charles river

FINAL REPORT

Test Facility Study No. 9800399

ZIKA mRNA: Mammalian Erythrocyte Micronucleus Test in Rat

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QUALITY ASSURANCE STATEMENT

Study Number: 9800399

This Study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows:

QA INSPECTION DATES

		Dates Findings Submitted to:	
Date(s) of Audit	Phase(s) Audited	Study Director	Study Director Management
14-Oct-2016	Final Study Plan	21-Oct-2016	21-Oct-2016
21-Oct-2016	Study Plan Amendment 1	21-Oct-2016	21-Oct-2016
21-Oct-2016	Study Plan Amendment 2	21-Oct-2016	21-Oct-2016
21-Oct-2016	Study Plan Amendment 3	21-Oct-2016	21-Oct-2016
26-Oct-2016	Study Plan Amendment 4	26-Oct-2016	26-Oct-2016
08-Nov-2016	Study Plan Amendment 5	08-Nov-2016	08-Nov-2016
08-Nov-2016	Study Plan Amendment 6	08-Nov-2016	08-Nov-2016
08-Nov-2016	Dose Preparation	08-Nov-2016	08-Nov-2016
08-Nov-2016	Blood Collection	08-Nov-2016	08-Nov-2016
09-Nov-2016	Necropsy	09-Jan-2017	09-Jan-2017
16-Dec-2016	Data Review - Analytical Chemistry	17-Dec-2016	17-Dec-2016
16-Dec-2016	Draft Phase Report - Dose Formulation Analysis	17-Dec-2016	17-Dec-2016
05-Jan-2017	Immunology	05-Jan-2017	05-Jan-2017
05-Jan-2017 - 06-Jan-2017 09-Jan-2017	Report Preparation	10-Jan-2017	10-Jan-2017
06-Jan-2017	Data Review - Animal Care	10-Jan-2017	10-Jan-2017
06-Jan-2017	Data Review - Veterinary Services	10-Jan-2017	10-Jan-2017
06-Jan-2017 09-Jan-2017	Data Review - Formulations	10-Jan-2017	10-Jan-2017
06-Jan-2017 09-Jan-2017	Data Review - Technical Operations	10-Jan-2017	10-Jan-2017
06-Jan-2017	Data Review - Clinical Pathology	10-Jan-2017	10-Jan-2017
06-Jan-2017 09-Jan-2017	Data Review - Necropsy	10-Jan-2017	10-Jan-2017
06-Jan-2017 09-Jan-2017	Data Review - In Vitro Sciences	10-Jan-2017	10-Jan-2017
06-Jan-2017 09-Jan-2017	Draft Phase Report - Deviation Log	10-Jan-2017	10-Jan-2017
06-Jan-2017 09-Jan-2017	Draft Report	10-Jan-2017	10-Jan-2017
09-Jan-2017	Study Plan Amendment 7	10-Jan-2017	10-Jan-2017
23-Jan-2017 - 24-Jan-2017	Data Review - Bioanalysis & Immunology	27-Jan-2017	27-Jan-2017
23-Jan-2017 - 24-Jan-2017	Report Preparation	27-Jan-2017	27-Jan-2017

QUALITY ASSURANCE STATEMENT - Study Number: 9800399

QA INSPECTION DATES

Date(s) of Audit	Phase(s) Audited	Dates Findings Submitted to: Study Director	Study Director Management
23-Jan-2017 - 24-Jan-2017	Draft Phase Report - Immunology	27-Jan-2017	27-Jan-2017
07-Mar-2017	Revised Draft Report	07-Mar-2017	07-Mar-2017
05-Jun-2017 - 07-Jun-2017	Data Review - Bioanalysis & Immunology	07-Jun-2017	07-Jun-2017
05-Jun-2017 - 07-Jun-2017	Report Preparation	07-Jun-2017	07-Jun-2017
05-Jun-2017 - 07-Jun-2017	Final Phase Report - Immunology	07-Jun-2017	07-Jun-2017
07-Jun-2017	Study Plan Amendment 8	07-Jun-2017	07-Jun-2017
07-Jun-2017	Final Phase Report - Dose Formulation Analysis	07-Jun-2017	07-Jun-2017
07-Jun-2017	Report Preparation	07-Jun-2017	07-Jun-2017
07-Jun-2017	Final Report	07-Jun-2017	07-Jun-2017

In addition to the above-mentioned audits, process-based and/or routine facility inspections were also conducted during the course of this study. Inspection findings, if any, specific to this study were reported by Quality Assurance to the Study Director and Management and listed as a Phase Audit on this Quality Assurance Statement.

The Final Report has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

[redacted]

Quality Assurance Auditor

[redacted]

Date

COMPLIANCE STATEMENT

The study was performed in accordance with the OECD Principles of Good Laboratory Practice and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- Characterization of the Test Item was performed by the Sponsor subcontractor according to established SOPs, controls, and approved test methodologies to ensure integrity and validity of the results generated; these analyses were not conducted in compliance with the GLP or GMP regulations.

This study was conducted in accordance with the procedures described herein. All deviations authorized/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.

[redacted] _____ Date: [redacted]

Study Director

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director	[redacted], MSc
Test Facility Management	[redacted] PhD, DABT

1.2. Individual Scientists (IS) at Test Facility

Analytical Chemistry	[redacted] BSc
Bioanalysis (mRNA Quantitation)	[redacted] DEC

Charles River Laboratories Montreal ULC
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Sherbrooke, Quebec

2. SUMMARY

The objective of this study was to determine the potential genotoxicity of ZIKA mRNA in SM102-containing lipid nanoparticles (mRNA-1706), when given by a single intravenous injection to rats using the bone marrow micronucleus test. In addition, the concentrations of ZIKA mRNA in the plasma were determined.

In order to determine the maximum tolerated dose, a dose-range finding test was performed prior to the main phase of the study, wherein male and female rats (3 animals/sex) were given a single intravenous injection. The doses evaluated were 2.6, 3.9, and 5.2 mg/kg for females, and 2.6, 5.2, and 10.3 mg/kg for males. Doses ≥ 3.9 mg/kg in the female rat resulted in signs of body weight loss therefore an MTD of 2.6 mg/kg was set for this gender. In males, a dose of 10.3 mg/kg resulted in mortality (2 out of 3 animals), but no clinical observations of note were seen at 5.2 mg/kg.

