

# Toxicology Review of FLUAD 65 Influenza Vaccines (IV)

BLA: 125510

Sponsor: Novartis Vaccines and Diagnostics, Inc.

Product: FLUAD 65 Influenza Vaccines (IV)

Proposed indication: Active immunization of persons 65 years of age and older against influenza disease

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## Introduction:

Influenza is a contagious respiratory illness caused by influenza viruses. Symptoms of the influenza are: fever (usually high), headache, extreme tiredness, dry cough, sore throat, runny or stuffy nose, muscle aches, stomach symptoms, such as nausea, vomiting, and diarrhea, also can occur but are more common in children than adults. It can cause mild to severe illness, and at times can lead to death.

Trivalent subunit influenza vaccines contain 3 influenza virus strains: two A strains and one B strain. The selection of the A and B influenza viruses for the vaccines is based on the annual WHO and CHMP strain recommendations for the northern and southern hemispheres, as published for the respective influenza season in the weekly epidemiological record (<http://www.who.int/wer/en/>). These viruses are the predominant strains considered most appropriate to the epidemiological situation in each hemisphere.

For more than 70 years, adjuvants have been used to increase the immunogenicity of vaccines. Several new adjuvants have been developed recently, especially for vaccines where increased immune responses may be desirable, such as those intended for special populations (e.g., elderly) or where purified antigens (e.g., subunit) are used. Because of the unacceptable reactogenicity profile for many adjuvants, only a handful (e.g., aluminum salts, the microfluidized squalene-in-water emulsion MF59™, and monophosphoryl lipids) have been licensed for human use. The MF59 adjuvant is an oil-in-water emulsion composed of a small amount of squalene (b) (4), stabilized by the addition of two emulsifiers a water-soluble surfactant (polysorbate 80, also known as Tween 80) and an oil-soluble surfactant (sorbitan trioleate, also known as Span 85), and a low ionic strength buffer.

Biodegradable squalene oil is a natural metabolite of cholesterol and a normal component of cell membranes. The induction of a local pro-inflammatory environment within the muscle, which promotes potent immune responses, has been reported as the mechanism of action of MF59.

The oil used in MF 59 adjuvant, squalene, is obtained from shark liver and is found in humans as a metabolite of cholesterol and as a normal component of cell membranes. The squalene particle size was reduced by (b) (4), which dramatically enhances the adjuvant activity of squalene, and stabilized by the addition of an aqueous soluble emulsifier (polysorbate 80) and an oil soluble emulsifier (sorbitan trioleate). The resulting formulation was designated MF59C.1. MF59C.1 adjuvant emulsion combined with the licensed inactivated subunit trivalent influenza virus vaccine, Agriflu constitutes the Novartis vaccine adjuvanted influenza vaccine, henceforth referred to as Fluad.

### **Clinical studies:**

Thirty-nine clinical studies in elderly aged ≥65 years, including both randomized controlled studies, and uncontrolled studies mostly done for the yearly seasonal strain update, were conducted for the clinical development of Fluad. Seven of these studies were extension studies with second or third consecutive immunizations (V7P3X1, V7P3X2, V7P5X1, V7P5X2, V7P7X1, V7P8X1, and V7P25X1). The clinical database for Fluad includes subjects from different countries and continents, and hemispheres and the studies were conducted during different influenza seasons from 1992/93 to 2009/10.

These studies were conducted over a period of about 20 years, consequently the formulation of Fluad changed over time, e.g. antigen and MF59 adjuvants were initially bedside mixed. Also, the original formulation was in water and over time included different amounts of thiomersal preservative. The current Fluad formulation is thiomersal free, is formulated in citrate buffer, and is presented in single-dose prefilled syringes containing premixed MF59 adjuvant and HA antigens. Data from all 39 studies are used to support the safety profile of Fluad in this application, whereas data from five studies (V7P5, V7P8, V7P17, V7P24, V7P34)

were considered pivotal to support Fludac improved immunogenicity compared with conventional non-adjuvanted influenza vaccines (both subunit and split). In four of these pivotal studies the comparator vaccine was Agriflu, the Novartis seasonal vaccine produced with the same production platform as Fludac but without adjuvant. The fifth comparator (Flushield in study V7P24) was a split vaccine. Immunogenicity results of study V7P3 have been presented in this application in support of the greater cross reactivity to heterovariant influenza strains conferred by Fludac.

