 11      | 64      | 421     | 7.6         | 8.42       | <1.7         | 1.8         | 55     | 42      | 13       | 3.3          | 141       | 4.7      |
|       | 15         | 202     | 41      | 13      | 84      | 671     | 6.6         | 8.61       | <1.7         | 1.2         | 52     | 39      | 12       | 3.2          | 140       | 4.0      |
|       | 31         | 261     | 23      | 8       | 60      | 741     | 6.6         | 8.42       | <1.7         | 1.3         | 52     | 40      | 13       | 3.0          | 140       | 4.2      |
|       | 32         | 238     | 32      | 11      | 94      | 774     | 6.0         | 9.30       | <1.7         | 1.2         | 51     | 39      | 12       | 3.3          | 142       | 4.0      |
|       | 33         | 260     | 17      | 13      | 57      | 428     | 6.4         | 7.92       | <1.7         | 1.1         | 55     | 43      | 12       | 3.5          | 141       | 4.1      |
| 3F    | 16         | 177     | 29      | 13      | 64      | 581     | 6.5         | 8.03       | <1.7         | 1.3         | 59     | 44      | 14       | 3.1          | 140       | 4.2      |
|       | 17         | 170     | 43      | 12      | 54      | 540     | 8.3         | 9.05       | <1.7         | 2.1         | 61     | 46      | 14       | 3.2          | 140       | 4.3      |
|       | 18         | 177     | 32      | 10      | 61      | 1231    | 5.8         | 9.12       | <1.7         | 1.7         | 58     | 45      | 13       | 3.4          | 141       | 4.1      |
|       | 34         | 156     | 19      | 13      | 55      | 1363    | 6.5         | 7.88       | <1.7         | 2.1         | 62     | 46      | 16       | 3.0          | 139       | 4.1      |
|       | 35         | 214     | 19      | 15      | 65      | 1086    | 6.8         | 9.14       | <1.7         | 1.1         | 56     | 45      | 12       | 3.8          | 142       | 4.1      |
|       | 36         | 149     | 30      | 10      | 49      | 475     | 8.4         | 8.59       | <1.7         | 1.2         | 59     | 45      | 13       | 3.4          | 141       | 4.8      |
## Appendix 16 (continued)

### Clinical Chemistry: Individual Values: Pretrial

| Group / sex | Animal No. | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|------------|-----------|-------------|-----------|-------------|
| 1F          | 10         | 2.42      | 3.69        | 59        |
|             | 11         | 1.78      | 3.62        | 54        |
|             | 12         | 1.71      | 3.67        | 60        |
|             | 28         | 2.14      | 3.72        | 68        |
|             | 29         | 1.65      | 3.67        | 53        |
|             | 30         | 1.99      | 3.59        | 64        |
| 2F          | 13         | 2.10      | 3.87        | 61        |
|             | 14         | 2.15      | 3.59        | 64        |
|             | 15         | 1.96      | 3.69        | 62        |
|             | 31         | 1.86      | 3.46        | 65        |
|             | 32         | 2.02      | 3.49        | 62        |
|             | 33         | 2.13      | 3.54        | 62        |
| 3F          | 16         | 1.89      | 3.74        | 59        |
|             | 17         | 1.57      | 3.70        | 47        |
|             | 18         | 1.98      | 3.63        | 57        |
|             | 34         | 2.04      | 3.78        | 70        |
|             | 35         | 2.36      | 3.73        | 68        |
|             | 36         | 2.09      | 3.75        | 54        |
Appendix 17
Clinical Chemistry: Individual Values: Day 66

| Group / Animal No. | ALP (U/L) | ALT (U/L) | AST (U/L) | LDH (U/L) | CPK (U/L) | Urea (mmol/L) | Glu (mmol/L) | T.Bil (µmol/L) | Chol (mmol/L) | TP (g/L) | Alb (g/L) | Glob (g/L) | AG-R (mmol/L) | Na (mmol/L) | K (mmol/L) |
|-------------------|-----------|-----------|-----------|-----------|-----------|---------------|--------------|----------------|---------------|----------|-----------|-----------|--------------|-----------|----------|
| 1M 1              | 70        | 37        | 16        | 67        | 793       | 8.7           | 8.48         | <1.7           | 0.4           | 54       | 43        | 11        | 3.8          | 144       | 4.2      |
| 2M 2              | 57        | 57        | 12        | 54        | 442       | 7.5           | 7.34         | <1.7           | 0.6           | 53       | 44        | 10        | 4.4          | 145       | 4.7      |
| 3M 3              | 54        | 33        | 11        | 54        | 442       | 7.4           | 7.79         | <1.7           | 0.5           | 60       | 48        | 12        | 3.8          | 146       | 4.7      |
| 2M 4              | 71        | 24        | 14        | 45        | 554       | 6.8           | 7.10         | <1.7           | 0.9           | 62       | 45        | 16        | 2.7          | 142       | 4.5      |
| 5M 5              | 72        | 42        | 9         | 51        | 367       | 7.0           | 7.02         | <1.7           | 0.7           | 62       | 48        | 14        | 3.4          | 143       | 4.3      |
| 6M 6              | 59        | 59        | 19        | 82        | 581       | 9.2           | 7.03         | <1.7           | 0.5           | 63       | 46        | 17        | 2.6          | 143       | 4.6      |
| 2M 22             | 62        | 31        | 13        | 81        | 482       | 6.5           | 7.79         | <1.7           | 0.6           | 61       | 47        | 14        | 3.5          | 144       | 4.6      |
| 3M 19             | 49        | 42        | 9         | 46        | 373       | 7.7           | 7.22         | <1.7           | 0.4           | 60       | 42        | 17        | 2.4          | 147       | 4.9      |
| 2M 24             | 56        | 34        | 13        | 46        | 810       | 8.5           | 7.42         | <1.7           | 0.4           | 60       | 46        | 14        | 3.2          | 146       | 4.8      |
| 3M 7              | 66        | 36        | 9         | 59        | 636       | 6.7           | 7.90         | <1.7           | 0.4           | 62       | 47        | 15        | 3.1          | 145       | 4.5      |
| 8M 8              | 60        | 36        | 14        | 197       | 1925      | 6.1           | 7.60         | <1.7           | 0.4           | 60       | 45        | 15        | 3.0          | 146       | 4.1      |
| 9M 9              | 55        | 38        | 17        | 79        | 576       | 6.9           | 6.85         | <1.7           | 0.4           | 62       | 47        | 15        | 3.2          | 146       | 4.1      |
| 2M 25             | 63        | 26        | 11        | 46        | 529       | 7.5           | 7.13         | <1.7           | 0.7           | 61       | 45        | 16        | 2.8          | 146       | 4.1      |
| 3M 26             | 87        | 86        | 19        | 58        | 412       | 6.9           | 8.03         | <1.7           | 0.4           | 62       | 46        | 16        | 2.9          | 145       | 4.5      |
| 2M 27             | 95        | 27        | 15        | 66        | 484       | 6.5           | 7.50         | <1.7           | 0.5           | 57       | 43        | 14        | 3.0          | 145       | 4.3      |
## Appendix 17  (continued)

### Clinical Chemistry: Individual Values: Day 66

| Group / sex | Animal No. | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|------------|-----------|-------------|-----------|-------------|
| 1M          | 1          | 1.20      | 3.53        | 91        |
|             | 2          | 1.44      | 3.55        | 70        |
|             | 3          | 1.30      | 3.78        | 67        |
|             | 19         | 1.18      | 3.58        | 63        |
|             | 20         | 1.21      | 3.60        | 52        |
|             | 21         | 1.29      | 3.58        | 58        |
| 2M          | 4          | 1.27      | 3.68        | 51        |
|             | 5          | 1.28      | 3.64        | 50        |
|             | 6          | 1.35      | 3.73        | 63        |
|             | 22         | 1.54      | 3.67        | 50        |
|             | 23         | 1.43      | 3.73        | 69        |
|             | 24         | 1.34      | 3.74        | 90        |
| 3M          | 7          | 1.46      | 3.79        | 64        |
|             | 8          | 1.48      | 3.62        | 69        |
|             | 9          | 1.35      | 3.71        | 63        |
|             | 25         | 1.22      | 3.58        | 66        |
|             | 26         | 1.39      | 3.70        | 80        |
|             | 27         | 1.24      | 3.51        | 64        |
### Appendix 17 (continued)

#### Clinical Chemistry: Individual Values: Day 66

| Group | 1 | 2 | 3 |
|-------|---|---|---|
| Test Item | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose) | 0 | 25 | 50 |

| Group / sex | Animal No. | ALP U/L | ALT U/L | AST U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R mmol/L | Na mmol/L | K mmol/L |
|-------------|-----------|---------|---------|---------|---------|---------|-------------|------------|--------------|-------------|--------|---------|----------|-------------|-----------|---------|
| 1F          | 10        | 89      | 19      | 8       | 49      | 639     | 6.2         | 6.57       | <1.7         | 1.1         | 57     | 46      | 11       | 4.4         | 145       | 4.2     |
|             | 11        | 109     | 22      | 8       | 37      | 341     | 6.9         | 6.79       | <1.7         | 1.2         | 58     | 46      | 13       | 3.7         | 143       | 4.4     |
|             | 12        | 64      | 36      | 9       | 42      | 1056    | 8.0         | 6.99       | <1.7         | 1.3         | 58     | 45      | 13       | 3.4         | 142       | 5.3     |
|             | 28        | 96      | 26      | 10      | 70      | 553     | 9.2         | 6.82       | <1.7         | 1.5         | 58     | 45      | 13       | 3.4         | 146       | 4.1     |
|             | 29        | 198     | 75      | 13      | 47      | 1315    | 5.9         | 7.82       | <1.7         | 1.3         | 58     | 45      | 13       | 3.5         | 143       | 4.4     |
|             | 30        | 69      | 32      | 9       | 36      | 679     | 10.6        | 7.54       | <1.7         | 1.3         | 60     | 47      | 13       | 3.5         | 143       | 4.5     |
| 2F          | 13        | 59      | 46      | 13      | 47      | 377     | 6.9         | 8.01       | 1.7          | 1.3         | 62     | 47      | 15       | 3.1         | 144       | 4.3     |
|             | 14        | 60      | 29      | 12      | 73      | 582     | 8.1         | 7.02       | <1.7         | 1.3         | 59     | 45      | 15       | 3.1         | 145       | 3.8     |
|             | 15        | 97      | 47      | 12      | 50      | 815     | 8.3         | 7.47       | <1.7         | 1.3         | 58     | 45      | 12       | 3.7         | 146       | 4.4     |
|             | 31        | 81      | 69      | 17      | 87      | 1088    | 9.3         | 7.71       | <1.7         | 1.2         | 54     | 41      | 13       | 3.1         | 145       | 4.2     |
|             | 32        | 71      | 52      | 12      | 47      | 615     | 7.1         | 7.95       | <1.7         | 1.3         | 57     | 45      | 12       | 3.7         | 144       | 4.3     |
|             | 33        | 89      | 29      | 16      | 54      | 431     | 8.2         | 7.30       | <1.7         | 0.7          | 60     | 45      | 15       | 3.1         | 145       | 3.9     |
| 3F          | 16        | 83      | 114     | 32      | 54      | 711     | 8.3         | 6.93       | <1.7         | 1.2         | 60     | 43      | 17       | 2.6         | 144       | 4.8     |
|             | 17        | 80      | 47      | 14      | 60      | 610     | 7.3         | 7.85       | <1.7         | 2.5         | 63     | 47      | 16       | 2.9         | 142       | 4.3     |
|             | 18        | 108     | 36      | 12      | 43      | 781     | 6.5         | 6.99       | <1.7         | 2.0         | 60     | 46      | 14       | 3.2         | 144       | 3.9     |
|             | 34        | 91      | 46      | 14      | 36      | 552     | 8.5         | 7.12       | 2.2          | 2.9         | 64     | 48      | 16       | 3.0         | 143       | 4.6     |
|             | 35        | 87      | 27      | 14      | 31      | 737     | 5.9         | 7.74       | <1.7         | 1.0         | 62     | 47      | 15       | 3.2         | 147       | 4.0     |
|             | 36        | 57      | 82      | 13      | 41      | 348     | 9.1         | 7.85       | 1.7          | 1.2         | 61     | 45      | 15       | 3.0         | 143       | 4.4     |
### Appendix 17  (continued)
Clinical Chemistry : Individual Values: Day 66

| Group  | Test Item | Dosage (µg/dose) |  |  |  |
|--------|-----------|-----------------|---|---|---|
| 1      | Control   | 0               | 2 | MenPF-1 | 25 |
| 2      | MenPF-1   | 50              |   |          |   |

| Group / sex | Animal No. | CI mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|------------|-----------|-------------|-----------|-------------|
| 1F          | 10         | 1.60      | 3.69        | 66        |
|             | 11         | 1.33      | 3.64        | 60        |
|             | 12         | 1.18      | 3.78        | 74        |
|             | 28         | 1.42      | 3.67        | 80        |
|             | 29         | 1.55      | 3.68        | 80        |
|             | 30         | 1.53      | 3.72        | 85        |
| 2F          | 13         | 1.59      | 3.82        | 79        |
|             | 14         | 1.48      | 3.51        | 81        |
|             | 15         | 1.41      | 3.89        | 75        |
|             | 31         | 1.95      | 3.46        | 114       |
|             | 32         | 1.40      | 3.57        | 69        |
|             | 33         | 1.49      | 3.49        | 76        |
| 3F          | 16         | 1.29      | 3.68        | 83        |
|             | 17         | 1.35      | 3.67        | 73        |
|             | 18         | 1.73      | 3.57        | 64        |
|             | 34         | 1.31      | 3.76        | 85        |
|             | 35         | 1.49      | 3.55        | 77        |
|             | 36         | 1.54      | 3.62        | 72        |
### Appendix 18
#### Clinical Chemistry: Individual Values: Day 92