The main phase study design was as follows:

Text Table 1
Experimental Design - Main Test

Group No.	Test Material	Dose Level (mg/kg[a,e])	Dose Volume (mL/kg)	Dose Conc. (mg/mL)[e]	Sampling Time[b] (hours)	No. of Animals			
						Main Study		Bioanalysis Study[d]	
						M	F	M	F
1	Negative control	0	5	0	24	5	5	3	3
					48	5	5	-	-
2	mRNA-1706	1.0/1.3	5	0.2 / 0.26	24	5	-	3	-
3	mRNA-1706	2.0/2.6	5	0.4 / 0.52	24	5	-	3	-
4	mRNA-1706	4.0/5.2	5	0.8 / 1.03	24	5	-	3	-
					48	5	-	-	-
9	mRNA-1706	0.5/0.6	5	0.1 / 0.129	24	-	5	-	3
10	mRNA-1706	1.0/1.3	5	0.2 / 0.26	24	-	5	-	3
11	mRNA-1706	2.0/2.6	5	0.4 / 0.52	24	-	5	-	3
					48	-	5	-	-
5	Positive control	c							

M = Males; F = Females; - = Not applicable.
[a] Where the high dose for each sex was the MTD.
[b] Bone marrow collection up to 30 minutes post-indicated sampling time was acceptable.
[c] Positive scoring control slides (taken from three male rats previously dosed with positive control cyclophosphamide at 20 mg/kg) were added to the slides for evaluation.
[d] Bioanalysis animals were used for quantification of exposure in plasma only.
[e] Values based on Summary of Analysis (SoA) issued on 11 Oct 2016/Values based on SoA issued on 03 May 2017 (Refer to memorandum in Appendix 2).

During the main test, there was no mortality. At doses ≤ 2.6 mg/kg, no notable Test Item-related clinical signs were observed for the male or female animals. At 5.2 mg/kg, in males, decreased weight gain, yellow staining of the fur at the urogenital and abdominal areas or redness of the tail skin were observed. Females also suffered a depression in weight gain, which increased with dose, 24 hours postdose, but recovered by 48 hrs.

Twenty-four (24) or 48 hours after administration, the animals were euthanized, both femurs were dissected from each animal and the bone marrow was collected. The bone marrow smears were fixed, temporarily stained with acridine orange, and examined under code using fluorescence microscopy. A total of 4000 immature erythrocytes (IE) per animal was examined for the presence of micronuclei indicative of chromosome damage. In addition, the proportion of immature erythrocytes in the total population (immature and mature erythrocytes, (ME)) was assessed for each animal as a measure of potential bone marrow toxicity. The data for the concurrent negative control (group mean % IE/(IE + ME) and micronucleated immature erythrocytes (MIE)) were within the ranges determined from laboratory historical data.

The positive control induced statistically significant increases in micronuclei. Therefore, the performance of the negative and positive control was consistent with a valid assay.

Animals treated with mRNA-1706 did not show any notable decreases in the proportion of IE at the 24-hour and 48-hour sampling times, indicating that there was minimal bone marrow toxicity.

Male animals treated with mRNA-1706 showed 2-fold over negative control, non-dose dependent (i.e. not observed at the mid dose), statistically significant increases in the incidence of MIE, at the 24-hour sampling time. The increased incidences observed were outside the mean distribution of the historical negative control data, and a positive trend test was obtained. A 3-fold increase in the number of MIE was obtained at the 48 hr sampling time at the high dose in males.

Female animals treated with mRNA-1706 showed statistically significant increases in the incidence of MIE, at the 48-hour sampling time only at the MTD dose level, however the increased incidences were within the historical control range. No statistically significant increases in the incidence of MIE were obtained at the 24-hour sampling time.

Thus, elevated incidences of MIE, meeting the criteria for a clear positive response, were noted at 5.2 mg/kg (48-hour sampling group) in males. mRNA-1706 was therefore considered positive for the induction of chromosome damage in rat immature erythrocytes.

The end of use stability of mRNA-1706 was determined under another study where concentration and particle size results were shown to be consistent with the Certificate of Analysis. The End of Use bulk Test Item analysis, however, demonstrated a purity of 53.1%, which is lower than the original purity results of 75%, provided by the Sponsor per the Certificate of Analysis. However, the purpose of this study was to test the in vivo genotoxicity risk of the of the lipid nanoparticle and mRNA construct distinct from the protein produced and thus any effect on purity of the mRNA is not expected to impact these results.

In conclusion, mRNA-1706 did induce chromosome damage in rat bone marrow immature erythrocytes, when tested up to the MTD, in accordance with regulatory guidelines.

3. INTRODUCTION

The objective of this study was to determine the potential genotoxicity of ZIKA mRNA in SM102-containing lipid nanoparticles (mRNA-1706), when given by a single intravenous injection to rats using the bone marrow micronucleus test. In addition, the concentrations of ZIKA mRNA in the plasma were determined.

The design of this study was based on ICH S2(R1) and S3a guidelines, and OECD guidelines 417 and 474.

The Study Director signed the study plan on 06 Oct 2016, and dosing was initiated on 17 Oct 2016 (dose range finding test) and 08 Nov 2016 (main test). The in-life phase of the study was completed on 10 Nov 2016. The experimental start date was 11 Oct 2016, and the experimental completion date was 11 Jan 2017. The study plan, study plan amendments and deviations are presented in Appendix 1.

4. MATERIALS AND METHODS

4.1. Test Item and Negative Control

4.1.1. Test Item

Identification:	ZIKA mRNA in SM102-containing lipid nanoparticles (also known as mRNA-1706)
Supplier:	Moderna Therapeutics, Inc.
Batch (Lot) No.:	MTDP16064
Concentration:	1.7 / 2.2* mg/mL
Date of Manufacture:	24 Aug 2016
Retest Date:	24 Aug 2017 (1 year after date of manufacture)
Physical Description:	White to off-white lipid nanoparticle dispersion
Storage Conditions:	Kept in a refrigerator set to maintain 4°C

* Concentration based on SoA released on 11 Oct 2016/Concentration based on SoA released on 03 May 2017 (refer to memorandum in Appendix 2)

4.1.2. Negative Control

Identification: Phosphate buffered saline (PBS), pH 7.2

Full details of the negative control including supplier, appearance, storage, expiry date, copy of certificate of analysis and formulation are retained as raw data.

4.2. Test Item Characterization

The Sponsor provided to the Test Facility documentation of the identity, strength, purity, composition, and stability for the Test Item. A Certificate of Analysis was provided to the Test Facility and is presented in Appendix 2.