**Stability Summary:**

Stability studies were performed on the same batches of vaccine and adjuvant control as used in study number 6560-106 (see appendix 3 of this study). CPG 7909, lot no. PLI009-04 is stable for 24 months.

**Toxicity studies submitted to support this BLA:**

General toxicology studies (studies submitted in amendment 0):

- 1- 4-week vaccine toxicity study with FLUDAC® + IC31® vaccine by two intramuscular injections in (b) (4) rabbits including a 2-week recovery period (486688).
- 2- 2-dose intramuscular injection toxicity study with MF59-adjuvanted influenza vaccine with and without CpG 7909 in rabbits (6560-106).
- 3- 4-week vaccine toxicity study with Fludac®, Fludac High B, and Fludac High H3+IC31® Influenza vaccine formulations by three intramuscular injections in (b) (4) rabbits followed by a 2-week recovery period (488182).
- 4- 30-day subacute toxicity study by intramuscular route in (b) (4) rabbits treated with the test articles AGRIPPAL S1 and AGRIPPAL S1 + MF59 (940292).
- 5- Biocine™ Env 2-3 vaccine II with MTP-PE adjuvant emulsion (CGP 19 835A) comparative intramuscular tolerability study in (b) (4) dogs-90-6231.
- 6- 8-month intramuscular toxicity study of Biocine® HIV gp120 antigen and Biocine® HIV Env 2-3 antigen in rabbits (2670-100).

Genotoxicology studies (studies submitted in amendment 0):

- 1- Micronucleus cytogenetic assay in mice (g96aq62-122).
- 2- Micronucleus cytogenetic assay in mice (g96aq61-122).
- 3- Bacterial reverse mutation assay (g96aq62-502).
- 4- Bacterial reverse mutation assay (g96aq61-502).

Reproductive Study (studies submitted in amendment 19):

- 1- A reproductive and developmental toxicity study with four intramuscular injections in (b) (4) rabbits

### **Summary of the toxicology studies:**

Précis:

#### **Study # 1**

In this repeated dose toxicology study, (b) (4) rabbits were treated with MF59, Agrippal S1, or Agrippal S1 + MF59. Animals were dosed with 0.5 mL of MF59 or test article on study days (SD's) 1 and 15. Test article were administered intramuscularly into the left and right vastus lateralis muscle. Terminal sacrifice necropsies were conducted on SD 16 and recovery sacrifice necropsies were conducted on SD 30. A dose (0.5mL) equivalent, on a body weight basis, to about 30 times the human dose was used.

#### **Study # 2**

In this repeated dose toxicology study, (b) (4) rabbits were treated with MF59-adjuvanted influenza vaccine, Fluad® or MF59-adjuvanted influenza + CpG 7909. Animals in groups 1 and 2 were dosed with 0.55 mL of control or test article on study days (SD's) 1 and 15. Test article were administered intramuscularly into the hindlimb of each animal by alternating legs. Terminal sacrifice necropsies were conducted on SD 17 and recovery sacrifice necropsies were conducted on SD 29. Test article dose volume was equivalent to the proposed clinical dose.

#### **Study # 3**

In this repeated dose toxicology study, (b) (4) rabbits were treated with Fluad® or IC31. Animals in groups 1 and 2 were dosed with 0.5 mL of control or test article on study days (SD's) 1 and 15. Test article were administered intramuscularly into the hindlimb of each animal by alternating legs. Terminal sacrifice necropsies were conducted on SD 17 and recovery sacrifice necropsies were conducted on SD 29. Test article dose volume was equivalent to the proposed clinical dose.

#### **Study # 4**

In this repeated dose toxicology study, (b) (4) rabbits were treated with Fluad®, Fluad High B, or Fluad High H3+IC31® influenza vaccine formulations. Animals were treated by intramuscular injections on study days (SD's) 1, 15, and 29. Terminal sacrifice necropsies were conducted on SD 31 and recovery sacrifice necropsies were conducted on SD 43. Test article dose volume was equivalent to the proposed clinical dose.