| Group / Animal No. | ALP U/L | ALT U/L | AS T U/L | LDH U/L | CPK U/L | Urea mmol/L | Glu mmol/L | T.Bil µmol/L | Chol mmol/L | TP g/L | Alb g/L | Glob g/L | AG-R | Na mmol/L | K mmol/L |
|--------------------|---------|---------|----------|---------|---------|-------------|------------|--------------|-------------|--------|--------|----------|------|----------|---------|
| 1M 19              | 64      | 38      | 14       | 70      | 588     | 7.5         | 6.16       | <1.7         | 0.3         | 56     | 44     | 12       | 3.7  | 147      | 3.5     |
| 20                | 49      | 14      | 10       | 62      | 517     | 7.4         | 6.01       | <1.7         | 0.4         | 57     | 45     | 13       | 3.5  | 144      | 4.3     |
| 21                | 53      | 26      | 18       | 61      | 413     | 6.1         | 7.69       | <1.7         | 0.3         | 55     | 44     | 11       | 4.1  | 145      | 4.3     |
| 2M 22              | 57      | 32      | 13       | 56      | 423     | 6.9         | 6.58       | <1.7         | 0.6         | 61     | 49     | 13       | 3.8  | 144      | 4.6     |
| 23                | 49      | 42      | 12       | 66      | 429     | 8.0         | 6.55       | <1.7         | 0.4         | 58     | 47     | 12       | 4.0  | 145      | 4.6     |
| 24                | 45      | 42      | 17       | 96      | 591     | 9.7         | 6.62       | <1.7         | 0.5         | 57     | 46     | 11       | 4.1  | 147      | 4.6     |
| 3M 25              | 59      | 18      | 8        | 63      | 465     | 7.3         | 6.52       | <1.7         | 0.7         | 58     | 46     | 12       | 3.7  | 143      | 4.4     |
| 26                | 67      | 50      | 21       | 83      | 476     | 5.9         | 7.33       | <1.7         | 0.3         | 58     | 45     | 13       | 3.4  | 143      | 4.2     |
| 27                | 181     | 27      | 17       | 70      | 382     | 6.6         | 7.10       | <1.7         | 0.4         | 56     | 45     | 12       | 3.9  | 145      | 4.3     |
## Appendix 18  (continued)
Clinical Chemistry : Individual Values: Day 92

| Group / sex | Test Item | Dosage (µg/dose) | 1  | 2  | 3  |
|-------------|-----------|------------------|----|----|----|
|             |           |                  | Cl mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
| 1M          | 19        | 0                | 1.19 | 3.51 | 76  |
|             | 20        | 25               | 1.05 | 3.74 | 62  |
|             | 21        | 50               | 1.33 | 3.54 | 63  |
| 2M          | 22        | 25               | 1.03 | 3.95 | 52  |
|             | 23        | 105              | 1.25 | 3.81 | 73  |
|             | 24        | 106              | 1.10 | 3.87 | 100 |
| 3M          | 25        | 101              | 1.23 | 3.64 | 71  |
|             | 26        | 103              | 1.21 | 3.56 | 78  |
|             | 27        | 104              | 1.26 | 3.61 | 73  |
## Appendix 18 (continued)

### Clinical Chemistry: Individual Values: Day 92

| Group / Test Item | 1  | 2  | 3  |
|-------------------|----|----|----|
| Test Item         | Control | MenPF-1 | MenPF-1 |
| Dosage (µg/dose)  | 0 | 25 | 50 |

| Group / Animal No. | ALP (U/L) | ALT (U/L) | AST (U/L) | LDH (U/L) | CPK (U/L) | Urea (mmol/L) | Glu (mmol/L) | T.Bil (µmol/L) | Chol (mmol/L) | TP (g/L) | Alb (g/L) | Glob (g/L) | AG-R mmol/L | Na (mmol/L) | K (mmol/L) |
|-------------------|-----------|-----------|-----------|-----------|-----------|---------------|--------------|---------------|--------------|----------|----------|-----------|------------|-----------|-----------|
| 1F 28              | 84        | 30        | 11        | 51        | 387       | 9.9           | 5.96         | <1.7          | 1.3          | 61       | 49       | 12        | 4.2        | 145       | 4.3       |
| 29                | 99        | 38        | 13        | 79        | 757       | 8.0           | 6.24         | <1.7          | 1.3          | 61       | 49       | 12        | 4.1        | 143       | 4.4       |
| 30                | 63        | 31        | 10        | 43        | 508       | 9.8           | 6.77         | <1.7          | 1.2          | 59       | 46       | 13        | 3.6        | 144       | 4.4       |
| 2F 31              | 63        | 36        | 14        | 35        | 446       | 10.0          | 7.53         | <1.7          | 1.1          | 53       | 42       | 12        | 3.6        | 144       | 4.4       |
| 32                | 58        | 50        | 18        | 40        | 423       | 9.1           | 7.27         | <1.7          | 1.1          | 56       | 44       | 13        | 3.5        | 143       | 4.3       |
| 33                | 65        | 31        | 19        | 161       | 610       | 8.0           | 6.97         | <1.7          | 0.8          | 55       | 44       | 11        | 3.9        | 141       | 4.3       |
| 3F 34              | 91        | 24        | 14        | 34        | 345       | 8.5           | 6.34         | 1.7           | 2.8          | 63       | 49       | 14        | 3.4        | 143       | 4.2       |
| 35                | 109       | 29        | 15        | 44        | 521       | 7.9           | 6.48         | <1.7          | 1.0          | 61       | 47       | 14        | 3.5        | 144       | 4.1       |
| 36                | 60        | 42        | 14        | 47        | 313       | 9.8           | 6.71         | <1.7          | 0.9          | 61       | 46       | 15        | 3.0        | 142       | 4.4       |
## Appendix 18 (continued)
### Clinical Chemistry: Individual Values: Day 92

| Group / sex | Animal No. | CI mmol/L | Phos mmol/L | Ca mmol/L | Crea µmol/L |
|-------------|------------|-----------|-------------|-----------|-------------|
| 1F          | 28         | 1.24      | 3.83        | 87        |             |
|             | 29         | 0.99      | 3.77        | 73        |             |
|             | 30         | 1.26      | 3.75        | 90        |             |
| 2F          | 31         | 1.36      | 3.49        | 125       |             |
|             | 32         | 1.18      | 3.51        | 84        |             |
|             | 33         | 1.50      | 3.47        | 90        |             |
| 3F          | 34         | 1.05      | 3.82        | 94        |             |
|             | 35         | 1.34      | 3.75        | 79        |             |
|             | 36         | 1.15      | 3.54        | 69        |             |
Appendix 19
Antibody Analysis

1 INTRODUCTION

MenPF-1 is a developmental vaccine against disease caused by Neisseria meningitidis (the meningococcus). The major antigens in this vaccine are the outer membrane proteins PorA and FetA. The vaccine contains outer membrane vesicles (OMVs) from a meningococcal strain genetically modified to over-express the FetA antigen.

MenPF-1 OMVs, adsorbed to aluminium hydroxide (Al(OH)₃) adjuvant, have been produced by the Norwegian Institute of Public Health (NIPH). A validation batch (Lot number FMOX1102) of the MenPF-1 vaccine was tested for in vivo toxicity in rabbits. The results of this study will be used to support an application by the University of Oxford for the use of MenPF-1 in a Phase 1 clinical trial in humans.

During the toxicology study, New Zealand White rabbits were given four doses of Al(OH)₃-only control inoculum or Al(OH)₃-adjuvanted MenPF-1 OMVs. Rabbits receiving MenPF-1 OMVs were given either a single human dose (25μg total protein) or double human dose (50μg total protein). Doses were given on days 1, 22, 43 and 64. Blood samples were collected from each rabbit pre-immunisation, before dosing on days 22, 64 and on day 92. Blood samples were processed, and extracted serum samples stored at -80°C.

The immunological testing of serum samples was performed by the National Institute of Biological Standards and Control (NIBSC). An in vitro Enzyme Linked Immunosorbent Assay (ELISA) was used at NIBSC to determine seroconversion of rabbits in the study. Seroconversion is defined as the development of detectable specific antibodies raised against the vaccine in response to immunisation. The ELISA was used to demonstrate seroconversion in the rabbits which should switch from MenPF-1 seronegative to MenPF-1 seropositive if successfully immunised. The binding of antibodies in pre- and post-vaccination sera to MenPF-1 OMVs was assessed using a validated assay of suitable sensitivity and specificity.
2 PROCEDURE – ENZYME LINKED IMMUNOSORBENT ASAY

All details of the test, including samples tested, dilutions made, critical timings, pipette serial numbers, and buffers and reagents used on the MenPF-1 Rabbit ELISA test were recorded on the test record form (Document S/N 6116).

a). A 2μg/ml solution of MenPF-1 OMV in coating buffer was prepared according to the following table:

| Number of Plates | Total Solution Volume (ml) | Volume Coating Buffer (ml) | Volume OMV stock (μl) |
|------------------|---------------------------|---------------------------|----------------------|
| 1                | 12                        | 11.947                    | 53                   |
| 2                | 24                        | 23.893                    | 107                  |
| 3                | 34                        | 33.849                    | 151                  |
| 4                | 45                        | 44.800                    | 200                  |
| 5                | 55                        | 54.756                    | 244                  |
| 6                | 65                        | 64.711                    | 289                  |

The appropriate wells of microtitre plates were coated with 100μl of solution, covered and incubated at 44°C for a minimum of 16 hours in a sealed container which was labelled to be identifiable to the test operator.

b). The ELISA plates were washed with Wash Buffer using the Skatran Plate washer. If the machine was switched off or the connected buffer had been changed from that required by this assay, a blank plate of pure water was first used to rinse the machine; the machine was then primed with the required Wash Buffer. All buffer changes were recorded on the test record form.

c). Plates were blocked with 100μl per well of Dilution Buffer, covered and incubated for a minimum of 1 hour (+10 minutes) at room temperature in a sealed container.

d). The ELISA plates were washed as in step b).

e). Dilutions of the sera to be tested, and the positive control, were prepared by dilution of the Buffer as follows:
- Positive control sera, diluted 1:500 (1:10 followed by 1:50);
- Negative control sera, diluted 1:100 (1:10 followed by 1:10);
- Test sera taken on day 0 (Test Sample 1), diluted 1:100 (1:10 followed by 1:10);
- Test sera taken on day 22 (Test Sample 2), diluted 1:300 (1:10 followed by 1:30);
Appendix 19  (continued)
Antibody Analysis

- Test sera taken on day 64 (Test Sample 3), diluted 1:900 (1:10 followed by 1:90);
- Test sera taken on day 92 (Test Sample 4), diluted 1:900 (1:10 followed by 1:90).

f). ELISA plates were then prepared. One 96-well plate was required to test all serum samples extracted from each rabbit, including the positive control serum, at a maximum of 8 dilutions for each serum sample. All samples were tested in duplicate columns (see example plate layout in Figure 1). Samples were assigned randomly to columns (see Appendix 1). For the standard assay, random plate layouts were generated and can be found on the MenPF-1 Rabbit ELISA test record form (retained in the study data). For rabbits for which less than four serum samples were available, columns listed as “Test Sample 4” were left blank. The template used in the assay on the MenPF-1 Rabbit ELISA was noted on the test record form (retained in the study data). A different template for each assay was used in rotation in the order 1 through to 8.

![Figure 1: Example plate layout (all samples and controls are randomised across the plate). See Appendix 1 for detailed plate layout templates.](image)

- Pre-diluted test sample or positive control.
  - TS = Test serum
  - +ve = Positive control serum
  - -ve = Negative control serum

h). Wells in rows B - H were filled with 100µl of Dilution Buffer; row A was left empty.
Appendix 19  (continued)
Antibody Analysis

i). 150μl of each diluted preparation was added to the wells in row A. 50μl of each sample was then removed and transferred to the appropriate wells in row B. Wells were mixed for a maximum of 5 times. 50μl volumes were then transferred to the next row (C). This procedure was repeated down the plate. Following mixing of row H, 50μl of the sample was discarded from each well. Each well in rows A through to H now contained 100μl volumes. Plates were then covered and incubated at room temperature in the sealed container for a minimum of 1 hours (+10 minutes).

j). ELISA plates were washed as in step b).

k). Goat anti-rabbit HRP conjugate was diluted 1:2000 in Dilution Buffer and 100μl added to all wells, covered and incubated for a minimum of 1 hour (+10 minutes) in the sealed container at room temperature.

l). ELISA plates were washed as in step b).

m). 100μl TMBblue substrate was then added to all wells and incubated at room temperature for up to ten minutes. Following colour development, 100μl 1M sulphuric acid was added to all the wells to stop the reaction. The plates were then read at 450 nm using a microplate reader

n). Raw data was printed immediately, signed and dated.

3 10. DATA ANALYSIS, VALIDITY AND DETERMINATION OF SEROCONVERSION

Absorbance levels across the dilution series from each test sample were used to directly compare the levels of IgG binding following immunisation of each rabbit to pre-trial sera, in order to determine whether each rabbit was seroconverted. All calculations were recorded on the print-out of the raw data and reviewed by the Responsible Scientist. No computer software was required for data analysis.

10.1. DATA ANALYSIS

a) Referring to the dilution series listed below, for each test sample, the highest dilution factor at which the absorbance at 450nm was higher than 0.70 was determined (where at least two consecutive dilutions were higher than the threshold, except where only a 1/100 dilution of a sample had absorbance higher than 0.70). The dilution factor was recorded as “IG”. If a sample did not result in absorbance higher than 0.70 at a dilution of 1:100, IG was recorded as 100. If higher or lower dilutions (to a minimum of 1/100) were required to determine IG, the test sample was repeated with appropriate dilutions.
Appendix 19 (continued)
Antibody Analysis

For duplicates of a single sample, if IG values were one dilution apart, a mean value was taken as the IG for that sample. If IG values for duplicates of a single sample were greater than one dilution apart, that sample was repeated.

| Row | Positive | Test Sample 1 /Negative | Test Sample 2 | Test Sample 3/4 |
|-----|----------|--------------------------|--------------|-----------------|
| Start dilution | A | 500 | 100 | 300 | 900 |
| Dilution series | B | 1500 | 300 | 900 | 2700 |
|                 | C | 4500 | 900 | 2700 | 8100 |
|                 | D | 13500 | 2700 | 8100 | 24300 |
|                 | E | 40500 | 8100 | 24300 | 72900 |
|                 | F | 121500 | 24300 | 72900 | 218700 |
|                 | G | 364500 | 72900 | 218700 | 656100 |
|                 | H | 1093500 | 218700 | 656100 | 1968300 |

b) For each test sample 2, 3 and 4, the increase in binding following vaccination was calculated as follows:

$$\Delta IG = \frac{IG_{(Test\ Sample\ n)}}{IG_{(Test\ Sample\ 1)}}$$

Where “n” = 2, 3 or 4.