4.3. Analysis of Test Item (End of Use)

The stability of the bulk Test Item was not determined during the course of this study. An end-of-use bulk test item analysis was conducted under CR SHB Study No. 5002045 to demonstrate stability of the test item during the dosing period. Results were reported under CR SHB Study No. 5002045.

4.4. Test Item and Negative Control Inventory and Disposition

Records of the receipt, distribution, and storage of the Test Item and negative control were maintained. All unused Test Item was returned to the Sponsor. Any remaining negative control was retained at the Test Facility or discarded upon expiry.

4.5. Dose Formulation and Analysis

4.5.1. Preparation of Negative Control

The negative control, PBS, was dispensed on the day of use for administration to Group 1 control animals and appropriate Test Item dilutions. As aliquots were prepared shortly before dosing, they did not require 4°C storage, and were kept in a controlled temperature area set to maintain 21°C pending transfer for dose administration.

Details of the dispensing of the negative control have been retained in the study records.

4.5.2. Preparation of Test Item

The Test Item, mRNA-1706, was diluted with PBS at appropriate concentrations to meet dose level requirements. The dosing formulations were prepared shortly before dosing, and were kept in a controlled temperature area set to maintain 21°C pending transfer for dose administration (except for Group 8 formulations which were stored at in a refrigerator set to maintain 4°C, and subsequently allowed to warm to room temperature for at least 30 minutes before dosing). The formulations were stirred gently and not vortexed. Any residual volumes of formulated Test Item were shipped back to the Sponsor for mRNA/lipid identity confirmation. The results were as expected and confirmed the mRNA/lipid identity used on the study.

Details of the preparation and dispensing of the Test Item have been retained in the study records.

4.5.3. Sample Collection and Analysis

Dose formulation samples were collected for analysis as indicated in the following table.

Text Table 2
Dose Formulation Sample Collection Schedule

Interval	Homogeneity	Concentration	Sampling From
Day of preparation (Main Test)	All Test Item concentrations[a]	Group 1 and all Test Item concentrations	Dosing container

[a] The homogeneity results obtained from the top, middle and bottom preparations were averaged and utilized as the concentration results.

Samples to be analyzed were transferred to the analytical laboratory at the Test Facility and were analyzed on the same day.

Any residual/retained analytical samples (and Test Item used in analysis) were discarded before issuance of the Final Report.

4.5.3.1. Analytical Method

Analyses were performed by IEX-HPLC using a validated analytical procedure (CR MTL Study No. 1801737).

4.5.3.2. Concentration and Homogeneity Analysis

Duplicate sets of samples (0.5 mL) for each sampling time point were transferred to the analytical laboratory; the remaining samples (triplicate 0.5 mL) were retained as backup samples. Concentration results were considered acceptable if mean sample concentration results were within or equal to ± 15% of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to ± 20%. Homogeneity results were considered acceptable if the relative standard deviation of the mean value at each sampling location was ≤ 5%. After acceptance of the analytical results, backup samples were discarded.

4.6. Test System

4.6.1. Receipt

Sprague Dawley Crl:CD (SD) rats were received from Charles River Canada Inc., St-Constant, QC, Canada.

For the dose range finding test, 12 males and 12 females were received on 11 Oct 2016. For the main test, 49 males and 50 females were received on 01 Nov 2016. Following arrival, each animal was given a general physical examination by a member of the veterinary staff to ensure normal health status.

For the dose range finding test, the animals were 48 to 56 days old at dosing, and the males weighed between 178 and 268 g and the females between 149 and 204 g. For the main test, the animals were 49 days old at dosing, and the males weighed between 166 and 208 g and the females between 152 and 181 g.

4.6.2. Justification for Test System and Number of Animals

The rat was chosen as the animal model for this study as it is an accepted rodent species for preclinical genotoxicity testing by regulatory agencies.

Young adult animals are chosen for use because of the high rate of cell division in the erythropoietic system and because of their general suitability for toxicological investigations.

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the Test Item. This study was designed such that it did not require an unnecessary number of animals to accomplish its objectives.

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.

4.6.3. Animal Identification

At study assignment, each animal was identified using indelible ink.

4.6.4. Environmental Acclimation

An acclimation period of 6-14 days was allowed between animal receipt and the start of treatment in order to accustom the animals to the laboratory environment.

4.6.5. Selection, Assignment, Replacement, and Disposition of Animals

Animals were assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. Males and females were randomized separately. Before the initiation of dosing, one assigned animal was found dead and was replaced by an alternate animal obtained from the same shipment and maintained under the same environmental conditions. Four alternate animals were used on the study during dosing due to injection technical difficulties. The disposition of all animals was documented in the study records.

4.6.6. Husbandry

4.6.6.1. Housing

Animals were group housed (up to 3 animals of the same sex and same dosing group together) in stainless steel cages equipped with an automatic watering valve. These housing conditions were maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The rooms in which the animals were kept were documented in the study records.

Animals were separated during designated procedures/activities. Each cage was clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex. Cages were arranged on the racks in group order. Where possible, negative control group animals were housed on a separate rack from the Test Item-treated animals.

4.6.6.2. Environmental Conditions

Temperatures of 19°C to 25°C with a relative humidity of 30% to 70% were maintained. A 12-hour light/12-hour dark cycle was maintained.

4.6.6.3. Food

PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) was provided ad libitum throughout the study, except during designated procedures.

The feed was analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Test Facility.

It is considered that there are no known contaminants in the feed that would have interfered with the objectives of the study.

4.6.6.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation and which has been further circulated through a pressure reducing system equipped with a 5-micron size bacteriostatic filter was freely available to each animal via an automatic watering system (except during designated procedures).

Periodic analysis of the water is performed, and results of these analyses are on file at the Test Facility.

It is considered that there are no known contaminants in the water that could have interfered with the outcome of the study.

4.6.6.5. Animal Enrichment

Animals were socially housed for psychological/environmental enrichment and were provided with items such as a hiding device and a chewing object, as well as nesting material (see Deviations), except when interrupted by study procedures/activities.

4.6.6.6. Veterinary Care

Veterinary care was available throughout the course of the study. All veterinary examinations and recommended therapeutic treatments, if any, were documented in the study records.