#### **Study # 5**

In this repeated dose toxicology study, (b) (4) rabbits were treated with MF59 with or without HIV gp120 or HIV Env 2-3. Animals were dosed with 0.5 mL of control or test article on study days (SD's) 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, and 232. The injection sites were alternated between left and right hindlegs on each successive dosing interval. Terminal sacrifice necropsies



were conducted on SD 233 and recovery sacrifice necropsies were conducted on SD 247. Test article dose volume was equivalent to the proposed clinical dose.

#### Study # 6 (Genotoxicology)

(b) (4) mice were used for the micronucleus assay for this study. A total of 130 mice were placed into 5 groups. Each group was split into 3 subgroups; each subgroup used the bone marrow from each mouse at one of the following times: 24, 48, or 72 hours post dose. Each group had 15 males and 15 females; each subgroup had 5 males and 5 females. Group 1 received 20 mL/kg of 0.9% normal saline IP. Group 2 (low dose) received 20 mL/kg (1250 mg/kg) of test article IP. Group 3 (mid dose) received 20 mL/kg (2500 mg/kg) of test article IP. Group 4 (high dose) received 20 mL/kg (5000 mg/kg) of test article IP. Group 5 (positive control) received 20 mL/kg (60 mg/kg) of cyclophosphamide (CP) IP; this group was assigned 5 males and 5 females and the bone marrow was read 24 hours post dose. Each animal was dosed a single time via a needle/syringe. No prominent toxicities were found.

#### Study # 7 (Genotoxicology)

In this Ames testing study, (b) (4)

(b) (4) were used. Doses used in the definitive study were 100, 333, 1000, 3333, and 5000 µg/plate. No positive responses due to MF 59C.1 exposure with any of the tester strains in the presence and absence of (b) (4) in this bacterial reverse mutation assay were reported.

#### Study # 8 (Reproductive)

In this repeated dose toxicology study, (b) (4) rabbits were treated with control or Fluvad<sup>®</sup> (MF59-adjuvanted trivalent influenza vaccine [aTIV]). Animals were dosed with 0.5 mL of control or test article 21 days (M-21) and 7 days (M-7) before mating, then on days 7 and 20 of gestation. Test article were delivered by intramuscular injections into the dorsal lumbar muscle. Immune responses due to test article treatment were reported. Test article dose volume was equivalent to the proposed clinical dose.

### **Toxicology Studies Review**

#### **Study # 1:**

Title and study number: 30-day subacute toxicity study by intramuscular route in (b) (4) rabbits treated with the test articles AGRIPPAL S1 and AGRIPPAL S1 + MF59. Study number 940292.

**Performing laboratory:** (b) (4)

**Study initiation date:** 08/19/1994**Final Report date:** 02/01/1995**Test article batch/lot:**

Test article	Batch No.	Purity	Expiration Date
Agrippal S1	73	NR	NR
Agrippal S1 + MF59	J 52P18H1	NR	NR
MF59	MIC 838	NR	NR

NR = Not reported.

**Animal species and strain:** (b) (4) rabbits**Breeder/supplier:** (b) (4)**Number of animals per group and sex:** Eighteen per sex per group**Age:** 3 months**Body weight range:** 2.739-3.273 kg**Route and site of administration:** Intramuscular (IM) injection into the left and right vastus lateralis muscle.**Volume of injection:** 0.5 mL**Frequency of administration and study duration:** Test article was administered on study days 1 (left side) and 15 (right side) at a dose volume of 0.5 mL. Study duration was 30 days.**Dose:** 0.5 mL**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND.**Means of administration:** IM injection**Report status:** Final

This study utilized a dose (0.5mL) equivalent on a body weight basis to about 30 times the human dose.

**Experimental design**

Group	Treatment	Dose volume (mL)	Treatment days	Number of animals (#/sex/group) Treatment phase (day 16)	Number of animals (#/sex/group) Recovery phase (day 30)
1	MF59	0.5	1 and 15	3	3
2	Agrippal S1	0.5	1 and 15	3	3
3	Agrippal S1 + MF59	0.5	1 and 15	3	3

Table 1: Experimental design (study #1).