For the positive control serum, $\Delta IG$ was calculated as follows:

$$\Delta IG = \frac{IG_{(Positive\ control\ serum)}}{IG_{(Negative\ control\ serum)}}$$

c) Values for $\Delta IG$ were recorded on the test record form.

4 10.2. VALIDITY REQUIREMENTS

In order for the test to be valid:

i). The maximum absorbance at 450nm for the positive control serum must be greater than 3.0 for both repeats.

ii). The minimum absorbance at 450nm for the positive control serum must be less than 0.7 for both repeats.

iii). The maximum absorbance at 450nm for the negative control serum must be greater than 0.7.

iv). The $\Delta IG$ value calculated for the positive control serum must be between 90 and 810.

Validity of the assay was recorded on the test record form.
Appendix 19 (continued)
Antibody Analysis

A test was repeated if it did not meet the validity requirements, if IG values were greater than one dilution apart for duplicates of any test sample, or if alternative dilutions were required to determine IG values for any test sample.

10.3. DETERMINATION OF SEROCONVERSION
When analysis of serum samples from all animals was completed, seroconversion was determined for each time point after initiation of the trial (Day 22, Day 64 and Day 92). For each post-vaccination serum sample, when IG ≥ 4 seroconversion was determined to have occurred.

5 11. RECORDING OF RESULTS
Copies of all raw and analysed data, as well as scanned copies of all test record forms, were stored in the bactMenPtox drive. Hard copies of all test record forms and raw data were stored in B38. On completion of analysis of all serum samples, all printed and electronic data were sent to Charles River Laboratories for review and incorporation into the toxicology report.
### 6. TABLES

**Table 1**  Geometric Mean AIG Values: Males – Main Study

| Group | Treatment (µg/dose of MenPE-1) | Day of Antibody Bled |
|-------|-------------------------------|----------------------|
|       |                               | Pre-trial | 22 | 64 | 92 |
| 1     | 0                             | -         | 1.44 | 1 | - |
| 2     | 25                            | -         | 81 | 1536 | - |
| 3     | 50                            | -         | 81 | 7479 | - |

- = Not applicable
### Table 2: Geometric Mean AIG Values: Females – Main Study

| Group | Treatment (µg/dose of MenPF-1) | Day of Antibody Bleed | Pre-trial | 22 | 64 | 92 |
|-------|--------------------------------|-----------------------|----------|----|----|----|
| 1     | 0                              | -                     | 1        | 2.08 | -  |
| 2     | 25                             | -                     | 71       | 441 | -  |
| 3     | 50                             | -                     | 102      | 5186 | -  |

* = Not applicable
### Table 3: Geometric Mean AIG Values: Males – Recovery Study

| Group | Treatment (µg/dose of MenP{I}) | Day of Antibody Bleed |
|-------|-------------------------------|-----------------------|
|       |                               | Pre-trial | 22 | 64 | 92 |
| 1     | 0                             | -         | 1.65 | 1.65 | 1.65 |
| 2     | 25                            | -         | 27 | 1669 | 1199 |
| 3     | 50                            | -         | 505 | 4880 | 2854 |

- = Not applicable
Appendix 19  (continued)
Antibody Analysis

| Group | Treatment (µg/dose of MenP¥1) | Day of Antibody Bleed | Pre-trial | 22  | 64  | 92  |
|-------|--------------------------------|-----------------------|-----------|-----|-----|-----|
| 1     | 0                              | -                     | 1         | 3   | 3   |
| 2     | 25                             | -                     | 24        | 951 | 1669|
| 3     | 50                             | -                     | 56        | 2407| 1325|

- = Not applicable

Table 4 Geometric Mean AIG Values: Females – Recovery Study
Appendix 19  (continued)
Antibody Analysis

7   APPENDICES

Appendix 1   ELISA PLATE LAYOUT TEMPLATES

Random plate layouts for Anti-MenPF-1 Rabbit Immunoglobulin ELISA

Test sample, reference, and positive control added to row A of 96 well plates as indicated below:

| Key: | Positive serum | Negative Serum | Test sample |
|------|----------------|----------------|-------------|
| +    |                |                |             |
| -    |                |                |             |

| Template 1 | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|            | TS2 | +   | -   | -   | TS1 | TS3 | TS4 | +   | TS2 | TS4 | TS3 | TS1 |
| Template 2 | -   | TS3 | TS4 | +   | -   | TS3 | TS1 | TS4 | +   | TS1 | TS2 | TS2 |
|            | TS3 | TS2 | TS1 | -   | TS4 | TS2 | TS1 | TS3 | +   | TS4 | -   | +   |
| Template 3 | TS1 | TS3 | -   | TS4 | +   | TS2 | +   | TS1 | TS2 | TS4 | -   | TS3 |
|            | -   | TS1 | TS4 | TS2 | -   | +   | TS3 | TS2 | TS4 | TS1 | TS3 | +   |
| Template 4 | -   | TS2 | TS4 | TS2 | TS1 | TS3 | +   | TS1 | TS3 | -   | TS4 | +   |
|            | TS4 | +   | TS4 | -   | TS2 | +   | TS3 | -   | TS1 | TS3 | TS1 |     |
| Template 5 | TS3 | TS3 | TS4 | TS1 | +   | TS1 | TS2 | +   | TS4 | -   | -   | TS2 |     |
| Template 6 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Template 7 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Template 8 |     |     |     |     |     |     |     |     |     |     |     |     |     |
**Appendix 19 (continued)**

**Antibody Analysis**

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**Appendix 2  Individual ΔIG Values : Males – Main Study**

| Group | Animal Number | Treatment (μg/dose of MenPF-1) | Day of Antibody Bleed |
|-------|---------------|--------------------------------|-----------------------|
|       |               | Pre-trial 22 | 64 | 92 |
| 1     | 1             | -          | 1  | 1  | -  |
|       | 2             | -          | 1  | 1  | -  |
|       | 3             | -          | 3  | 1  | -  |
| 2     | 4             | -          | 243| 2187| -  |
|       | 5             | -          | 27 | 729 | -  |
|       | 6             | -          | 81 | 2187| -  |
| 3     | 7             | -          | 27 | 1458| -  |
|       | 8             | -          | 243| 13122| -  |
|       | 9             | -          | 81 | 21870| -  |

- = Not applicable
Appendix 3  Individual ΔIG Values : Females – Main Study

| Group | Animal Number | Treatment (μg/dose of Men(PI-1)) | Pre-trial | Day of Antibody Bleed |
|-------|---------------|----------------------------------|-----------|-----------------------|
| 1     | 10            | 0                                | -         | 1                     | 1 | - |
|       | 11            |                                  | -         | 1                     | 3 | - |
|       | 12            |                                  | -         | 1                     | 3 | - |
| 2     | 13            | 25                               | -         | 9                     | 9 | - |
|       | 14            |                                  | -         | 27                    | 729 | - |
|       | 15            |                                  | -         | 1458                  | 13122 | - |
| 3     | 16            | 50                               | -         | 81                    | 13122 | - |
|       | 17            |                                  | -         | 162                   | 810 | - |
|       | 18            |                                  | -         | 81                    | 13122 | - |

- = Not applicable
### Appendix 4  Individual ΔIG Values: Males – Recovery Study

| Group | Animal Number | Treatment (μg/dose of MenPF-1) | Pre-trial | Day of Antibody Bleed |
|-------|---------------|---------------------------------|-----------|-----------------------|
| 1     | 19            | 0                               | -         | 4.5                   |
|       | 20            |                                 | -         | 1                     |
|       | 21            |                                 | -         | 1                     |
| 2     | 22            | 25                              | -         | 27                    |
|       | 23            |                                 | -         | 9                     |
|       | 24            |                                 | -         | 81                    |
| 3     | 25            | 50                              | -         | 1093.5                |
|       | 26            |                                 | -         | 486                   |
|       | 27            |                                 | -         | 243                   |

- = Not applicable
### Appendix 5  Individual ΔIG Values : Females – Recovery Study

| Group | Animal Number | Treatment (µg/dose of Men(PF-1)) | Day of Antibody Bleed |
|-------|---------------|----------------------------------|-----------------------|
|       |               |                                  | Pre-trial | 22 | 64 | 92 |
| 1     | 28            | 0                                | -         | 1  | 1  | 1  |
|       | 29            |                                  | -         | 1  | 27 | 27 |
|       | 30            |                                  | -         | 1  | 1  | 1  |
| 2     | 31            | 25                               | -         | 6  | 1458 | 729 |
|       | 32            |                                  | -         | 27 | 2430 | 4374 |
|       | 33            |                                  | -         | 81 | 243 | 1458 |
| 3     | 34            | 50                               | -         | 27 | 486 | 486 |
|       | 35            |                                  | -         | 243 | 13122 | 729 |
|       | 36            |                                  | -         | 27 | 2387 | 6561 |

- = Not applicable
### Individual Necropsy and Histological Findings: Day 66

#### Abbreviations Used

| Abbreviation | Organ/Structure |
|--------------|-----------------|
| ADR          | Adrenal Gland   |
| AOR          | Aorta           |
| APP          | Appendix        |
| BRA          | Brain           |
| CAE          | Caecum          |
| CER          | Cervix          |
| COL          | Colon           |
| DUO          | Duodenum        |
| EPI          | Epididymis      |
| EYE          | Eye             |
| FEM          | Femur           |
| GALT         | Gut Associated Lymphoid Tissue |
| GBL          | Gallbladder     |
| HEA          | Heart           |
| ILE          | Ileum           |
| JEJ          | Jejunum         |
| KID          | Kidney          |
| LAC          | Lacrimal Gland  |
| LIV          | Liver           |
| LUN          | Lung            |
| LNIN         | Inguinal Lymph Node |
| LULU         | Lumbar Lymph Node |
| LNMA         | Mandibular Lymph Nodes |
| LNMS         | Mesenteric Lymph Nodes |
| MAM          | Mammary Gland   |
| OES          | Oesophagus      |
| OPT          | Optic Nerve     |
| OVA          | Ovary           |
| OVD          | Oviduct         |
| PAR          | Parathyroid Gland |
| PCEN         | Pancreas (Endocrine) |
| PCEX         | Pancreas (Exocrine) |
| PIT          | Pituitary Gland |
| PRO          | Prostate        |
| REC          | Rectum          |
| sac          | Sacculus Rutundus |
| SCI          | Sciatic Nerve   |
| SEM          | Seminal Vesicle |
| SGSM         | Salivary gland (Submaxillary) |
| SKI          | Skin and Subcutis |
| SKM          | Skeletal Muscle |
| SPL          | Spleen          |
| SPL          | Spleen          |
| SPN          | Spinal Cord     |
| STO          | Stomach         |
| STR          | Sternal         |
| TES          | Testis          |
| THM          | Thymus          |
| THR          | Thyroid Gland   |
| TOG          | Tongue          |
| TRA          | Trachea         |
| URE          | Ureter          |
| URB          | Urinary Bladder |
| UTE          | Uterus          |
| VAG          | Vagina          |
Appendix 20 (continued)
Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO | FINDINGS |
|------------|----------|
| 1          | Terminal Kill  
             Day of Necropsy: 66 |

Necropsy Findings:
- Tissues not listed below were normal

Histological Findings:
- Aspermia, unilateral
- Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
- Basophilic tubules, multifocal, minimal
- Agonal congestion/haemorrhage, (relates to necropsy finding)
- Only one examined
- Only anterior lobe examined
- Seminiferous epithelial degeneration, unilateral, moderate, (relates to necropsy finding)

Organs Examined and No Abnormality Detected:
- ADR, AOR, APP, BRA, CAE, COL, DUO, EYE, FEM, GBL, HEA, ILE, JEJ, LAC, LIV, LUN, LNIN, LNLU, LNMA, LNMS, OES, OPT, PCEN, PCEX, PAR, PIT, PRO, REC, SGSM, SCI, SEM, SKM, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, GALT, sac
Appendix 20  (continued)
Individual Necropsy and Histological Findings: Day 66

| PROJECT NUMBER: | 520419 |
| TREATMENT: | Group 1 (0 ug/dose) MALES |

| ANIMAL NO: | FINDINGS: |
|---|---|
| 2 | Terminal Kill Day of Necropsy: 66 |

Necropsy Findings:
- General Comments: Tissues not listed below were normal
- Lung: Discolouration, dark, all lobes, red
- Testis: Small, right
- Thymus: Discolouration, dark, left lobe, red

Histological Findings:
- Epididymis: Aspermia, unilateral
- Injection Site 1: Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells) Inflammation, mononuclear cell, moderate Myofibre necrosis, mild
- Lung: Agonal congestion/haemorrhage, (relates to necropsy finding)
- Lymph Node (Lumbar): Macrophage accumulation, minimal, (with multinucleated cells and intracytoplasmic foreign material) Erythrocytosis/erythrophagocytosis, minimal
- Parathyroid Gland: Tissue absent from section. No more available
- Testis: Immaturity, unilateral, (relates to necropsy finding) Seminiferous epithelial degeneration, unilateral, focal, mild
- Thymus: Agonal congestion/haemorrhage, (relates to necropsy finding)
Appendix 20  (continued)
Individual Necropsy and Histological Findings: Day 66

PROJECT NUMBER: 520419
TREATMENT: Group 1 (0 ug/dose) MALES

ANIMAL NO: FINDINGS:

2 (CONTINUED)

HISTOLOGICAL FINDINGS:
ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
ADR, AOR, APP, BRA, CAE, COL, DUO, EYE, FEM, GBL, HEA, ILE, JEJ, KID, LAC, LIV, LUN, LNIN, LNMA, LNMS, OES, OPT, PCEN, PCEX, PIT, PRO, REC, SGSM, SCI, SEM, SKM, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, GALT, sac

3 Terminal Kill
Day of Necropsy: 66

NECROPSY FINDINGS:
GENERAL COMMENTS: Tissues not listed below were normal
LUNG: Discolouration, dark, all lobes