4.7. Experimental Design

Text Table 3
Experimental Design - Dose Range Finding Test

Group No.	Test Material	Dose Level (mg/kg)[b]	Dose Volume (mL/kg)	Dose Concentration (mg/mL)[b]	No. of Animals	
					Male	Female
6	mRNA-1706	2.0/2.6 [a]	5	0.4/0.52	3	3
7	mRNA-1706	4.0/5.2	5	0.8/1.03	3	3
8	mRNA-1706	8.0/10.3 (M) 3.0/3.9 (F)	5/1.9 (M/F)	1.6/2.06	3	3

[a] The dose for initial testing was determined taking into account any relevant information supplied by the Sponsor.
[b] Values based on Summary of Analysis (SoA) issued on 11 Oct 2016/Values based on SoA issued on 03 May 2017 (Refer to memorandum in Appendix 2).

Text Table 4
Experimental Design - Main Test

Group No.	Test Material	Dose Level (mg/kg[a,e])	Dose Volume (mL/kg)	Dose Conc. (mg/mL)[e]	Sampling Time[b] (hours)	No. of Animals			
						Main Study		Bioanalysis Study[d]	
						M	F	M	F
1	Negative control	0	5	0	24	5	5	3	3
					48	5	5	-	-
2	mRNA-1706	1.0/1.3	5	0.2/0.26	24	5	-	3	-
3	mRNA-1706	2.0/2.6	5	0.4/0.52	24	5	-	3	-
4	mRNA-1706	4.0/5.2	5	0.8/1.03	24	5	-	3	-
					48	5	-	-	-
9	mRNA-1706	0.5/0.6	5	0.1/0.129	24	-	5	-	3
10	mRNA-1706	1.0/1.3	5	0.2/0.26	24	-	5	-	3
11	mRNA-1706	2.0/2.6	5	0.4/0.52	24	-	5	-	3
					48	-	5	-	-
5	Positive control	[c]							

M = Males; F = Females; - = Not applicable.
[a] Where the high dose for each sex was the MTD.
[b] Bone marrow collection up to 30 minutes post-indicated sampling time was acceptable.
[c] Positive scoring control slides (taken from three male rats previously dosed with positive control cyclophosphamide at 20 mg/kg) were added to the slides for evaluation.
[d] Bioanalysis animals were used for quantification of exposure in plasma only.
[e] Values based on Summary of Analysis (SoA) issued on 11 Oct 2016/Values based on SoA issued on 03 May 2017 (Refer to memorandum in Appendix 2).

4.7.1. Administration of Test Item and Negative Control

The Negative Control and Test Item were administered once by intravenous injection (1-2 minutes slow bolus, see Deviations) into the tail vein, 48 or 24 hours prior to euthanasia. The dose was given using a syringe/needle or butterfly set up. The dose volume for each animal was based on the most recent body weight measurement. The first day of dosing was designated as Day 1.

4.7.2. Justification of Route and Dose Levels

The subcutaneous is the intended route of human exposure. The intravenous route was chosen to mimic maximum exposure for any dose route. In order to determine the high dose to be used in the main micronucleus test, a dose range finding test was performed. The initial dose level was selected based on Sponsor internal data where single intravenous doses with modified mRNA construct(s) in various lipid nanoparticle formulations were well-tolerated in rats up to 1 mg/kg. Based on previous Sponsor experience, a second dose is not tolerated. Therefore, a single dose study design was used for the present study and the initial dose for the dose range finding test was 2.6 mg/kg (2 mg/kg originally as per SoA issued on 11 Oct 2016).

The high dose for the main test was based on results of the dose range finding test and was the estimated maximum tolerated dose (MTD).

In addition, analysis of plasma samples from satellite animals in the main test was conducted to quantify in vivo exposure to the test compound.

4.8. In-life Procedures, Observations, and Measurements

The in-life procedures, observations, and measurements listed below were performed for main study animals, including spare animals, where appropriate. Bioanalysis animals were weighed according to Section 4.8.4 and any abnormal clinical observations were recorded.

4.8.1. Mortality/Moribundity Checks

Throughout the study, animals were observed for general health/mortality and moribundity twice daily, once in the morning and once in the afternoon. Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings.

4.8.2. Cage Side Observations (Dose Range Finder Animals Only)

Cage side observations were performed hourly (± 8 minutes from the time of dosing of each animal) for the first four hours following dosing on Day 1 (see Deviations) and once 24 h and 48 h following dosing (± 30 minutes).

Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings.

4.8.3. Detailed Clinical Observations

The animals were removed from the cage, and a detailed clinical observation was performed once during the pretreatment period (for all animals) and once 3 hours (± 30 minutes) following dosing on the day of treatment (for main study animals only).

4.8.4. Body Weights

Animals were weighed individually prior to randomization, on the day prior to treatment initiation (for calculation of individual dose volumes) and at end of the test. Body weights measured on the day prior to treatment initiation and at end of the test are included in the report (for dose range finding and main study animals only).

4.9. Laboratory Evaluations

4.9.1. Bioanalysis

4.9.1.1. Bioanalytical Sample Collection

Blood was collected by jugular venipuncture. Samples were collected according to the following table.

Text Table 5
Blood (Plasma) Sample Collection Schedule

Group No.	No. of Males/Females	Sample Collection Time Points (Time Postdose on Day 1)
		15 min
1	3/3	X
2	3/-	X
3	3/-	X
4	3/-	X
9	-/3	X
10	-/3	X
11	-/3	X

X = Sample was collected.

Target Volume: 0.5 mL

Anticoagulant: K_2EDTA

4.9.1.2. Bioanalytical Sample Processing

Bioanalytical samples were processed to plasma; blood samples were kept on wet ice prior to processing. The samples were centrifuged within 30 minutes in a refrigerated centrifuge (set to maintain 4°C) for 15 minutes and set at 3000xg. Immediately after plasma collection, the samples were aliquoted into 2 x 50 µL aliquot and a leftover. Aliquots were snap frozen in liquid nitrogen, put on dry ice and then stored in a freezer set to maintain -80°C until analysis.

Plasma samples were transferred on dry ice to CR SHB for analysis.

4.9.1.3. Bioanalytical Sample Analysis

Plasma samples were used for ZIKA mRNA quantitation by the Immunology department (CR SHB) using a validated bDNA method (validation Study No. 3001357). The procedure followed during the course of this study along with the assay for acceptance criteria was detailed in the appropriate analytical procedure. Samples were analyzed in duplicate.

Any residual/retained bioanalytical samples were discarded prior to the issuance of the Final Report.