**Methods:**

Randomization procedure: Yes

Statistical analysis plan: Yes

The following parameters were evaluated: Cageside observation (twice a day), clinical signs (daily), injection site irritation (4 hours after each injection and daily

thereafter), body weights (day -6, prior to dosing [days 1 and 15], weekly thereafter, and on day 30), food consumption (not measured), ophthalmoscopy (day -4 and before sacrifice), body temperature (once during the pre-dose phase, before and 48 hours after each dose, weekly thereafter [days 8 and 22] and before sacrifice), haematology, clinical chemistry, and coagulation (day -5, 48 hours after dosing, and before sacrifice), gross anatomy at termination (SDs 16 and 30), organ weights and histopathology on a selection of tissues. Blood samples for antibody-determination (not measured).

Parameters	Frequency of Testing
Cageside observation <sup>1</sup>	Twice a day
Clinical observations <sup>2</sup>	Daily
Body weight	Day -6, prior to dosing [days 1 and 15], weekly thereafter, and on day 30
Food consumption	Not measured
Body temperature	Once during the pre-dose phase, before and 48 hours after each dose, weekly thereafter [days 8 and 22], and before sacrifice
Ophthalmologic exam	Day -4 and before sacrifice
Clinical chemistry*	Day -5, 48 hours after dosing, and before sacrifice
Hematology*	Day -5, 48 hours after dosing, and before sacrifice
Coagulation*	Day -5, 48 hours after dosing, and before sacrifice
Immunological response	Not measured
Evaluation of site of inoculation (e.g., the dermal Draize scoring method)	4 hours after each injection and daily thereafter
Necropsy	SDs 16 and 30
Tissues for histopathology	SDs 16 and 30

\* Collected from the auricular vein.

Table 2: Parameters evaluated (study #1).

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	

<sup>1</sup> Cageside observations include mortality, morbidity, general health and signs of toxicity.

<sup>2</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Organ/Tissue	Collected	Not collected
Bone (sternum & femur)		X
Bone marrow smear (femoral)	!	
Brain	!*	
Cecum	!	
Colon	!	
Duodenum	!	
Epididymides	!*	
Esophagus	!	
Eyes (with optic nerve)	!	
Femur, including articular surface	!	
Gall bladder	!	
Lesions	!	
Harderian gland		X
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands	!	
Liver	!*	
Lung (with bronchi)	!	
Lymph nodes (mesenteric)	!	
Lymph nodes (cervical)	!	
Ovaries	!*	
Pancreas	!	
Peyer's patch		X
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands (submandibular)	!	
Sciatic nerve	!	
Seminal vesical		X
Skeletal muscle	!	
Skin and mammary gland	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Sternum with bone marrow	!	
Stomach	!	
Testes	!*	

Organ/Tissue	Collected	Not collected
Thymus	!*	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters		X
Uterus	!	
Urinary bladder	!	
Vagina	!	
Vertebrae	!	
Zymbal's gland (if applicable)		X

Table 3: Tissues collected (study #1).

Table of Histology – Tissues identified in the table above were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

## Results:

Morbidity and mortality: All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Lactic dehydrogenase Week 3 M&F $\downarrow \leq 0.7$ G2	Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Total bile acids
B) HEPATOBILIARY	Alkaline phosphatase (ALP) Week 3 M&F $\uparrow \geq 1.7$ G2	Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides Week 3 M&F $\uparrow \geq 1.6$ G2 Week 3 M&F $\uparrow \geq 1.6$ G3	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase

Table 4: Clinical chemistry results (study #1).

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>3</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Monocyte count SD -1 M&F $\uparrow \geq 2.1$ G2 Week 1 M&F $\downarrow \leq 0.5$ G2 Week 3 M&F $\downarrow \leq 0.0$ G3  Eosinophils count Week 1 M&F $\downarrow \leq 0.5$ G3 Week 5 M&F $\downarrow \leq 0.7$ G2	Basophils count Lymphocyte count Macrophage Large Unstained Cells (LUC) Total Leukocytes (WBC) Neutrophil count
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Table 5: Hematology results (study #1).

**Systemic toxicity:**

There were no test article-related effects on clinical observations, body weight, body temperature, ophthalmic examinations, macroscopic, and microscopic findings were reported.