HISTOLOGICAL FINDINGS:
INJECTION SITE 1: Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
Inflammation, mononuclear cell, mild
KIDNEY: Basophilic tubules, focal, minimal
LUNG: Agonal congestion/haemorrhage, (relates to necropsy finding)
LYMPH NODE (LUMBAR): Erythrocytosis/erythrophagocytosis, minimal
PARATHYROID GLAND: Only one examined
PITUITARY GLAND: Tissue lost at necropsy

(Continued)
## Appendix 20  (continued)
### Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 3          | (CONTINUED) |

**HISTOLOGICAL FINDINGS:**

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**

ADR, AOR, APP, BRA, CAE, COL, DUO, EPI, EYE, FEM, GBL, HEA, ILE, JEJ, LAC, LIV, LUN, LNIN, LNMA, LNMS, OES, OPT, PCEN, PCEX, PAR, PRO, REC, SGS, SCI, SEM, SKM, SKI, SPN, SPL, STR, STO, TES, THM, THR, TOG, TRA, URE, URB, GALT, sac

Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
Appendix 20 (continued)
Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 10         | Terminal Kill |
|            | Day of Necropsy: 66 |

**Necropsy Findings:**
- **General Comments:** Tissues not listed below were normal
- **Lung:** Discolouration, dark, all lobes, red
- **Lymph Node (inguinal):** Not found at necropsy, right
- **Lymph Node (lumbar):** Discolouration, dark, red

**Histological Findings:**
- **Adrenal Gland:** Cortical vacuolated cell focus, minimal
- **Injection Site 1:** Macrophage accumulation, intramuscular, mild, (with cytoplasmic foreign material and multinucleated giant cells), Inflammation, mononuclear cell, mild, Myofibre necrosis, minimal, Regeneration, myofibre, minimal, (relates to necropsy finding)
- **Lung:** Agonal congestion/haemorrhage, (relates to necropsy finding)
- **Lymph Node (inguinal):** Only one examined
- **Lymph Node (lumbar):** Erythrocytosis/erythrophagocytosis, mild, (relates to necropsy finding)
- **Lymph Node (mesenteric):** Tissue lost during processing
- **Parathyroid Gland:** Tissue absent from section, No more available

**Organs Examined and No Abnormality Detected:**
- AOR, APP, BRA, CAE, CER, COL, DUO, EYE, FEM, GBL, HEA, ILE, JEJ, KID, LAC, LIV, LUN, LNIN, LNMA, MAM, OES, OPT, OVA, OVD, PCEN, PCEX, PIT, REC, SGSM, SCI...

Pathology File Ref.: MCLIS_520419_MAIN_LL_KEEP2.SPL (continued)
Appendix 20  (continued)
Individual Necropsy and Histological Findings: Day 66

PROJECT NUMBER: 520419
TREATMENT: Group 1 (0 ug/dose) FEMALES

ANIMAL NO: FINDINGS:

10  (CONTINUED)

HISTOLOGICAL FINDINGS:
ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
SKM , SKI , SPN , SPL , STR , STO ,
THM , THR , TOG , TRA , URE , URB ,
UTE , VAG , GALT, sac

11  Terminal Kill
Day of Necropsy: 66

NECROPSY FINDINGS:
GENERAL COMMENTS : Tissues not listed below were normal
LUNG : Discolouration, all lobes, red,
(mottled)
LYMPH NODE (MESENTERIC) : Discolouration, dark, red

HISTOLOGICAL FINDINGS:
ADRENAL GLAND : Only one medulla examined
HEART : Inflammatory cell foci, myocardial,
minimal
INJECTION SITE 1 : Inflammation, polymorphonuclear
leukocytic, dermal, focal, minimal
Macrophage accumulation,
intramuscular, moderate, (with
cytoplasmic foreign material and
multinucleated giant cells)
LUNG : Agonal congestion/haemorrhage,
(relates to necropsy finding)
LYMPH NODE (LUMBAR) : Macrophage accumulation, mild, (with
multinucleated cells and
intracytoplasmic foreign material)

(Continued)
### Appendix 20  (continued)
**Individual Necropsy and Histological Findings: Day 66**

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 11 (CONTINUED) | |
| **HISTOLOGICAL FINDINGS:** | |
| Lymph Node (Mesenteric): | Erythrocytosis/erythrophagocytosis, minimal, (relates to necropsy finding) |
| Pituitary Gland: | Only anterior lobe examined |
| ORGANS EXAMINED AND NO ABNORMALITY DETECTED: | |
| ADR, AOR, APP, BRA, CAE, CER, COL, DUO, EYE, FEM, GBL, ILE, JEJ, KID, LAC, LIV, LUN, LNIN, LNMA, MAM, OES, OPT, OVA, OVD, PCEN, PCEX, PAR, PIT, REC, SGSM, SCI, SKM, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, UTE, VAG, GALT, sac |

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 12 | Terminal Kill |
| Day of Necropsy: 66 |
| **NECROPSY FINDINGS:** | |
| General Comments: | Tissues not listed below were normal |
| Lung: | Spongy, all lobes |
| Discolouration, dark, all lobes, red |
| **HISTOLOGICAL FINDINGS:** | |
| Adrenal Gland: | Medulla not present |
| Injection Site 1: | Macrophage accumulation, intramuscular, mild, (with cytoplasmic foreign material and multinucleated giant cells) |
| Inflammation, mononuclear cell, mild |

Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
### Appendix 20  (continued)
### Individual Necropsy and Histological Findings: Day 66

**PROJECT NUMBER:** 520419  
**TREATMENT:** Group 1 (0 ug/dose) FEMALES

| ANIMAL NO | FINDINGS |
|-----------|---------|
| 12        | (CONTINUED) |

**HISTOLOGICAL FINDINGS:**  
- **LACRIMAL GLAND:** Tissue absent from section. No more available  
- **LUNG:** Agonal congestion/haemorrhage, (relates to necropsy findings)  
- **MAMMARY GLAND:** Duct ectasia, mild  
- **PARATHYROID GLAND:** Tissue absent from section. No more available

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**
ADR, AOR, APP, BRA, CAE, CER, COL, DUO, EYE, FEM, GBL, HEA, ILE, JEJ, KID, LIV, LUN, LNIN, LNLU, LNMA, LNMS, OES, OPT, OVA, OVD, PCEN, PCEX, PIT, REC, SGSM, SCI, SKM, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, UTE, VAG, GALT, sac

Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
### Appendix 20  (continued)
### Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 4         | Terminal Kill  
Day of Necropsy: 66  |

**Necropsy Findings:**
- **General Comments:** Tissues not listed below were normal
- **Lung:** Discolouration, dark, all lobes

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 5         | Terminal Kill  
Day of Necropsy: 66  |

**Necropsy Findings:**
- **General Comments:** Tissues not listed below were normal
- **Lung:** Discolouration, all lobes, (mottled)
- **Lymph Node (Lumbar):** Discolouration, dark, right, red

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 6         | Terminal Kill  
Day of Necropsy: 66  |

**Necropsy Findings:**
- **General Comments:** Tissues not listed below were normal
- **Lung:** Discolouration, dark, all lobes
## Individual Necropsy and Histological Findings: Day 66

**PROJECT NUMBER:** 520419  
**TREATMENT:** Group 2 (25 ug/dose) FEMALES

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 13        | Terminal Kill  
Day of Necropsy: 66 |

**NECROPSY FINDINGS:**  
**GENERAL COMMENTS:** Tissues not listed below were normal  
**LUNG:** Discolouration, dark, all lobes, red

| 14        | Terminal Kill  
Day of Necropsy: 66 |

**NECROPSY FINDINGS:**  
**GENERAL COMMENTS:** All tissues normal

| 15        | Terminal Kill  
Day of Necropsy: 66 |

**NECROPSY FINDINGS:**  
**GENERAL COMMENTS:** Tissues not listed below were normal  
**LUNG:** Discolouration, dark, all lobes, red  
Spongy, all lobes  
**LYMPH NODE (MESENTERIC):** Discolouration, dark, red
### Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 7         | Terminal Kill |
|           | Day of Necropsy: 66 |

#### Necropsy Findings:
- **GENERAL COMMENTS**: Tissues not listed below were normal
- **ADRENAL GLAND**: Not found at necropsy, left
- **LUNG**: Discolouration, dark, all lobes
- **LYMPH NODE (LUMBAR)**: Not found at necropsy, left

#### Histological Findings:
- **ADRENAL GLAND**: Only one examined
  - Macrophage accumulation, intramuscular, mild, (with cytoplasmic foreign material and multinucleated giant cells)
  - Inflammation, polymorphonuclear leukocytic, moderate
  - Myofibre necrosis, mild
  - Fibrosis, interstitial, mild
- **KIDNEY**: Tubular mineralisation, medullary, minimal
  - Basophilic tubules, multifocal, minimal
- **LACRIMAL GLAND**: Tissue lost during processing
- **LUNG**: Agonal congestion/haemorrhage, (relates to necropsy finding)
  - Osseous metaplasia, focal, minimal
- **LYMPH NODE (LUMBAR)**: Only one examined
- **LYMPH NODE (MESENTERIC)**: Erythrocytosis/erythrophagocytosis, minimal
- **THYROID GLAND**: Inflammatory cell foci, minimal
- **URINARY BLADDER**: Mineral deposits, epithelial, surface, multifocal, minimal

(Continued)
Appendix 20  (continued)
Individual Necropsy and Histological Findings: Day 66

PROJECT NUMBER: 520419
TREATMENT: Group 3 (50 ug/dose) MALES

ANIMAL NO: FINDINGS:

7 (CONTINUED)

HISTOLOGICAL FINDINGS:
ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
ADR, AOR, APP, BRA, CAE, COL, DUO, EPI, EYE, FEM, GBL, HEA, ILE, JEJ, LIV, LNIN, LNLU, LNMA, OES, OPT, PCEN, PCEX, PAR, PIT, PRO, REC, SGSM, SCI, SEM, SKM, SKI, SPN, SPL, STR, STO, TES, THM, TOG, TRA, URE, GALT, sac

8 Terminal Kill
Day of Necropsy: 66

NECROPSY FINDINGS:
GENERAL COMMENTS: Tissues not listed below were normal
LUNG: Discolouration, all lobes, (mottled)
LYMPH NODE (LUMBAR): Enlargement, left, (20 x 6 x 4 mm)

HISTOLOGICAL FINDINGS:
INJECTION SITE 1: Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
Inflammation, mononuclear cell, mild
Regeneration, myofibre, mild

LUNG: Inflammatory cell foci, minimal
Agonal congestion/haemorrhage, (relates to necropsy finding)

LYMPH NODE (INGUINAL): Only one examined
LYMPH NODE (LUMBAR): Erythrocytosis/erythrophagocytosis, minimal

(continues)
### Appendix 20 (continued)
**Individual Necropsy and Histological Findings: Day 66**

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 8          | (CONTINUED) |
|            | HISTOLOGICAL FINDINGS: |
|            | LYMPH NODE (LUMBAR): Macrophage accumulation, minimal, (with multinucleated cells and intracytoplasmic foreign material) Lymphoid hyperplasia, moderate, (relates to necropsy finding) |
|            | PARATHYROID GLAND: Tissue absent from section. No more available |

**PROJECT NUMBER:** 520419  
**TREATMENT:** Group 3 (50 ug/dose) MALES  

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 9          | Terminal Kill  
Day of Necropsy: 66 |

**NECROPSY FINDINGS:**  
**GENERAL COMMENTS:** Tissues not listed below were normal  
**LYMPH NODE (LUMBAR):** Enlargement, left, (8 x 4 x 3 mm)  

**HISTOLOGICAL FINDINGS:**  
**INJECTION SITE 1:** Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
## Appendix 20  (continued)
### Individual Necropsy and Histological Findings: Day 66

| PROJECT NUMBER: | 520419 |
|-----------------|--------|
| TREATMENT:      | Group 3 (50 ug/dose) MALES |
| ANIMAL NO:      | FINDINGS: |
| 9 (CONTINUED)   | |

**HISTOLOGICAL FINDINGS:**

**INJECTION SITE 1:**
- Fibrosis, interstitial, marked
- Inflammation, polymorphonuclear leukocytic, marked
- Myofibre necrosis, moderate

**KIDNEY:**
- Nephropathy, focal, minimal

**LUNG:**
- Inflammatory cell foci, minimal

**LYMPH NODE (LUMBAR):**
- Erythrocytosis/erythrophagocytosis, minimal
- Lymphoid hyperplasia, mild, (relates to necropsy finding)
- Macrophage accumulation, minimal, (with intracytoplasmic foreign material)

**PARATHYROID GLAND:**
- Tissue absent from section. No more available

**SKELETAL MUSCLE:**
- Inflammatory cell foci, minimal

**TESTIS:**
- Segmental hypoplasia, focal, mild

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**
- ADR, AOR, APP, BRA, CAE, COL, DUO, EPI, EYE, FEM, GBL, HEA, ILE, JEJ, LAC, LIV, LNIN, LNMA, LNMS, OES, OPT, PCEN, PCEX, PIT, PRO, REC, SGM, SCI, SEM, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, GALT, sac

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Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
### Appendix 20 (continued)

**Individual Necropsy and Histological Findings: Day 66**

| PROJECT NUMBER: | 520419 |
|-----------------|---------|
| TREATMENT:      | Group 3 (50 ug/dose) FEMALES |

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 16         | Terminal Kill |
|            | Day of Necropsy: 66 |

**Necropsy Findings:**
- **GENERAL COMMENTS:** Tissues not listed below were normal
- **LYMPH NODE (LUMBAR):** Discolouration, dark, left
  - Enlargement, left, (10 x 5 x 3 mm)
- **LYMPH NODE (MESENTERIC):** Discolouration, dark, red

**Histological Findings:**
- **ADRENAL GLAND:** Only one medulla examined
  - Macrophage accumulation, intramuscular, minimal, (with cytoplasmic foreign material and multinucleated giant cells)
  - Inflammation, polymorphonuclear leukocytic, moderate
  - Myofibre necrosis, mild
  - Regeneration, myofibre, minimal
  - Fibrosis, interstitial, mild
- **LIVER:** Inflammatory cell infiltration, periportal, minimal
- **LUNG:** Inflammatory cell foci, perivascular, mild
- **LYMPH NODE (LUMBAR):** Only one examined
  - Erythrocytosis/erythrophagocytosis, mild, (relates to necropsy finding discolouration dark)
  - Lymphoid hyperplasia, moderate, (relates to necropsy finding enlargement)
  - Macrophage accumulation, minimal, (with intracytoplasmic foreign material)

Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
Appendix 20  (continued)
Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 16         | (CONTINUED) |
| Mustard     | Erythrocytosis/erythrophagocytosis, minimal, (relates to necropsy finding) |
|            | Only one examined |
|            | Inflammatory cell foci, minimal |
|            | (CONTINUED) |
|            | ADR, AOR, APP, BRA, CAE, CER, COL, DUO, EYE, GBL, HEA, ILE, KID, LAC, LNIN, LNMA, MAM, OES, OPT, OVA, OVD, PCEN, PCEX, PAR, PIT, REC, SGSM, SCI, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, UTE, VAG, GALT, sac |

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 17         | Terminal Kill |
|            | Day of Necropsy: 66 |

| NECROPSY FINDINGS: | GENERAL COMMENTS: |
|--------------------|--------------------|
| LUNG:              | Tissues not listed below were normal |
|                    | Discolouration, all lobes, red, (mottled) |

| HISTOLOGICAL FINDINGS: | ADRENAL GLAND: |
|------------------------|---------------|
|                        | Diffuse cortical cell hypertrophy, mild |

Pathology File Ref.: MI_CLIS_520419_MAIN_LL_KEEP2.SPL
Appendix 20  (continued)
Individual Necropsy and Histological Findings: Day 66

PROJECT NUMBER: 520419
TREATMENT: Group 3 (50 ug/dose) FEMALES

ANIMAL NO: FINDINGS:

17  (CONTINUED)

HISTOLOGICAL FINDINGS:
INJECTION SITE 1: Macrophage accumulation, intramuscular, focal, minimal, (with cytoplasmic foreign material and multinucleated giant cells)
Inflammation, mononuclear cell, minimal, (with focal polymorphonuclear cells)
KIDNEY: Tubular mineralisation, medullary, minimal
Basophilic tubules, multifocal, minimal
LIVER: Oval cell hyperplasia, minimal
LUNG: Agonal congestion/haemorrhage, (relates to necropsy finding)
LYMPH NODE (INGUINAL): Only one examined
LYMPH NODE (LUMBAR): Tissue absent from section. No more available
PARATHYROID GLAND: Tissue absent from section. No more available
SKELETAL MUSCLE: Inflammatory cell foci, minimal
GUT ASSOCIATED LYMPHOID TISSUE: Inflammation, Peyer's patch, focal, minimal

ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
AOR, APP, BRA, CAE, CER, COL, DUO, EYE, FEM, GBL, HEA, ILE, JEJ, LAC, LUN, LNIN, LNMA, LNMS, MAM, OES, OPT, OVA, OVD, PCEN, PCEX, PIT, REC, SGS, SCI, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, UTE, VAG, sac

Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
## Appendix 20 (continued)
### Individual Necropsy and Histological Findings: Day 66

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 18         | Terminal Kill |
|            | Day of Necropsy: 66 |

**NECROPSY FINDINGS:**
- Tissues not listed below were normal
- Lung: Discolouration, dark, all lobes, red
- Oviduct: Cyst, right, clear, (one, 5 mm)

**HISTOLOGICAL FINDINGS:**
- Aorta: Mineralisation, medial, mild
- Injection Site 1: Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
- Inflammation, polymorphonuclear leukocytic, mild
- Regeneration, myofibre, minimal
- Fibrosis, interstitial, minimal
- Mineralisation, minimal
- Kidney: Tubular mineralisation, cortical, minimal
- Basophilic tubules, minimal
- Lung: Agonal congestion/haemorrhage, (relates to necropsy finding)
- Lymph Node (inguinal): Only one examined
- Lymph Node (lumbar): Erythrocytosis/erythrophagocytosis, minimal
- Macrophage accumulation, minimal, (with multinucleated cells and intracytoplasmic foreign material)
- Lymphoid hyperplasia, mild
- Oviduct: Cyst, (relates to necropsy finding)
- Parathyroid gland: Only one examined
- Pituitary gland: Posterior lobe not present
- Skeletal muscle: Inflammatory cell foci, minimal

(Continued)
## Appendix 20 (continued)
### Individual Necropsy and Histological Findings: Day 66

| PROJECT NUMBER: | 520419 |
|-----------------|--------|
| TREATMENT:      | Group 3 (50 ug/dose) FEMALES |
| ANIMAL NO:      | FINDINGS: |
| 18              | (CONTINUED) |

**HISTOLOGICAL FINDINGS:**

ORGANS EXAMINED AND NO ABNORMALITY DETECTED:

ADR, APP, BRA, CAE, CER, COL, DUO, EYE, FEM, GBL, HEA, ILE, JEJ, LAC, LIV, LUN, LNIN, LNMA, LNMS, MAM, OES, OPT, OVA, PCEN, PCEX, PAR, PIT, REC, SGS, SCI, SKI, SPN, SPL, STR, STO, THM, THR, TOG, TRA, URE, URB, UTE, VAG, GALT, sac

Pathology File Ref.: MICLIS_520419_MAIN_LL_KEEP2.SPL
Appendix 21
Individual Necropsy and Histological Findings: Day 92

Abbreviations Used

INJ1 = Injection Site 1
INJ2 = Injection Site 2
LNIN = Inguinal Lymph Node
LULU = Lumbar Lymph Node
### Appendix 21  (continued)
#### Individual Necropsy and Histological Findings: Day 92

| PROJECT NUMBER: | 520419 |
|-----------------|--------|
| TREATMENT:      | Group 1 (0 ug/dose) MALES |

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 19         | Recovery Kill |
|            | Day of Necropsy: 92 |

#### NECROPSY FINDINGS:
- **GENERAL COMMENTS:** Tissues not listed below were normal
- **ADRENAL GLAND:** Discolouration, both, dark, red
- **LUNG:** Discolouration, dark, all lobes, red
- **TRACHEA:** Fluid accumulation, pale, (frothy)

#### HISTOLOGICAL FINDINGS:
- **INJECTION SITE 1:** Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
- **LYMPH NODE (LUMBAR):** Only one examined
  - Macrophage accumulation, minimal, (with multinucleated cells and intracytoplasmic foreign material)

#### ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
- LNIN

| 20         | Recovery Kill |
|            | Day of Necropsy: 92 |

#### NECROPSY FINDINGS:
- **GENERAL COMMENTS:** Tissues not listed below were normal
- **LUNG:** Spongy, all lobes
  - Discolouration, all lobes, (mottled)
- **LYMPH NODE (LUMBAR):** Discolouration, both, dark, red
- **TRACHEA:** Fluid accumulation, pale, (froth filled)

(Continued)
Appendix 21  (continued)
Individual Necropsy and Histological Findings: Day 92

PROJECT NUMBER: 520419
TREATMENT: Group 1 (0 ug/dose) MALES

ANIMAL NO: FINDINGS:

20  (CONTINUED)

HISTOLOGICAL FINDINGS:
INJECTION SITE 1 : Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
LYMPH NODE (LUMBAR) : Erythrocytosis/erythropagocytosis, moderate, (relates to necropsy finding)
Macrophage accumulation, mild, (with multinucleated cells and intracytoplasmic foreign material)

ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
LNIN

21  Recovery Kill
Day of Necropsy: 92

NECROPSY FINDINGS:
GENERAL COMMENTS : All tissues normal

HISTOLOGICAL FINDINGS:
INJECTION SITE 1 : Macrophage accumulation, intramuscular, mild, (with cytoplasmic foreign material and multinucleated giant cells)
LYMPH NODE (LUMBAR) : Macrophage accumulation, mild, (with multinucleated cells and intracytoplasmic foreign material)

(CONTINUED)
## Appendix 21  (continued)
### Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 21 (CONTINUED) | |

**HISTOLOGICAL FINDINGS:**

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**

LNIN

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Pathology File Ref.: MICLIS_520419_BEC_11_KEEP2.SPL

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### Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 28        | Recovery Kill  
            | Day of Necropsy: 92 |

#### NECROPSY FINDINGS:
- **GENERAL COMMENTS**: Tissues not listed below were normal
- **LUNG**: Discolouration, all lobes, (mottled)
- **Spongy, all lobes**
- **TRACHEA**: Fluid accumulation, pale, (froth filled)

#### HISTOLOGICAL FINDINGS:
- **LYMPH NODE (LUMBAR)**: Macrophage accumulation, minimal, (with multinucleated cells and intracytoplasmic foreign material)

#### ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
- INJ1, LNIN

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 29        | Recovery Kill  
            | Day of Necropsy: 92 |

#### NECROPSY FINDINGS:
- **GENERAL COMMENTS**: Tissues not listed below were normal
- **LUNG**: Discolouration, all lobes, (mottled)
- **Spongy, all lobes**
- **TRACHEA**: Fluid accumulation, dark, red, (froth filled)

Pathology File Ref.: MICLIS_520419_REC_LL_KEEP2.SPL
## Appendix 21 (continued)
### Individual Necropsy and Histological Findings: Day 92

**PROJECT NUMBER:** 520419  
**TREATMENT:** Group 1 (0 ug/dose) FEMALES

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 29        | (CONTINUED) |
| **HISTOLOGICAL FINDINGS:** | |
| INJECTION SITE 1 : | Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells) |
| LYMPH NODE (LUMBAR) : | Tissue absent from section. No more available |
| **ORGANS EXAMINED AND NO ABNORMALITY DETECTED:** | |
| LNIN |

30  
Recovery Kill  
Day of Necropsy: 92  

**NECROPSY FINDINGS:**  
**GENERAL COMMENTS :** Tissues not listed below were normal  
**LUNG :** Discolouration, (mottled)  

**HISTOLOGICAL FINDINGS:**  
**LYMPH NODE (LUMBAR) :** Only one examined  

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**  
INJ1, LNIN, LNLU
## Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO | FINDINGS:                                      |
|-----------|-----------------------------------------------|
| 22        | Recovery Kill                                 |
|           | Day of Necropsy: 92                          |
|           | NECROPSY FINDINGS:                            |
|           | Tissues not listed below were normal          |
|           | LUNG : Discolouration, dark, all lobes, red   |
|           | LYMPH NODE (MANDIBULAR) :                     |
|           | Discolouration, dark, red                     |
| 23        | Recovery Kill                                 |
|           | Day of Necropsy: 92                          |
|           | NECROPSY FINDINGS:                            |
|           | Tissues not listed below were normal          |
|           | LUNG : Discolouration, dark, all lobes, red   |
|           | TRACHEA : Fluid accumulation, pale, (frothy)  |
| 24        | Recovery Kill                                 |
|           | Day of Necropsy: 92                          |
|           | NECROPSY FINDINGS:                            |
|           | Tissues not listed below were normal          |
|           | LUNG : Discolouration, dark, all lobes, red   |
### Appendix 21  (continued)

#### Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 31        | Recovery Kill Day of Necropsy: 92 |
| **Necropsy Findings:** | **General Comments:** |
| **Lung:** | Tissues not listed below were normal  |
|           | Spongy, all lobes  |
|           | Discolouration, all lobes, (mottled)  |
| **Oviduct:** | Cyst, right, clear, (one, 3 mm)  |
| **Trachea:** | Fluid accumulation, pale, (froth filled)  |
| 32        | Recovery Kill Day of Necropsy: 92 |
| **Necropsy Findings:** | **General Comments:** |
| **Lung:** | Tissues not listed below were normal  |
|           | Discolouration, all lobes, (mottled)  |
|           | Spongy, all lobes  |
| **Trachea:** | Fluid accumulation, dark, red, (froth filled)  |
| 33        | Recovery Kill Day of Necropsy: 92 |
| **Necropsy Findings:** | **General Comments:** |
| **Ovary:** | Tissues not listed below were normal  |
|           | Foci, dark, both, few, (2 mm)  |

Pathology File Ref.: MICLIS_520419_REC_LL_KEEP2.SPL
**Appendix 21 (continued)**

**Individual Necropsy and Histological Findings: Day 92**

| ANIMAL NO: | FINDINGS: |
|-------------|-----------|
| 25          | Recovery Kill  |
|             | Day of Necropsy: 92 |

**NECROPSY FINDINGS:**

**GENERAL COMMENTS:** Tissues not listed below were normal

**LUNG:** Discolouration, dark, all lobes, red

**LYMPH NODE (LUMBAR):** Discolouration, right, dark, red

**THYROID GLAND:** Small, right

**HISTOLOGICAL FINDINGS:**

**INJECTION SITE 1:**

- Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
- Fibrosis, interstitial, mild
- Regeneration, myofibre, minimal
- Inflammation, with necrosis, minimal

**LYMPH NODE (LUMBAR):**

- Erythrocytosis/erythrophagocytosis, mild, (relates to necropsy finding)
- Macrophage accumulation, minimal, (with multinucleated cells and intracytoplasmic foreign material)

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**

- LNIN
Appendix 21  (continued)
Individual Necropsy and Histological Findings: Day 92

PROJECT NUMBER: 520419
TREATMENT: Group 3 (50 ug/dose) MALES

ANIMAL NO: FINDINGS:

26  Recovery Kill
    Day of Necropsy: 92

NECROPSY FINDINGS:
GENERAL COMMENTS : Tissues not listed below were normal
LYMPH NODE (LUMBAR) : Discolouration, both, dark, red

HISTOLOGICAL FINDINGS:
LYMPH NODE (LUMBAR) : Erythrocytosis/erythrophagocytosis, mild, (relates to necropsy finding)

ORGANS EXAMINED AND NO ABNORMALITY DETECTED:
INJ1, LNIN

27  Recovery Kill
    Day of Necropsy: 92

NECROPSY FINDINGS:
GENERAL COMMENTS : Tissues not listed below were normal
LUNG : Spongy, all lobes
LYMPH NODE (INGUINAL) : Discolouration, dark, all lobes, red
LYMPH NODE (LUMBAR) : Discolouration, right, dark, red
TRACHEA : Fluid accumulation, dark

HISTOLOGICAL FINDINGS:
INJECTION SITE 1 :
    Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells)
    Fibrosis, interstitial, mild
    Inflammation, with necrosis, mild

Pathology File Ref.: MICLIS_520419_REC_LL_KEEP2.SPL
### Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 27 (CONTINUED) | |

**HISTOLOGICAL FINDINGS:**
- **INJECTION SITE 1:** Regeneration, myofibre, minimal
  - Myofibre necrosis, minimal
- **LYMPH NODE (INGUINAL):** No histological correlation with necropsy finding
- **LYMPH NODE (LUMBAR):** Erythrocytosis/erythrophagocytosis, minimal, (relates to necropsy finding)
  - Only one examined

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**
- INJ2, LNIN

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Pathology File Ref.: MICLIS_520419_REC_LL_KEEP2.SPL
### Appendix 21  (continued)
### Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 34        | Recovery Kill  
            | Day of Necropsy: 92 |

**NECROPSY FINDINGS:**

**GENERAL COMMENTS:** Tissues not listed below were normal.

**LUNG:** Discolouration, dark, all lobes, red. Spongy, all lobes.

**TRACHEA:** Fluid accumulation, pale, (froth filled).

**HISTOLOGICAL FINDINGS:**

**INJECTION SITE 1:** Macrophage accumulation, intramuscular, moderate, (with cytoplasmic foreign material and multinucleated giant cells).