4.10. Terminal Procedures

4.10.1. Unscheduled Deaths

Two male animals were found dead during the Dose Range Finding Test. Another animal suffered an accidental death during the pre-treatment period of the Main Test. These animals were not subjected to necropsy and the carcasses were discarded without examination. No animals died during the course of the Main Test.

4.10.2. Scheduled Euthanasia

The dose range finding and the bioanalysis animals were euthanized by carbon dioxide asphyxiation, after completion of the observation period and blood collection schedule, respectively. The carcasses were discarded without further examination.

Main study animals surviving until scheduled euthanasia were not fasted prior to termination. At time points specified in the experimental design table (24 or 48 h, ± 30 minutes), the animals underwent exsanguination from the abdominal aorta following isoflurane anesthesia.

4.10.3. Necropsy

Both femurs, were dissected from each main study animal. Animals did not undergo further examination and carcasses were disposed of according to the Test Facility standard operating procedures.

The proximal head was removed from each femur while keeping the distal head intact, and as much tissue as possible was removed from the bones. The bone marrow from both femurs of each animal was pooled/eluted in 5 mL Hanks' Balance Salts Solution by aspiration through an appropriate size needle fitted to a plastic syringe. The resulting cell suspensions were processed for the micronucleus test.

5. MICRONUCLEUS TEST

5.1. Smear Preparation

The cell suspensions were centrifuged for 5 minutes. Each resulting cell pellet were resuspended in 2 mL of filtered fetal bovine serum and re-centrifuged. The cell pellet was resuspended in a small volume of fetal bovine serum to facilitate smearing in the conventional manner on glass microscope slides. Several smears were prepared from each animal.

5.2. Fixation and Staining of the Slides

The smears were fixed in methanol for at least 10 minutes and then stained with the fluorescent metachromatic dye, acridine orange. Note that stained smears deteriorate (especially during examination); since they were regarded as temporary preparations, they were discarded following confirmation of results by the Study Director. However, one fixed, unstained slide per animal was mounted with a coverslip for protection and archived with the raw data for the study for potential retrospective examination. Any remaining reserve slides were discarded after the experimental phase of the study.

Positive control slides, taken from three male rats previously dosed with positive control (cyclophosphamide, 20 mg/kg at 10 mL/kg given 24 hours prior to sampling) as part of a GLP-compliant study were added to the slides for evaluation. Data from these animals were entered under the study under a positive control group called Group 5 for use in statistical evaluation. Arbitrary animal numbers were given based on the standard animal identification scheme. No slides from the positive control Group 5 were archived.

5.3. Microscopic Examination

The slides were encoded to minimize potential operator bias and then examined by fluorescence microscopy using a blue excitation filter and a yellow barrier filter. A total of 4000 immature erythrocytes per animal were examined for the presence of micronuclei. One smear was examined per animal, the remaining smears being held temporarily as reserves in case of technical problems with the first smear.

Micronuclei were identified by the following criteria:

- Large enough to discern morphological characteristics
- Should possess a generally rounded shape with a clearly defined outline
- Should be brightly stained and similar in color to the nuclei of other cells (bright yellow/green)
- Should lie in the same focal plane as the cell
- Lack internal structure, *i.e.* they are pyknotic
- There should be no micronucleus-like debris in the area surrounding the cell

In addition, the proportion of immature erythrocytes was assessed by examination of a total of at least 500 erythrocytes per animal, if available. The incidence of any micronucleated mature erythrocytes observed during this assessment was recorded as a check for potential micronucleus-like artifacts.

6. ACCEPTANCE CRITERIA

The following criteria were applied for assessment of assay acceptability:

1. The concurrent negative control data are considered acceptable for addition to the laboratory historical control database.
2. The concurrent positive controls or scoring controls should induce responses that are compatible with those generated in the historical positive control database and produce a statistically significant increase compared with the concurrent negative control.
3. The appropriate number of doses and cells has been analyzed (4000 immature erythrocytes per animal).
4. The maximum dose tested is one that allows maximum exposure up to 2000 mg/kg/day for non-toxic compounds, or the limit of solubility or MTD based on available toxicity data, in accordance with current guidelines.

7. EVALUATION AND INTERPRETATION OF RESULTS

Means were calculated, where applicable, according to sampling time point. Otherwise, data were presented as individual values by animal.

Providing that all acceptability criteria are fulfilled, a Test Item is considered clearly **positive** if:

1. At least one of the Test Item treatment groups exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent negative control,
2. The increase is dose-related at least at one sampling time when evaluated with an appropriate trend test (trend test not applicable if only the highest dose is examined at a particular sampling time),

3. Any of the results are outside the distribution of the historical negative control data (e.g. 95% control limits).

When all of these criteria are met, the Test Item is then considered able to induce chromosomal damage or damage to the mitotic apparatus in the tissue studied in this test system.

Providing that all acceptability criteria are fulfilled, a Test Item is considered clearly **negative** if:

1. None of the Test Item treatment groups exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent negative control,
2. All results are inside the distribution of the historical negative control data (e.g. 95% control limits),
3. Direct or indirect evidence supportive of exposure of, or toxicity to, the target tissue(s) has been demonstrated.

The Test Item is then considered unable to induce chromosomal damage in the tissue studied in this test system.

Bone marrow cell toxicity is normally indicated by a substantial decrease in the proportion of immature erythrocytes (proportion of immature erythrocytes less than 20% of that for the concurrent control group). If any treated group of animals shows evidence of severe bone marrow depression (group mean proportion of immature erythrocytes less than 20% of that for the concurrent control group), then any apparent increase in the frequency of micronucleated immature erythrocytes is interpreted with caution.

If no definite judgment could be made to fit the above criteria, the results of the assay were concluded as equivocal.

8. STATISTICAL ANALYSIS

All statistical tests were conducted at the 5% significance level. The pairwise comparisons were reported at the 0.1%, 1%, and/or 5% levels. Result was considered to be significant if $p \leq 0.05$.

Numerical data collected on scheduled occasions were analyzed as indicated according to sex and occasion. Descriptive statistics number, mean and standard deviation were reported whenever possible.

For males, Group 1 was compared to the positive control group (Group 5), and to each of Test Item Groups 2, 3, and 4 (Test Item Group 4 only for the 48-hour sampling time using Group 1 48-hour sampling time). These pairwise comparisons were implemented using Fisher's Exact one-sided test, in order to check if the micronucleated immature erythrocytes incidence in Group 5 and in each of Groups 2, 3, and 4 was significantly higher than the micronucleated immature erythrocytes incidence in Group 1.