Lactic dehydrogenase (LDH) levels were decreased in group 2 (males and females mean value) on week 3. Alkaline phosphatase (ALP) levels were increased in group 2 (males and females mean value) on week 3. Triglyceride levels were increased in groups 2 and 3 (males and females mean value) on week 3. Beta globulin levels were significantly decreased in group 2 (males and females mean value) on day 3.

Monocyte levels were increased in group 2 (males and females mean value) on week -1. Monocyte levels were decreased in groups 2 and 3 (males and females

<sup>3</sup> With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

mean value) on weeks 1 and 3, respectively. Eosinophil levels were decreased in groups 3 and 2 (males and females mean value) on weeks 1 and 5, respectively. Mean corpuscular hemoglobin concentration and partial thromboplastin time were decreased in group 3. Prothrombin time was decreased in both treated groups 48 hours after the 2<sup>nd</sup> injection.

**Organ Weight:**

Body and Organs Weight	MALES- FEMALES (DOSING PHASE/RECOVERY PHASE)  1 (CONTROL)	MALES- FEMALES (DOS NG PHASE/RECOVERY PHASE)  2	MALES- FEMALES (DOS NG PHASE/RECOVERY PHASE)  3
	NUMBER OF ANIMALS 3/3	NUMBER OF ANIMALS 3/3	NUMBER OF ANIMALS 3/3
BODY WEIGHT (terminal)	3090/3450	3240/3370	3180/3470
BRAIN	9.11/9.19	9.87/9.33	9.39/9.00
ADRENALS	0.19/0.21	0.21/0.22	0.20/0.21
EPIDIDYMIDES	2.28/2.23	1.92*/2.25	2.19/2.44
HEART	6.61/7.63	7.15/6.98	6.74/7.35
KIDNEYS	14.7/16.5	15.3/15.8	15.0/16.1
LIVER	63.9/69.6	69.6/71.0	67.7/69.9
SPLEEN	1.25/1.43	1.50/1.32	1.46/1.45
TESTES	4.07/5.58	4.45/5.49	4.31/4.78
THYROID and PARATHYROID	ND	ND	ND
THYMUS	3.01/3.89	3.72/4.19	3.24/4.01
OVARIES	0.201/0.204	0.232/0.268	0.318*/0.254
UTERUS	ND	ND	ND

Absolute weights are expressed as mean (grams). \*Different from controls at  $P \leq 0.05$ . ND = Not determined.

Table 6: Organs weight (study #1).

Epididymides weight was significantly reduced (16%) in group 2 males at the dosing phase. At the dosing phase, spleen weight was increased 20% and 17% in groups 2 and 3, respectively. At the recovery phase, testes weight was decreased 14% in group 3. At the dosing phase, thymus weight was increased 24% in group 2. At the dosing phase, ovaries weight was increased 15% and 58% (statistically significant) in groups 2 and 3, respectively. At the recovery phase, ovaries weight was increased 31% and 25% in groups 2 and 3, respectively.



## Gross Pathology:

Group	Findings (intermediate phase)
1M&F	Slight hemorrhagic area at the right injection site (1/6)
2M&F	Slight hemorrhagic area at the right injection site (1/6); slight dark firm area (1/6)
3M&F	Slight hemorrhagic area at the right injection site (4/6)

Table 7: Macroscopic findings (study # 1).

Microscopic finding in intermediate phase// final (recovery) phase animals are listed below:

Groups	Findings (intermediate phase// final (recovery) phase)
1M&F	<p>Slight decreased spermatid elements in epididymides (3/6); slight aggregate of mononuclear cells adjacent to degenerative or necrotic cells in liver (5/6); slight vacuolization consistent with fatty change hepatocellular in liver (3/6); slight inflammation-subacute periportal in liver (4/6); slight mineralization in ovaries (1/6); slight decreased spermatogenesis tubules in testes (2/6); slight multinucleated spermatid tubules in testes (1/6)// slight aggregate of mononuclear cells adjacent to degenerative or necrotic cells in liver (4/6); slight necrosis hepatocellular in liver (1/6); slight vacuolization consistent with fatty change hepatocellular in liver (2/6); slight inflammation-subacute periportal in liver (6/6); slight erythrophagocytosis sinus in mesenteric lymph nodes (1/6); slight inflammation-subacute interstitium in thymus (1/6)</p> <p>Right injection site: Slight hemorrhage interstitium (3/6); moderate acute inflammation interstitium (1/6); slight inflammation-subacute interstitium (5/6); slight degeneration muscle (6/6)// slight acute inflammation interstitium (1/6); slight inflammation-subacute interstitium (4/6); slight degeneration muscle (4/6)</p> <p>Left injection site: Slight hemorrhage interstitium (2/6); slight inflammation-subacute interstitium (6/6); slight degeneration muscle (4/6)// slight inflammation-subacute interstitium (3/6); slight degeneration muscle (2/6)</p>
2M&F	Slight decreased spermatid elements in epididymides (3/6); slight aggregate of mononuclear cells adjacent to degenerative or necrotic cells in liver (5/6); slight vacuolization consistent with fatty change hepatocellular in liver (1/6); slight

Groups	Findings (intermediate phase// final (recovery) phase)
	<p>inflammation-subacute periportal in liver (5/6); slight acute inflammation in lungs (1/6); slight erythrophagocytosis sinus in mesenteric lymph nodes (1/6); slight mineralization in ovaries (2/6); slight decreased spermatogenesis tubules in testes (1/6); slight multinucleated spermatids tubules in testes (2/6)// slight aggregate of mononuclear cells adjacent to degenerative or necrotic cells in liver (5/6); slight pigment-laden macrophages in liver (1/6); slight necrosis hepatocellular in liver (2/6); slight vacuolization consistent with fatty change hepatocellular in liver (2/6); slight inflammation-subacute periportal in liver (6/6); slight inflammation-subacute interstitium in thymus (1/6)</p> <p>Right injection site: Slight hemorrhage interstitium (1/6); moderate acute inflammation interstitium (4/6); slight inflammation-subacute interstitium (2/6); moderate degeneration muscle (5/6)// slight hemorrhage interstitium (1/6); moderate acute inflammation interstitium (1/6); slight inflammation-subacute interstitium (2/6); slight degeneration muscle (3/6)</p> <p>left injection site: Slight hemorrhage interstitium (2/6); slight acute inflammation interstitium (1/6); slight inflammation-subacute interstitium (3/6); slight degeneration muscle (4/6)// slight inflammation-subacute interstitium (1/6); slight degeneration muscle (3/6)</p>
3M&F	<p>Slight decreased spermatid elements in epididymides (1/6); slight aggregate of mononuclear cells adjacent to degenerative or necrotic cells in liver (6/6); slight vacuolization consistent with fatty change hepatocellular in liver (1/6); slight inflammation-subacute periportal in liver (5/6); slight mineralization in ovaries (2/6); slight extramedullary hematopoiesis in spleen (1/6); slight dilatation tubules in testes (1/6); slight multinucleated spermatids tubules in testes (1/6)// slight aggregate of mononuclear cells adjacent to degenerative or necrotic cells in liver (4/6); slight inflammation-subacute periportal in liver (6/6); slight erythrophagocytosis sinus in mesenteric lymph nodes (2/6)</p> <p>Right injection site: Slight hemorrhage interstitium (4/6); severe acute inflammation interstitium (3/6); moderate inflammation-subacute interstitium (3/6); slight degeneration muscle (6/6)// slight inflammation-subacute interstitium (6/6); slight</p>

Groups	Findings (intermediate phase// final (recovery) phase)
	degeneration muscle (4/6)  left injection site: Slight hemorrhage interstitium (1/6); moderate acute inflammation interstitium (1/6); slight inflammation-subacute interstitium (5/6); slight degeneration muscle (5/6)// slight inflammation-subacute interstitium (4/6)

Table 8: Microscopic findings (study # 1).

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

### Body temperature

No test-article related changes in body temperature were reported. At day 15, a group 2 mean temperature was slightly higher than in the control group. No temperature above 40° C was reported.

Group	Males	Females
1Control	0	0
2	0	0
3	0	0

Table 9: Body temperature (study # 1).

Table of occurrences for body temperature  $\geq 40^{\circ}$  C.