Inflammation, with necrosis, mild.

Myofibre necrosis, minimal.

Regeneration, myofibre, minimal.

Fibrosis, interstitial, minimal.

**LYMPH NODE (LUMBAR):** Tissue absent from section. No more available.

**ORGANS EXAMINED AND NO ABNORMALITY DETECTED:**

LNIN

| ANIMAL NO | FINDINGS |
|-----------|----------|
| 35        | Recovery Kill  
            | Day of Necropsy: 92 |

**NECROPSY FINDINGS:**

**GENERAL COMMENTS:** Tissues not listed below were normal.

**LUNG:** Spongy, all lobes.

Discolouration, all lobes, (mottled).

(Continued)
### Individual Necropsy and Histological Findings: Day 92

| ANIMAL NO: | FINDINGS: |
|------------|-----------|
| 35         | (CONTINUED) |
|            | NECROPSY FINDINGS: |
|            | LYMPH NODE (MANDIBULAR): Discolouration, dark, red |
|            | TRACHEA: Fluid accumulation, pale, red, (frothy) |
|            | HISTOLOGICAL FINDINGS: |
|            | INJECTION SITE 1: Macrophage accumulation, intramuscular, minimal, (with cytoplasmic foreign material and multinucleated giant cells) Inflammation, mononuclear cell, minimal |
|            | LYMPH NODE (INGUINAL): Macrophage accumulation, minimal, (with cytoplasmic foreign material and multinucleated giant cells) |
|            | ORGANS EXAMINED AND NO ABNORMALITY DETECTED: LNLU |

36  
Recovery Kill  
Day of Necropsy: 92  

NECROPSY FINDINGS:  
GENERAL COMMENTS: All tissues normal  

HISTOLOGICAL FINDINGS:  
LYMPH NODE (LUMBAR): Tissue absent from section. No more available  

ORGANS EXAMINED AND NO ABNORMALITY DETECTED: INJ1, LNIN
### Appendix 22

#### Absolute Organ Weights (g) : Individual Values: Day 66

| Group / Test Item | Dosage (µg/dose) | 1  | 2  | 3  |
|-------------------|------------------|----|----|----|
| Control           | 0                |    |    |    |
| MenPF-1           | 25               |    |    |    |
| MenPF-1           | 50               |    |    |    |
| Body Weight (kg)  |                  |    |    |    |
| Adrenals          | 0.2527           | 10.77 | 2.0622 | 10.03 | 16.87 | 79.66 | 24.15 | 0.031 | 0.84 |
| Brain             | 0.2860           | 9.70 | 2.0353 | 7.78 | 18.64 | 88.53 | 20.96 | 0.029 | 1.51 |
| Epididymides      | 0.3589           | 10.11 | 2.3263 | 9.08 | 21.10 | 126.91 | 22.51 | -     | 0.56 |
| Heart             | 0.3187           | 9.49 | 2.6223 | 9.58 | 22.72 | 163.34 | 24.14 | 0.032 | 1.27 |
| Kidneys           | 0.3187           | 9.49 | 2.6223 | 9.58 | 22.72 | 163.34 | 24.14 | 0.032 | 1.27 |
| Liver             | 0.3416           | 9.94 | 2.8897 | 10.86 | 18.92 | 114.52 | 30.80 | 0.044 | 1.57 |
| Lung              | 0.1259           | 9.85 | 2.9247 | 9.32 | 20.67 | 132.93 | 23.03 | 0.027 | 1.05 |
| Pituitary         | 0.2550           | 9.75 | 2.1088 | 8.05 | 18.64 | 91.68 | 28.60 | 0.026 | 0.82 |
| Prostate          | 0.2526           | 9.54 | 2.3121 | 9.00 | 18.28 | 112.96 | 15.91 | 0.026 | 0.78 |

- Animal 3 - Pituitary gland weight not recorded in error at necropsy
- Animal 7 - Left adrenal gland lost at necropsy; excluded from statistical analysis
Appendix 22  (continued)
Absolute Organ Weights (g) : Individual Values: Day 66

| Group / sex | Animal No. | Spleen | Testes | Thymus | Thyroid |
|-------------|------------|--------|--------|--------|---------|
| 1M          | 1          | 1.00   | 3.43   | 3.03   | 0.346   |
|             | 2          | 0.95   | 3.87   | 3.55   | 0.530   |
|             | 3          | 1.21   | 5.90   | 2.02   | 0.341   |
| 2M          | 4          | 1.02   | 5.11   | 2.92   | 0.229   |
|             | 5          | 1.04   | 6.52   | 2.99   | 0.301   |
|             | 6          | 1.14   | 5.25   | 3.31   | 0.229   |
| 3M          | 7          | 1.35   | 4.72   | 4.23   | 0.281   |
|             | 8          | 1.40   | 5.85   | 3.47   | 0.261   |
|             | 9          | 1.14   | 6.10   | 2.89   | 0.392   |

Group Item:
- Control
- MenPF-1
Dosage (µg/dose):
- 0
- 25
- 50
### Appendix 22  (continued)

#### Absolute Organ Weights (g) : Individual Values: Day 66

| Group / sex | Animal No. | Body Weight (kg) | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen |
|-------------|------------|------------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|
| 1F          | 10         | 3.9              | 0.2162   | 10.39 | 8.11  | 22.07   | 143.53| 28.53| 0.350   | 0.056     | 1.45   |
|             | 11         | 4.1              | 0.2991   | 9.68  | 11.66 | 24.29   | 135.89| 27.33| 0.391   | 0.035     | 1.88   |
|             | 12         | 3.9              | 0.2307   | 10.81 | 9.46  | 19.03   | 112.30| 27.94| 0.399   | 0.026     | 2.28   |
| 2F          | 13         | 3.6              | 0.3718   | 10.06 | 8.75  | 20.37   | 101.47| 23.54| 0.355   | 0.039     | 1.71   |
|             | 14         | 3.7              | 0.3533   | 9.35  | 10.23 | 16.68   | 90.64 | 12.53| 0.391   | 0.030     | 1.58   |
|             | 15         | 4.4              | 0.3075   | 9.64  | 10.93 | 23.76   | 177.00| 28.46| 0.568   | 0.038     | 2.11   |
| 3F          | 16         | 3.2              | 0.3428   | 9.27  | 7.61  | 16.37   | 74.32 | 11.55| 0.415   | 0.042     | 1.78   |
|             | 17         | 4.1              | 0.2856   | 9.74  | 8.44  | 23.33   | 149.32| 19.58| 0.427   | 0.023     | 1.11   |
|             | 18         | 4.2              | 0.3600   | 9.71  | 11.23 | 27.39   | 166.01| 32.79| 0.671   | 0.029     | 1.27   |
## Appendix 22  (continued)
### Absolute Organ Weights (g) : Individual Values: Day 66

| Group / sex | Test Item | Dosage (µg/dose) | 1  | 2   | 3   |
|-------------|-----------|-----------------|----|-----|-----|
| 1F          | Control   | 0               | 2.61 | 0.437 | 8.76 |
| 11          | MenPF-1   | 25              | 4.01 | 0.273 | 8.15 |
| 12          | MenPF-1   | 50              | 2.68 | 0.378 | 7.25 |
| 2F          | Control   | 0               | 3.05 | 0.450 | 9.14 |
| 13          | MenPF-1   | 25              | 4.01 | 0.273 | 8.15 |
| 14          | MenPF-1   | 50              | 2.68 | 0.378 | 7.25 |
| 3F          | Control   | 0               | 2.74 | 0.428 | 7.09 |
| 16          | MenPF-1   | 25              | 4.01 | 0.273 | 8.15 |
| 17          | MenPF-1   | 50              | 2.68 | 0.378 | 7.25 |
| 18          | MenPF-1   | 120             | 5.11 | 0.427 | 9.60 |
## Appendix 23
### Absolute Organ Weights (g) : Individual Values: Day 92

| Group / sex | Test Item | Dosage (µg/dose) | 1   | 2   | 3   |
|------------|-----------|------------------|-----|-----|-----|
| 1M         | Control   | 0                | 3.8 | 0.2218 | 10.55 |
|            | MenPF-1   | 25               | 9.99 | 2.1469 | 19.05 |
|            | MenPF-1   | 50               | 20.95 | 108.62 | 25.63 |
| 2M         | Control   | 0                | 3.4 | 0.2673 | 9.91 |
|            | MenPF-1   | 25               | 1.9302 | 8.72 | 19.06 |
|            | MenPF-1   | 50               | 10.34 | 104.81 | 29.27 |
| 3M         | Control   | 0                | 3.3 | 0.2885 | 9.11 |
|            | MenPF-1   | 25               | 1.7122 | 8.62 | 16.32 |
|            | MenPF-1   | 50               | 9.01 | 109.57 | 23.60 |

| Group / sex | Animal No. | Body Weight (kg) | Adrenals | Brain | Epididy- mides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate |
|------------|------------|------------------|----------|-------|----------------|-------|---------|-------|------|-----------|----------|
| 1M         | 19         | 3.8              | 0.2218   | 10.55 | 2.1469        | 9.99  | 20.95   | 108.62| 25.63| 0.028     | 1.00     |
|            | 20         | 3.4              | 0.2673   | 9.91  | 1.9302        | 8.72  | 19.06   | 104.81| 29.27| 0.015     | 0.87     |
|            | 21         | 3.4              | 0.2975   | 9.86  | 2.5220        | 10.34 | 21.11   | 113.16| 15.30| 0.016     | 1.10     |
| 2M         | 22         | 3.7              | 0.3432   | 9.51  | 1.8618        | 9.98  | 22.64   | 147.33| 31.12| 0.024     | 0.80     |
|            | 23         | 3.4              | 0.2885   | 10.44 | 1.7122        | 8.62  | 16.32   | 109.57| 23.60| 0.021     | 0.92     |
|            | 24         | 3.3              | 0.3394   | 9.31  | 2.3734        | 9.01  | 14.90   | 89.90 | 31.27| 0.021     | 0.67     |
| 3M         | 25         | 3.3              | 0.2986   | 9.91  | 2.3776        | 8.18  | 16.26   | 102.19| 30.25| 0.014     | 0.88     |
|            | 26         | 3.0              | 0.4134   | 10.38 | 2.3246        | 8.64  | 16.38   | 77.62 | 12.40| 0.044     | 1.48     |
|            | 27         | 3.5              | 0.3338   | 10.22 | 1.6869        | 8.29  | 17.61   | 98.74 | 26.90| 0.032     | 0.82     |
### Appendix 23 (continued)

**Absolute Organ Weights (g) : Individual Values: Day 92**

| Group / sex | Animal No. | Spleen | Testes | Thymus | Thyroid |
|-------------|------------|--------|--------|--------|---------|
| 1M          | 19         | 0.62   | 6.28   | 4.44   | 0.222   |
|             | 20         | 0.93   | 5.76   | 3.25   | 0.176   |
|             | 21         | 1.47   | 7.02   | 2.62   | 0.357   |
| 2M          | 22         | 1.09   | 4.77   | 4.34   | 0.351   |
|             | 23         | 1.22   | 4.57   | 1.42   | 0.186   |
|             | 24         | 1.24   | 6.08   | 3.76   | 0.292   |
| 3M          | 25         | 1.45   | 5.16   | 2.91   | 0.207   |
|             | 26         | 1.19   | 5.80   | 1.77   | 0.231   |
|             | 27         | 0.81   | 5.30   | 3.52   | 0.184   |
### Appendix 23 (continued)
Absolute Organ Weights (g) : Individual Values: Day 92

| Group / Animal No. | No. | Dosage (µg/dose) | Test Item | Body Weight (kg) | Body Weight (kg) | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen |
|--------------------|-----|-----------------|-----------|-----------------|-----------------|----------|-------|-------|---------|-------|------|---------|-----------|-------|
| 1F 28               |     | 0               | Control   | 4.2             | 10.43           | 0.2788   | 8.63  | 19.75 | 115.85  | 35.29 | 0.400| 0.027   | 1.91      |       |
| 29                 |     | 25              | MenPF-1   | 4.3             | 9.84            | 0.2719   | 9.89  | 21.29 | 126.72  | 36.34 | 0.585| 0.034   | 1.48      |       |
| 30                 |     | 50              | MenPF-1   | 5.0             | 11.17           | 0.3647   | 8.90  | 25.45 | 145.41  | 21.54 | 0.490| 0.017   | 2.10      |       |
| 2F 31               |     | 0               | Control   | 3.9             | 8.79            | 0.2977   | 8.75  | 17.10 | 103.43  | 19.49 | 0.269| 0.031   | 2.11      |       |
| 32                 |     | 25              | MenPF-1   | 4.2             | 9.54            | 0.2550   | 8.88  | 20.04 | 94.36   | 28.78 | 0.379| 0.019   | 1.18      |       |
| 33                 |     | 50              | MenPF-1   | 3.5             | 10.15           | 0.2172   | 8.21  | 17.61 | 87.88   | 19.79 | 0.536| 0.042   | 2.13      |       |
| 3F 34               |     | 0               | Control   | 4.2             | 8.66            | 0.3504   | 11.87 | 18.50 | 139.43  | 37.57 | 0.462| 0.004   | 1.17      |       |
| 35                 |     | 25              | MenPF-1   | 4.2             | 10.18           | 0.2493   | 9.82  | 20.89 | 130.71  | 32.19 | 0.743| 0.028   | 1.55      |       |
| 36                 |     | 50              | MenPF-1   | 4.6             | 9.57            | 0.3062   | 9.90  | 22.21 | 149.16  | 20.95 | 0.409| 0.031   | 1.22      |       |
### Appendix 23 (continued)
### Absolute Organ Weights (g) : Individual Values: Day 92