As two out of three incidences of micronucleated immature erythrocytes were found to be significantly higher ($p \leq 0.05$), for the Test Item-treated 24-hour sampling time dataset, the significance of a dose-related increase in micronucleated immature erythrocytes incidence, across the control group (Group 1) and each of the three Test Item treated groups (Groups 2, 3, 4) was evaluated using Cochran-Armitage's one-sided test.

For females, Group 1 was compared to each of Test Item Groups 9, 10, and 11 (Test Item Group 11 only for the 48-hour sampling time using Group 1 48-hour sampling time). These pairwise comparisons were implemented using Fisher's Exact one-sided test, in order to check if the micronucleated immature erythrocytes incidence in each of Groups 9, 10, and 11 was significantly higher than the micronucleated immature erythrocytes incidence in Group 1.

As none of the incidences of micronucleated immature erythrocytes were found to be significantly higher ($p \leq 0.05$) for the Test Item-treated 24-hour sampling time dataset, no trend test was performed (i.e. Cochran-Armitage's one-sided test).

9. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized 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.

Text Table 6
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Provantis	8	Collection and/or analysis of in-life; clinical pathology, terminal data, and statistical analysis.
Dispense	8	Test Material receipt, accountability and/or formulation activities.
Excel (Microsoft)	2007	Calculations for the micronucleus test results
Mesa Laboratories AmegaView CMS	v3.0 Build 1208.8	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO_2, as appropriate.
Johnson Controls Metasys	7.0	Building Automation System. Control of HVAC and other building systems, as well as temperature/humidity control and trending in selected laboratories and animal rooms.

10. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, documentation, study plan, study plan amendments, specimens, and final report from this study were archived at the Test Facility by no later than the date of final report issue. One year after issue of the audited draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

Electronic data generated by the Test Facility were archived as noted above, except that the data collected using Provantis 8 and reporting files stored on SDMS, which were archived at the Charles River Laboratories facility location in Wilmington, MA.

11. RESULTS

11.1. Dose Formulation Analyses

(Appendix 3)

The mean sample concentration results for the Test Item dosing formulations were within the acceptance criterion of ± 15% of theoretical concentration (94.3% - 96.0%). Each individual sample concentration result was within ± 20% of theoretical concentrations. Homogeneity results were within the acceptance criterion of the relative standard deviation of the mean value at each sampling location being ≤ 5% (0.6% - 3.6%). No Test Item was detected in the control samples (Group 1).

11.2. End of Use Bulk Test Item Analysis

Concentration and particle size results obtained under CR SHB Study No. 5002045 were consistent with the Certificate of Analysis.

The End of Use bulk Test Item analysis, however, demonstrated a purity of 53.1%, which is lower than the original purity results of 75%, provided by the Sponsor per the Certificate of Analysis.

Although the purity analysis indicates RNA degradation, the concentration and particle size were within specification. The concentration of the dose formulations was based on the total RNA content. However, the purpose of this study was to test the in vivo genotoxicity risk of the of the lipid nanoparticle and mRNA construct distinct from the protein produced and thus any effect on purity of the mRNA is not expected to impact these results.

11.3. Mortality

(Appendix 4)

During the dose range finding test, two males dosed at 10.3 mg/kg of mRNA-1706 were found dead within 24 hours following dosing. Another animal assigned for TK processing suffered an accidental death during the pre-treatment period of the Main Test and was replaced accordingly. No other mortalities occurred during this study.

11.4. Clinical Observations

(Table 1 and Appendix 5)

During the dose range finding test, no Test-Item related clinical signs were noted for the animals that survived until their scheduled euthanasia.

Following administration of mRNA-1706 at 1.3 and 2.6 mg/kg, no notable Test Item-related clinical signs were observed for the male animals. Yellow staining of the fur at the urogenital and abdominal areas or redness of the tail skin was observed for a couple of the male animals dosed at 5.2 mg/kg. Additionally, an animal displayed skin scabbing of the pinnae while another was missing part of the pinnae. Following administration of mRNA-1706 at 0.6, 1.3, and 2.6 mg/kg, no clinical signs were observed for the females except for one animal of the high dose group that displayed thinning of the fur of the left forepaw and forelimb.

11.5. Body Weights and Body Weight Gains

(Table 2, Table 3, Appendix 6, and Appendix 7)

Body weights were measured prior to randomization for placement of animals in groups, prior to treatment initiation for the calculation of dose volume, and at termination, and the mean and individual data are presented. Body weight gains are also presented.

During the dose range finding test, there was some evidence of weight depression for the females (on the verge of weight loss) following administration of mRNA-1706 at 5.2 mg/kg. Weight loss was noted for the female animals following administration of mRNA-1706 at 3.9 mg/kg. Weight loss was noted for the only surviving male animal which was dosed at 10.3 mg/kg.

In the main test, Test Item-treated male animals suffered a depression in weight gain, which increased with dose, when compared to the negative control group, 24 hours postdose. A depression in weight gain was also observed 48 hours postdose for the high dose group of animals. The females also suffered a depression in weight gain, which increased with dose, 24 hours postdose. No depression in weight gain was observed 48 hours postdose for the high dose group of female animals.

11.6. Bioanalytical Evaluations

(Table 4, Appendix 8, and Appendix 9)

Bioanalysis evaluations for mRNA-1706 were conducted using plasma prepared from blood collected at 15 minutes postdose on Day 1, and results indicate that mRNA-1706 was quantifiable for all Test Item dose groups.

11.7. Microscopic Examination

(Table 5 and Appendix 10)

The data (as characterized by group mean % IE/(IE + ME) and MIE) for the concurrent negative control animals used in the main phase were within the laboratory historical ranges. The positive control, cyclophosphamide, induced statistically significant increases in micronuclei. Therefore, the performance of both the negative and positive control was consistent with a valid assay. Appendix 11 summarizes the vehicle/negative control micronucleus assay results obtained in previous experiments (laboratory historical control ranges).

11.7.1. Proportion of Immature Erythrocytes (% IE/(IE + ME))

Animals treated with mRNA-1706 did not show any notable decreases in the proportion of IE at the 24-hour and 48-hour sampling times, indicating that there was minimal bone marrow toxicity. All group mean values for the proportion of immature erythrocytes were within the historical vehicle/negative control range of 20-62%.

11.7.2. Micronucleated Immature Erythrocytes (MIE)

Male animals treated with mRNA-1706 showed 2-fold over negative control, non-dose dependent (i.e. not observed at the mid dose), statistically significant increases in the incidence of MIE, at the 24-hour sampling time. The increased incidences observed were outside the mean distribution of the historical negative control data, and a positive trend test was obtained.

A 3-fold increase in the number of MIE was obtained at the 48 hr sampling time at the high dose in males.

Female animals treated with mRNA-1706 showed statistically significant increases in the incidence of MIE, at the 48-hour sampling time only at the MTD dose level, however the increased incidences were within the historical control range. No statistically significant increases in the incidence of MIE were obtained at the 24-hour sampling time.

Thus, elevated incidences of MIE, meeting the criteria for a clear positive response, were noted at 5.2 mg/kg (48-hour sampling group) in males. mRNA-1706 was therefore considered positive for the induction of chromosome damage in rat immature erythrocytes.

11.7.3. Micronucleated Mature Erythrocytes (MME)

Animals treated with mRNA-1706 did not show any notable increases in the individual and group mean incidence of micronucleated mature erythrocytes (MME). The incidence of MME for all groups was uniformly low, confirming the absence of micronucleus-like artifacts.

12. CONCLUSION

In conclusion, mRNA-1706 did induce chromosome damage in rat bone marrow immature erythrocytes, when tested up to the MTD, in accordance with regulatory guidelines.

Table 1

Summary of Clinical Observations - Main Test

9800399

Day numbers relative to Start Date

Sex: Male

	0 mg/kg	1.0/1.3 mg/kg	2.0/2.6 mg/kg	4.0/5.2 mg/kg
Swollen Firm				
Number of Observations	1	1	.	1
Number of Animals	1	1	.	1
Days from - to	1 1	1 1	.	1 1
Skin, Red				
Number of Observations	.	.	.	2
Number of Animals	.	.	.	2
Days from - to	.	.	.	1 1
Skin, Scab				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	1 1
Fur, Staining, Yellow				
Number of Observations	.	.	.	2
Number of Animals	.	.	.	2
Days from - to	.	.	.	1 1
Pinna Partly Missing				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	1 1

Table 1

Summary of Clinical Observations - Main Test

9800399

Day numbers relative to Start Date

Sex: Female

	0 mg/kg	0.5/0.6 mg/kg	1.0/1.3 mg/kg	2.0/2.6 mg/kg
Fur, Thin Cover				
Number of Observations	.	.	.	1
Number of Animals	.	.	.	1
Days from - to	.	.	.	1 1

Table 2

Summary of Body Weight - Dose Range Finding Test

9800399

Bodyweight (g)

Sex: Male		Day(s) Relative to Start Date	
		-1	3
2.0/2.6	Mean	183.3	201.0
mg/kg	SD	9.2	1.0
	N	3	3
4.0/5.2	Mean	213.0	226.3
mg/kg	SD	15.7	17.6
	N	3	3
8.0/10.3M	Mean	254.0	228.0
3.0/3.9F	SD	13.5	-
mg/kg	N	3	1

Table 2

Summary of Body Weight - Dose Range Finding Test

9800399

Bodyweight (g)

Sex: Female		Day(s) Relative to Start Date	
		-1	3
2.0/2.6	Mean	158.7	165.3
mg/kg	SD	10.0	9.3
	N	3	3
4.0/5.2	Mean	168.7	169.3
mg/kg	SD	2.9	2.1
	N	3	3
8.0/10.3M	Mean	192.3	183.7
3.0/3.9F	SD	13.2	18.1
mg/kg	N	3	3

Table 2

Summary of Body Weight - Main Test

9800399

Bodyweight (g)

Sex: Male		Day(s) Relative to Start Date		
		-1	2	3
0	Mean	190.4	209.2	220.2
mg/kg	SD	13.2	12.5	18.6
	N	10	5	5
1.0/1.3	Mean	186.0	196.8	-
mg/kg	SD	5.4	8.8	-
	N	5	5	-
Group 2	%Diff	-2.3	-5.9	-
2.0/2.6	Mean	187.0	198.6	-
mg/kg	SD	6.5	6.2	-
	N	5	5	-
Group 3	%Diff	-1.8	-5.1	-
4.0/5.2	Mean	186.4	185.6	197.8
mg/kg	SD	13.7	20.4	12.8
	N	10	5	5
Group 4	%Diff	-2.1	-11.3	-10.2

Table 2

Summary of Body Weight - Main Test

9800399

Bodyweight (g)

Sex: Female		Day(s) Relative to Start Date		
		-1	2	3
0	Mean	166.9	183.0	169.6
mg/kg	SD	10.2	2.4	4.4
	N	10	5	5
0.5/0.6	Mean	164.0	170.8	-
mg/kg	SD	5.2	7.2	-
	N	5	5	-
Group 9	%Diff	-1.7	-6.7	-
1.0/1.3	Mean	162.6	165.8	-
mg/kg	SD	7.3	8.8	-
	N	5	5	-
Group 10	%Diff	-2.6	-9.4	-
2.0/2.6	Mean	164.2	163.6	177.2
mg/kg	SD	10.3	13.0	12.8
	N	10	5	5
Group 11	%Diff	-1.6	-10.6	4.5

Table 3

Summary of Body Weight Gains (g) - Dose Range Finding Test

9800399

Bodyweight Gain (Interval)

Sex: Male		Day(s) Relative to Start Date
		-1 → 3
2.0/2.6	Mean	17.7
mg/kg	SD	9.3
	N	3
4.0/5.2	Mean	13.3
mg/kg	SD	2.5
	N	3
8.0/10.3M	Mean	-13.0
3.0/3.9F	SD	-
mg/kg	N	1

Table 3

Summary of Body Weight Gains (g) - Dose Range Finding Test

9800399

Bodyweight Gain (Interval)

Sex: Female		Day(s) Relative to Start Date
		-1 → 3
2.0/2.6	Mean	6.7
mg/kg	SD	3.1
	N	3
4.0/5.2	Mean	0.7
mg/kg	SD	2.1
	N	3
8.0/10.3M	Mean	-8.7
3.0/3.9F	SD	6.8
mg/kg	N	3

Table 3

Summary of Body Weight Gains (g) - Main Test

9800399

Bodyweight Gain (Interval)