| Group / sex | Animal No. | Thymus (g) | Thyroid (g) | Uterus (g) |
|-------------|------------|------------|-------------|------------|
| 1F          | 28         | 3.95       | 0.270       | 6.80       |
|             | 29         | 3.66       | 0.390       | 10.62      |
|             | 30         | 5.88       | 0.402       | 10.32      |
| 2F          | 31         | 2.39       | 0.350       | 7.60       |
|             | 32         | 3.87       | 0.431       | 10.09      |
|             | 33         | 1.93       | 0.269       | 11.40      |
| 3F          | 34         | 3.08       | 0.416       | 9.15       |
|             | 35         | 2.60       | 0.240       | 11.85      |
|             | 36         | 3.56       | 0.454       | 8.24       |
## Appendix 24
### Relative Organ Weights (% Body Weights) : Individual Values: Day 66

| Group / sex | Animal No. | Adrenals | Brain | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate | Spleen |
|-------------|------------|----------|-------|--------------|-------|---------|-------|------|-----------|----------|--------|
| 1M          | 1          | 0.0082   | 0.347 | 0.06652      | 0.324 | 0.544   | 2.570 | 0.779| 0.0010    | 0.027    | 0.032  |
|             | 2          | 0.0087   | 0.294 | 0.06168      | 0.236 | 0.565   | 2.683 | 0.635| 0.0009    | 0.046    | 0.029  |
|             | 3          | 0.0112   | 0.316 | 0.07270      | 0.284 | 0.659   | 3.966 | 0.703| 0.018     | 0.018    | 0.038  |
| 2M          | 4          | 0.0078   | 0.278 | 0.05435      | 0.260 | 0.685   | 4.078 | 0.817| 0.0011    | 0.030    | 0.029  |
|             | 5          | 0.0097   | 0.288 | 0.07946      | 0.290 | 0.688   | 4.950 | 0.732| 0.0010    | 0.038    | 0.032  |
|             | 6          | 0.0101   | 0.292 | 0.08499      | 0.319 | 0.556   | 3.368 | 0.906| 0.0013    | 0.046    | 0.034  |
| 3M          | 7          | 0.0035   | 0.274 | 0.08124      | 0.259 | 0.574   | 3.693 | 0.640| 0.0008    | 0.029    | 0.038  |
|             | 8          | 0.0080   | 0.305 | 0.06590      | 0.252 | 0.583   | 2.865 | 0.894| 0.0008    | 0.026    | 0.044  |
|             | 9          | 0.0074   | 0.281 | 0.06800      | 0.265 | 0.538   | 3.322 | 0.468| 0.0008    | 0.023    | 0.034  |

Animal 3 - Pituitary gland weight not recorded in error at necropsy
Animal 7 - Left adrenal gland lost at necropsy; excluded from statistical analysis
| Group / sex | Animal No. | Testes | Thymus | Thyroid |
|-------------|------------|--------|--------|---------|
| 1M          | 1          | 0.111  | 0.098  | 0.0112  |
|             | 2          | 0.117  | 0.108  | 0.0161  |
|             | 3          | 0.184  | 0.063  | 0.0107  |
| 2M          | 4          | 0.146  | 0.083  | 0.0065  |
|             | 5          | 0.198  | 0.091  | 0.0091  |
|             | 6          | 0.154  | 0.097  | 0.0067  |
| 3M          | 7          | 0.131  | 0.118  | 0.0078  |
|             | 8          | 0.183  | 0.108  | 0.0082  |
|             | 9          | 0.179  | 0.085  | 0.0115  |
### Appendix 24 (continued)

**Relative Organ Weights (% Body Weights): Individual Values: Day 66**

| Group / Animal No. | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen | Thymus |
|--------------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|--------|
| 1F 10              | 0.0055   | 0.266 | 0.208 | 0.566   | 3.680 | 0.732| 0.0090  | 0.0014    | 0.037  | 0.067  |
| 11                 | 0.0073   | 0.236 | 0.284 | 0.592   | 3.314 | 0.667| 0.0095  | 0.0009    | 0.046  | 0.098  |
| 12                 | 0.0059   | 0.277 | 0.243 | 0.488   | 2.879 | 0.716| 0.0102  | 0.0007    | 0.058  | 0.069  |
| 2F 13              | 0.0103   | 0.279 | 0.243 | 0.566   | 2.819 | 0.654| 0.0099  | 0.0011    | 0.048  | 0.085  |
| 14                 | 0.0096   | 0.253 | 0.276 | 0.451   | 2.450 | 0.339| 0.0106  | 0.0008    | 0.043  | 0.087  |
| 15                 | 0.0070   | 0.219 | 0.248 | 0.540   | 4.023 | 0.647| 0.0129  | 0.0009    | 0.048  | 0.099  |
| 3F 16              | 0.0107   | 0.290 | 0.238 | 0.512   | 2.323 | 0.361| 0.0130  | 0.0013    | 0.056  | 0.086  |
| 17                 | 0.0070   | 0.238 | 0.206 | 0.569   | 3.642 | 0.478| 0.0104  | 0.0006    | 0.027  | 0.073  |
| 18                 | 0.0086   | 0.231 | 0.267 | 0.652   | 3.953 | 0.781| 0.0160  | 0.0007    | 0.030  | 0.122  |
## Appendix 24 (continued)
### Relative Organ Weights (% Body Weights) : Individual Values: Day 66

| Group / sex | Animal No. | Thyroid | Uterus |
|-------------|------------|---------|--------|
| 1F | 10 | 0.0112 | 0.225 |
|     | 11 | 0.0067 | 0.199 |
|     | 12 | 0.0097 | 0.186 |
| 2F | 13 | 0.0125 | 0.254 |
|     | 14 | 0.0124 | 0.326 |
|     | 15 | 0.0084 | 0.219 |
| 3F | 16 | 0.0134 | 0.222 |
|     | 17 | 0.0086 | 0.205 |
|     | 18 | 0.0102 | 0.229 |
## Appendix 25
Relative Organ Weights (% Body Weights) : Individual Values: Day 92

| Group / Sex | Animal No. | Adrenals | Brain | Epididymides | Heart | Kidneys | Liver | Lung | Pituitary | Prostate | Spleen |
|-------------|------------|----------|-------|--------------|-------|---------|-------|------|-----------|----------|--------|
| 1M          | 19         | 0.0058   | 0.278 | 0.05650      | 0.263 | 0.551   | 2.858 | 0.674| 0.0007    | 0.026    | 0.016  |
|             | 20         | 0.0079   | 0.291 | 0.05677      | 0.256 | 0.561   | 3.083 | 0.861| 0.0004    | 0.026    | 0.027  |
|             | 21         | 0.0088   | 0.290 | 0.07418      | 0.304 | 0.621   | 3.328 | 0.450| 0.0005    | 0.032    | 0.043  |
| 2M          | 22         | 0.0093   | 0.257 | 0.05032      | 0.270 | 0.612   | 3.982 | 0.841| 0.0006    | 0.022    | 0.029  |
|             | 23         | 0.0085   | 0.307 | 0.05036      | 0.254 | 0.480   | 3.223 | 0.694| 0.0006    | 0.027    | 0.036  |
|             | 24         | 0.0103   | 0.282 | 0.07192      | 0.273 | 0.452   | 2.724 | 0.948| 0.0006    | 0.020    | 0.038  |
| 3M          | 25         | 0.0091   | 0.300 | 0.07205      | 0.248 | 0.493   | 3.097 | 0.917| 0.0004    | 0.027    | 0.044  |
|             | 26         | 0.0138   | 0.346 | 0.07749      | 0.288 | 0.546   | 2.587 | 0.413| 0.0015    | 0.049    | 0.040  |
|             | 27         | 0.0095   | 0.292 | 0.04820      | 0.237 | 0.503   | 2.821 | 0.769| 0.0009    | 0.023    | 0.023  |
Appendix 25  (continued)
Relative Organ Weights (% Body Weights) : Individual Values: Day 92

| Group / sex | Animal No. | Testes  | Thymus  | Thyroid |
|-------------|------------|---------|---------|---------|
| 1M          | 19         | 0.165   | 0.117   | 0.0058  |
|             | 20         | 0.169   | 0.096   | 0.0052  |
|             | 21         | 0.206   | 0.077   | 0.0105  |
| 2M          | 22         | 0.129   | 0.117   | 0.0095  |
|             | 23         | 0.134   | 0.042   | 0.0055  |
|             | 24         | 0.184   | 0.114   | 0.0088  |
| 3M          | 25         | 0.156   | 0.088   | 0.0063  |
|             | 26         | 0.193   | 0.059   | 0.0077  |
|             | 27         | 0.151   | 0.101   | 0.0053  |
## Appendix 25 (continued)
### Relative Organ Weights (% Body Weights) : Individual Values: Day 92

| Group / Animal sex | Adrenals | Brain | Heart | Kidneys | Liver | Lung | Ovaries | Pituitary | Spleen | Thymus |
|--------------------|----------|-------|-------|---------|-------|------|---------|-----------|--------|--------|
| 1F                 | 28       | 0.0066| 0.248 | 0.205   | 0.470 | 2.758| 0.840   | 0.0095    | 0.0006 | 0.045  | 0.094  |
|                    | 29       | 0.0063| 0.229 | 0.230   | 0.495 | 2.947| 0.845   | 0.0136    | 0.0008 | 0.034  | 0.085  |
|                    | 30       | 0.0073| 0.178 | 0.223   | 0.509 | 2.908| 0.431   | 0.0098    | 0.0003 | 0.042  | 0.118  |
| 2F                 | 31       | 0.0076| 0.225 | 0.224   | 0.438 | 2.652| 0.500   | 0.0069    | 0.0008 | 0.054  | 0.061  |
|                    | 32       | 0.0061| 0.227 | 0.211   | 0.477 | 2.247| 0.685   | 0.0090    | 0.0005 | 0.028  | 0.092  |
|                    | 33       | 0.0062| 0.290 | 0.235   | 0.503 | 2.511| 0.565   | 0.0153    | 0.0012 | 0.061  | 0.055  |
| 3F                 | 34       | 0.0083| 0.206 | 0.283   | 0.440 | 3.320| 0.895   | 0.0110    | 0.0001 | 0.028  | 0.073  |
|                    | 35       | 0.0059| 0.242 | 0.234   | 0.497 | 3.112| 0.766   | 0.0177    | 0.0007 | 0.037  | 0.062  |
|                    | 36       | 0.0067| 0.208 | 0.215   | 0.483 | 3.243| 0.455   | 0.0089    | 0.0007 | 0.027  | 0.077  |
Relative Organ Weights (% Body Weights) : Individual Values: Day 92

| Group / sex | Animal No | Thyroid | Uterus |
|-------------|-----------|---------|--------|
| 1F          | 28        | 0.0064  | 0.162  |
|             | 29        | 0.0091  | 0.247  |
|             | 30        | 0.0080  | 0.206  |
| 2F          | 31        | 0.0090  | 0.195  |
|             | 32        | 0.0103  | 0.240  |
|             | 33        | 0.0077  | 0.326  |
| 3F          | 34        | 0.0099  | 0.218  |
|             | 35        | 0.0057  | 0.282  |
|             | 36        | 0.0099  | 0.179  |
FINAL REPORT

Study Phase: Pathology

Test Facility Study No. 520419

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

SPONSOR:
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Final Pathology Report

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1. RESPONSIBLE PERSONNEL

Study Pathologist: Lise Bertrand, DVM, MSc, DESV, Dipl ECVP
Charles River, Edinburgh, UK

2. SUMMARY

2.1. Main Study (Day 66)

Intramuscular administration of MenPF-1 vaccine to rabbits on 4 occasions at 50 µg/dose resulted in the accumulation of foreign material-laden macrophages and giant cells at the injection site for all animals. Polymorphonuclear inflammation was noted in 2/3 males and 2/3 females, and mononuclear inflammation was noted in 1/3 males and 1/3 females. Myofibre necrosis and/or regeneration, interstitial fibrosis and/or mineralisation were also observed in treated injection sites.

Lumbar lymph node enlargement was observed at necropsy in 2/3 males and 1/3 females, with corresponding lymphoid hyperplasia. Accumulation of foreign material-laden macrophages and giant cells was noted in the lumbar lymph nodes of 2/3 males and 2/2 females.

2.2. Recovery Study (Day 92)

After a 4 week recovery period, a number of findings persisted in treated injection sites (inflammation with or without necrosis, myofibre necrosis and/or regeneration, and/or interstitial fibrosis).

Accumulation of foreign material-laden macrophages and giant cells was noted in the lumbar and inguinal lymph nodes of 1/3 males and 1/3 females, respectively.

3. INTRODUCTION

This report presents the pathology findings in rabbits assigned to the study entitled A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period (Study No. 520419). The objective of this study was to determine the potential toxicity of MenPF-1 Vaccine, a prophylactic vaccine for the prevention of infection from bacterial meningitis, when given by intramuscular injection for 4 occasions over a 9 week period to rabbits, to evaluate the potential reversibility of any findings, and to provide data to support the use of MenPF-1 in humans.

The study was sponsored by Oxford Vaccine Group, UK where Andrew J Pollard, FRCPCH, PhD, served as the Sponsor representative. Bruce Robertson, BSc, Charles River, Edinburgh, UK served as the Study Director.
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4. MATERIALS AND METHODS

Experimental procedures applicable to pathology investigations are summarised in Text Table 1 and Text Table 2.

### Text Table 1
**Experimental Design**

| Group Number | Number of Animals | Dosage (µg/dose) | Conc. (µg/mL) | Dose Volume (mL/dose) |
|--------------|------------------|-----------------|---------------|-----------------------|
| M | F | M | F | Test Item | | |
| 1 | 3 | 3 | 3 | 3 | MOX Control | 0 | 0 | 0.5 mL |
| 2 | 3 | 3 | 3 | 3 | MenPF-1 | 25 | 50 | 0.5 mL |
| 3 | 3 | 3 | 3 | 3 | MenPF-1 | 50 | 50 | 2 x 0.5 mL |

All animals were submitted for necropsy on Day 66 (Scheduled Euthanasia (Day 66) / Main Study) or Day 92 (Scheduled Euthanasia (Day 92) / Recovery Study). Necropsies were performed and organ weights were collected by Charles River, Edinburgh personnel. Except as noted in Text Table 2 tissues were collected in 10% neutral buffered formalin.