Sex: Male		Day(s) Relative to Start Date	
		-1 → 2	-1 → 3
0	Mean	19.2	29.4
mg/kg	SD	3.3	3.8
	N	5	5
1.0/1.3	Mean	10.8	-
mg/kg	SD	6.4	-
	N	5	-
2.0/2.6	Mean	11.6	-
mg/kg	SD	2.3	-
	N	5	-
4.0/5.2	Mean	1.6	9.0
mg/kg	SD	8.7	6.6
	N	5	5

Table 3

Summary of Body Weight Gains (g) - Main Test

9800399

Bodyweight Gain (Interval)

Sex: Female		Day(s) Relative to Start Date	
		-1 → 2	-1 → 3
0	Mean	7.4	11.4
mg/kg	SD	1.5	6.3
	N	5	5
0.5/0.6	Mean	6.8	-
mg/kg	SD	2.9	-
	N	5	-
1.0/1.3	Mean	3.2	-
mg/kg	SD	2.6	-
	N	5	-
2.0/2.6	Mean	1.6	10.8
mg/kg	SD	3.6	4.4
	N	5	5

Table 4

Summary of mRNA Values

Day 1 - 15 min Post Dose
Males

Group 1 - Negative Control
Group 2 - mRNA-1706 1.0 / 1.3 mg/kg
Group 3 - mRNA-1706 2.0 / 2.6 mg/kg
Group 4 - mRNA-1706 4.0 / 5.2 mg/kg

Group	Summary Information	mRNA ng/mL
1	Mean	0.020
	SD	0.000
	N	3
2	Mean	12790.350
	SD	1886.867
	N	3
3	Mean	22322.723
	SD	16941.922
	N	3
4	Mean	40579.573
	SD	36977.049
	N	3

Table 4

Summary of mRNA Values

Day 1 - 15 min Post Dose
Females

Group 1 - Negative Control
Group 9 - mRNA-1706 0.5 / 0.6 mg/kg
Group 10 - mRNA-1706 1.0 / 1.3 mg/kg
Group 11 - mRNA-1706 2.0 / 2.6 mg/kg

Group	Summary Information	mRNA ng/mL
1	Mean	0.020
	SD	0.000
	N	3
9	Mean	6840.633
	SD	835.950
	N	3
10	Mean	13950.843
	SD	2756.722
	N	3
11	Mean	25842.473
	SD	3222.387
	N	3

Table 5

Summary of Micronucleus Results

Sampling Time - 24 Hours (± 30 minutes) After the Final Administration
Males

Group 1 - Negative Control
Group 2 - mRNA-1706 1.0 / 1.3 mg/kg
Group 3 - mRNA-1706 2.0 / 2.6 mg/kg
Group 4 - mRNA-1706 4.0 / 5.2 mg/kg
Group 5 - CP 20 mg/kg

Group	% IE/(IE+ME)	% MIE	% MME	Incidence MIE [a] Mean	±	SD
1	54.6	0.155	0.00	6.2	±	2.3
2	55.8	0.315	0.00	12.6 C	±	5.1
3	53.8	0.170	0.00	6.8	±	3.6
4	40.6	0.295	0.00	11.8 a, B	±	4.9
5	43.3	2.142	0.00	85.7 C	±	9.7

%IE/(IE+ME) Proportion of immature erythrocytes
%MIE Percentage of micronucleated immature erythrocytes
%MME Percentage of micronucleated mature erythrocytes
MIE Number of micronucleated cells observed per 4000 immature erythrocytes examined
CP Cyclophosphamide monohydrate
a Statistical analysis were performed on incidence of MIE
Significantly different from control (Group 1) value: A - $p \leq 0.05$ B - $p \leq 0.01$ C - $p \leq 0.001$ (Fisher's)
Significantly different from control (Group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Cochran-Armitage's)

Table 5

Summary of Micronucleus Results

Sampling Time - 24 Hours (± 30 minutes) After the Final Administration
Females

Group 1 - Negative Control
Group 9 - mRNA-1706 0.5 / 0.6 mg/kg
Group 10 - mRNA-1706 1.0 / 1.3 mg/kg
Group 11 - mRNA-1706 2.0 / 2.6 mg/kg

Group	% IE/(IE+ME)	% MIE	% MME	Incidence MIE [a] Mean	±	SD
1	43.9	0.135	0.00	5.4	±	4.7
9	51.4	0.200	0.00	8.0	±	3.7
10	44.6	0.205	0.00	8.2	±	3.7
11	45.0	0.135	0.00	5.4	±	2.1

%IE/(IE+ME) Proportion of immature erythrocytes
%MIE Percentage of micronucleated immature erythrocytes
%MME Percentage of micronucleated mature erythrocytes
MIE Number of micronucleated cells observed per 4000 immature erythrocytes examined
a Statistical analysis were performed on incidence of MIE

Table 5

Summary of Micronucleus Results

Sampling Time - 48 Hours (± 30 minutes) After the Final Administration
Males

Group 1 - Negative Control

Group 4 - mRNA-1706 4.0 / 5.2 mg/kg

Group	% IE/(IE+ME)	% MIE	% MME	Incidence MIE [a] Mean	±	SD
1	53.6	0.140	0.00	5.6	±	3.9
4	36.6	0.480	0.06	19.2 C	±	7.6

%IE/(IE+ME)	Proportion of immature erythrocytes
%MIE	Percentage of micronucleated immature erythrocytes
%MME	Percentage of micronucleated mature erythrocytes
MIE	Number of micronucleated cells observed per 4000 immature erythrocytes examined
a	Statistical analysis were performed on incidence of MIE

Significantly different from control (Group 1) value: A - $p \leq 0.05$ B - $p \leq 0.01$ C - $p \leq 0.001$ (Fisher's)

Table 5

Summary of Micronucleus Results

Sampling Time - 48 Hours (± 30 minutes) After the Final Administration
Females

Group 1 - Negative Control

Group 11 - mRNA-1706 2.0 / 2.6 mg/kg

Group	% IE/(IE+ME)	% MIE	% MME	Incidence MIE [a] Mean	±	SD
1	46.2	0.135	0.00	5.4	±	1.9
11	35.5	0.210	0.00	8.4 A	±	1.5

%IE/(IE+ME)	Proportion of immature erythrocytes
%MIE	Percentage of micronucleated immature erythrocytes
%MME	Percentage of micronucleated mature erythrocytes
MIE	Number of micronucleated cells observed per 4000 immature erythrocytes examined
a	Statistical analysis were performed on incidence of MIE

Significantly different from control (Group 1) value: A - $p \leq 0.05$ B - $p \leq 0.01$ C - $p \leq 0.001$ (Fisher's)