### Text Table 2
**Tissue Collection and Examination**

| Tissue | Weigh | Collect | Microscopic Evaluation | Comment |
|--------|-------|---------|------------------------|---------|
| Administration site | X | X | | Injection Site 1 and/or 2 (as appropriate) with additional muscle. |
| Animal identification | - | X | - | - |
| Artery, aorta | - | X | X | From thoracic segment. |
| Bone marrow smear | - | X | - | One bone marrow smear was collected from the femur (for possible examination). Bone marrow smears were allowed to air dry and were not fixed in formalin. |
| Bone marrow, femur | - | X | X | Collected with bone, femur |
| Bone marrow, sternum | - | X | X | Collected with bone, sternum |
| Bone, femur with articulating surface | - | X | X | Distal end to include femoral tibial joint. |
| Bone, sternum | - | X | X | |
| Brain | X | X | X | Forebrain, midbrain, cerebellum, and medulla oblongata. |
| Cervix | - | X | X | Collected with uterus. |
| Epididymis | X | X | X | Separate weights and examination. |
| Eye | X | X | X | Separate examination; Preserved in Davidson's fixative. |
| Gallbladder | - | X | X | |
| Gland, adrenal | X | X | X | Separate weights and examination. |
| Gland, testicular | - | X | X | Only 1 required for examination. |
| Gland, mammary | - | X | X | Collected with thoracic skin and included nipple; mammary gland was examined in females only. |
| Gland, parathyroid | - | X | X | Collected with thyroid; Examined only if present in the routine section of thyroid. |
| Gland, pituitary | X | X | X | |
| Gland, prostate | X | X | X | |
| Tissue                   | Weigh | Collect | Microscopic Evaluation | Comment                                                                                           |
|-------------------------|-------|---------|------------------------|----------------------------------------------------------------------------------------------------|
| Gland, salivary         | -     | X       | X                      | Submandibular: Only 1 required for examination.                                                      |
| Gland, seminal vesicle  | -     | X       | X                      |                                                                                                    |
| Gland, thyroid          | X     | X       | X                      | Separate weights and examination: weight included parathyroid.                                       |
| Gross lesions/masses    | -     | X       |                        |                                                                                                    |
| Gut-associated lymphoid tissue | -   | X       | X                      | Collected with small intestine.                                                                     |
| Heart                   | X     | X       | X                      |                                                                                                    |
| Kidney                  | X     | X       | X                      | Separate weights and examination.                                                                  |
| Large intestine, appendix | -   | X       | X                      |                                                                                                    |
| Large intestine, caecum  | -     | X       | X                      |                                                                                                    |
| Large intestine, colon   | -     | X       | X                      |                                                                                                    |
| Large intestine, rectum  | -     | X       | X                      |                                                                                                    |
| Large intestine, saccus rotundus | - | X       | X                      |                                                                                                    |
| Liver                   | X     | X       | X                      | Gallbladder drained before weighing.                                                                |
| Lung                    | X     | X       | X                      | Infused with 10% neutral buffered formalin after weighing.                                           |
| Lymph node, mandibular  | -     | X       | X                      | Only 1 required for examination.                                                                    |
| Lymph node, mesenteric  | -     | X       | X                      |                                                                                                    |
| Lymph node, lumbar      | -     | X       | X                      |                                                                                                    |
| Lymph node, inguinal    | -     | X       | X                      |                                                                                                    |
| Muscle, skeletal        | -     | X       | X                      | From thigh                                                                                         |
| Nerve, optic            | -     | X       | X                      | Preserved in Davidson’s fixative: Examined only if present in the routine section of the eye.       |
| Nerve, sciatric         | -     | X       | X                      | Only 1 required for examination.                                                                    |
| Oesophagus              | -     | X       | X                      |                                                                                                    |
| Ovary                   | X     | X       | X                      | Separate weights and examination.                                                                  |
| Oviduct                 | -     | X       | X                      | Only 1 required for examination. Collected with uterus.                                             |
| Pancreas                | -     | X       | X                      |                                                                                                    |
| Skin                    | -     | X       | X                      |                                                                                                    |
| Small intestine, duodenum | -   | X       | X                      | Collected with mammary gland.                                                                     |
| Small intestine, ileum   | -     | X       | X                      |                                                                                                    |
| Small intestine, jejunum | -   | X       | X                      |                                                                                                    |
| Spinal cord             | -     | X       | X                      | Cervical, thoracic, lumbar.                                                                        |
| Spleen                  | X     | X       | X                      |                                                                                                    |
| Stomach                 | -     | X       | X                      | Fundus and pylorus                                                                                  |
| Testis                  | X     | X       | X                      | Separate weights and examination: Preserved in Modified Davidson’s fixative.                       |
| Thymus                  | X     | X       | X                      |                                                                                                    |
| Tongue                  | -     | X       | X                      |                                                                                                    |
| Trachea                 | -     | X       | X                      |                                                                                                    |
| Urater                  | -     | X       | X                      | Only 1 required for examination.                                                                   |
| Urinary bladder         | -     | X       | X                      |                                                                                                    |
| Uterus                  | X     | X       | X                      |                                                                                                    |
| Vagina                  | -     | X       | X                      |                                                                                                    |

X = procedure conducted; - = not applicable.

Tissues required for microscopic evaluation were trimmed, processed routinely, embedded in paraffin, cut 4-6 μm thick, mounted on glass slides, and stained with hematoxylin and eosin (H&E) by Charles River, Edinburgh personnel. Microscopic evaluation was conducted by
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the undersigned Board-certified Veterinary Pathologist on all protocol-specified tissues from Main Study animals in Groups 1 and 3; and on injection site and lumbar and inguinal lymph nodes from Recovery animals in Groups 1 and 3.

Tissues were evaluated by light microscopy, and the results were entered directly into a validated pathology computer program (PLACES 2000, Instem) for preparation of data tables.

4.1.  Peer Review

All tissues from Animals 7, 9, 17 and 18; and all injection sites from Animals 19-21, 25-30, and 34-36 were examined by a second pathologist. Any differences in recording, grading or description of the findings were discussed by the Study Pathologist and Peer Reviewing Pathologist. The data in this report reflect the consensus view of the Study Pathologist and Reviewing Pathologist.

4.2.  Computerized Systems

The data described in this report were generated by direct computer entry using PLACES 2000 Software version 1 supplied by Instem.

The files referred to in this report are listed below:

PLAFOR_520419_MACMAIN_LL_KEEP2
PLAFOR_520419_MACREC_LL_KEEP1
PLAFOR_520419_MICMAIN_LBE_KEEP1
PLAFOR_520419_MICREC_LBE_KEEP1

5.  RESULTS AND DISCUSSIONS

5.1.  Gross Pathology

5.1.1.  Scheduled Euthanasia (Day 66)

Test article-related gross pathology findings are summarised in Text Table 3.

| Group                        | Males | Females |
|------------------------------|-------|---------|
| Dose (μg/dose)               |       |         |
| No. animals examined         | 1     | 2       |
|                              | 2     | 3       |
|                              | 3     | 3       |
| Lumbar lymph node (No. examined) | 3     | 3       |
| Enlargement, left            | 2     | 2       |

Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1 vaccine.
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5.1.2.  Scheduled Euthanasia (Day 92)
Test article-related gross findings noted at the terminal euthanasia were not observed at the end of the recovery period. Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1 vaccine.

5.2.  Organ Weights
No test article-related organ weight changes were noted at the end of the treatment and recovery periods. There were isolated organ weight values that were statistically different from their respective controls. However, no patterns, trends, or correlating data to suggest these values were toxicologically relevant. Thus, the organ weight differences observed were considered incidental and unrelated to administration of MenPF1 vaccine.

5.3.  Histopathology
5.3.1.  Scheduled Euthanasia (Day 66)
Test article-related microscopic findings are summarised in Text Table 4.
The accumulation of macrophages, observed both in the injection sites and lumbar lymph nodes, was characterised by aggregates of macrophages containing an abundant, pale basophilic, amorphous cytoplasmic material considered to be aluminium hydroxide. These macrophages were admixed with variable numbers of multinucleated giant cells.

The lymphoid hyperplasia correlated with the enlarged lumbar lymph nodes observed at necropsy.

Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1.
Appendix 26  (continued)
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A number of changes were observed in the clinical chemistry, haematology and coagulation group mean values, when compared to their respective controls: there were increased total proteins and globulins, and decreased albumin/globulin ratio in all treated males and in females given 50 μg/dose; increased neutrophil counts in males given 50 μg/dose; increased monocyte counts in all treated male groups; and increased fibrinogen in treated groups from both sexes. These differences correlated with the inflammatory reaction observed in the injection sites.

5.3.2.  Scheduled Euthanasia (Day 92)

Some of the microscopic findings noted at the terminal euthanasia were observed at the end of the period off dose (Day 92) and are summarised in Text Table 5.

| Text Table 5  | Summary Microscopic Findings – Scheduled Euthanasia (Day 92) |
|--------------|---------------------------------------------------------------|
| Group        | Males | 1 | 2 | 3 | Females | 1 | 2 | 3 |
| Dose (μg/dose) | 0 | 50 | 0 | 50 |
| No. animals examined | 3 | 3 | 3 | 3 |
| Injection site I (No. Examined) | | | | | | | | |
| Macrophage accumulation, intramuscular | (3) | (2) | (1) | (2) |
| Minimal | 0 | 0 | 0 | 1 |
| Mild | 1 | 0 | 0 | 0 |
| Moderate | 2 | 2 | 1 | 1 |
| Inflammation, with necrosis | (0) | (2) | (0) | (1) |
| Minimal | 0 | 1 | 0 | 0 |
| Mild | 0 | 1 | 0 | 1 |
| Inflammation, mononuclear cell | (0) | (0) | (0) | (1) |
| Minimal | 0 | 0 | 0 | 1 |
| Myofibre necrosis | (0) | (1) | (0) | (1) |
| Minimal | 0 | 1 | 0 | 0 |
| Myofibre regeneration | (0) | (2) | (0) | (1) |
| Minimal | 0 | 2 | 0 | 1 |
| Fibrosis, interstitial | (0) | (2) | (0) | (1) |
| Minimal | 0 | 0 | 0 | 1 |
| Mild | 0 | 2 | 0 | 0 |
| Inguinal lymph node (No. Examined) | 3 | 3 | 3 | 3 |
| Macrophage accumulation | (0) | (0) | (0) | (1) |
| Minimal | 0 | 0 | 0 | 1 |
| Lumbar lymph node (No. Examined) | 3 | 3 | 2 | 1 |
| Macrophage accumulation | (3) | (1) | (1) | (0) |
| Minimal | 1 | 1 | 1 | 0 |
| Mild | 2 | 0 | 0 | 0 |

* Numbers in parentheses represent the number of animals with the finding.

No treatment related findings were noted in Injection Site 2 (Animal 27).

Other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbit, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of MenPF-1.
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6. CONCLUSIONS

Intramuscular administration of MenPF-1 vaccine to rabbits on 4 occasions at 50 µg/dose resulted in the accumulation of foreign material-laden macrophages and giant cells at the injection site for all animals. Polymorphonuclear inflammation was noted in 2/3 males and 2/3 females, and mononuclear inflammation was noted in 1/3 males and 1/3 females. Myofibre necrosis and/or regeneration, interstitial fibrosis and/or mineralisation were also observed in treated injection sites.

Lumbar lymph node enlargement was observed at necropsy in 2/3 males and 1/3 females, with corresponding lymphoid hyperplasia. Accumulation of foreign material-laden macrophages and giant cells was noted in the lumbar lymph nodes of 2/3 males and 2/2 females.

After a 4 week recovery period, a number of findings persisted in treated injection sites (inflammation with or without necrosis, myofibre necrosis and/or regeneration, interstitial fibrosis).

Accumulation of foreign material-laden macrophages and giant cells was noted in the lumbar and inguinal lymph nodes of 1/3 males and 1/3 females, respectively.
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7. REPORT APPROVAL

Date: 09 FEB 2012

Lise Bertrand, DVM, MSc, DESV, Dipl ECVP
Study Pathologist
Charles River, Edinburgh, UK
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PEER-REVIEW CERTIFICATE
CHARLES RIVER STUDY NO. 520419

A 9 Week Study of MenPF-1 Vaccine by Intramuscular Injection in Rabbits with a 4 Week Recovery Period

EXPERIMENTAL DESIGN: MenPF-1 Vaccine is a prophylactic vaccine for the prevention of infection from bacterial meningitis. The objective of this study was to determine the potential toxicity of MenPF-1 Vaccine when given by intramuscular injection for 4 occasions over a 9 week period to rabbits to evaluate the potential reversibility of any findings.

PURPOSE: The purpose of this peer review was to assess the overall quality and consistency of the microscopic data and determine the validity of the study pathologist’s conclusions. The peer review for this study was conducted in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as accepted by Regulatory Authorities throughout the European Community, United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI).

METHODS:
1. Review of all tissues from animal numbers: 7 and 9 (Group 3 males), and 17 and 18 (Group 3 females).
2. Review of injection site 1 from all recovery animals.
3. Following review of the histologic sections and corresponding histopathology-related study data, findings were discussed with the study pathologist.

RESULTS:
Slides examined were of good quality with minimal artefacts (e.g. occasional minor folding, chattering, cracking during processing: occasional bone fragments from necropsy).

Any differences of opinion were resolved and mutual agreement on terminology and diagnoses were achieved. The histopathology tables and corresponding narrative contained in the pathology report reflect diagnoses and conclusions agreed to by the peer reviewer and study pathologist. No further action is recommended.

PRP Signature
PRP Petrina Rogerson. BMVS MRCVS

Date
16 Dec 2011

SP Signature
SP Lise Bertrand, DVM MSc DESV Dipl ECVP

Date
6 Dec 2011

cc J-Drive
Study File (Original)

PAT/198
(Revised 1 July 2011)