### Local toxicity:

No test article-related effects at the injection sites, including Draize scoring, were reported in any group.

Serology: Not determined.

Test article related effects are listed in the table below:

Test article related effects
↑ Triglyceride ↓ Monocytes ↓ Eosinophils ↑ Spleen weight ↑ Thymus weight ↑ Ovary weight

Assessment:

There were no test article-related effects on clinical observations, body weight, body temperature, ophthalmic examinations, macroscopic, and microscopic findings were reported.

Triglyceride is a blood lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so. They are formed by combining glycerol with three fatty acid molecules. High levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease<sup>4</sup> and stroke.<sup>5</sup>

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The decreases in the monocyte count reported in this study might be related to test article-treatment. Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood.

Spleen weight increase might be related to the intended immune response. The spleen plays important roles in regard to red blood cells and the immune system<sup>6</sup>. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal<sup>7</sup>. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation. The increase in thymus weight might be related to the immune responses due to test article-treatment.

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<sup>4</sup> "Boston scientists say triglycerides play key role in heart health". The Boston Globe. Retrieved 2014-06-18.

<sup>5</sup> Drummond *et al.* (2014) *Nutrition for Foodservice and Culinary Professionals* 8th Ed., John Wiley & Sons.

<sup>6</sup> Spleen, Internet Encyclopedia of Science.

<sup>7</sup> Mebius RE, Kraal G. (2005). Structure and function of the spleen. *Nat Rev Immunol.* 5(8):606-16.

The increase in ovary weight was not associated with any macroscopic or microscopic findings. Since the mineralization in ovaries was reported in the control group, this finding was not considered test article related.

**GLP study deviations or amendments:** Sections for study deviations or amendments were not included in this study.

**Conclusions:** Based on nonclinical toxicity assessments of this study there are no significant safety issues to report.

## Study # 2:

Title and study number: 2-Dose intramuscular injection toxicity study with MF59-adjuvanted influenza vaccine with and without CpG 7909 in rabbits. Study number 6560-106.

**Performing laboratory:** (b) (4)

**Study initiation date:** 06/09/2006

**Final Report date:** 12/06/2007

### Test article batch/lot:

Test article	Lot No.	Purity	Expiration Date
MF59-Adjuvanted Influenza Vaccine (Fluad®)	056801	Evidence of HA bands and absence or traces of NP and M components	07/31/2006
CpG 7909:			
CTS Lot No.	046264		
Vendor Lot No.	PLI009-04	96.8%	03/31/2007
0.9% Sodium Chloride Injection, USP	33-193-JT 37-180-JT	Meets requirements, USP	09/01/2007 01/01/2008

**Animal species and strain:** Male and female Hra: (b) (4) rabbits

**Breeder/supplier:** (b) (4)

**Number of animal per group and sex:** Eight per sex per group

**Age:** 13.5 weeks

**Body weight range:** 2251-2521 grams for males and 2212-2521 grams for females

**Route and site of administration:** Intramuscular (IM) injection into the epaxial muscle (longissimus dorsi)

**Volume of injection:** 0.55 mL

**Frequency of administration and study duration:** Test article was administered on study days 1 (right side) and 15 (left side) at a dose volume of 0.55 mL. Study duration was 29 days.

**Dose:** Total 45 ug antigens/trivalent dose and 500 ug of CPG 7909

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study (see appendix 3). CPG 7909, lot no. PLI009-04 is stable for 24 months.

**Means of administration:** IM injection

**Report status:** Final

This study utilized the clinical dose of Fluad® (MF59-adjuvanted influenza vaccine) and the highest anticipated clinical dose of CpG 7909 (500 µg). The frequency of dosing was compressed relative to the proposed clinical regimen but was sufficient to allow for an appropriate immune response.

#### Experimental design

Group	Dose composition Influenza antigens (µg/strain)	Dose composition MF59 (mL)	Dose composition CpG 7909 (µg)	Dose volume (mL)	Number of animals (#/sex/group). Treatment phase	Number of animals (#/sex/group). Recovery phase
1 (Control) MF59- adjuvanted influenza vaccine; Fluad®	15*	0.25	0	0.55	4	4
2 MF59- adjuvanted influenza + CpG 7909	15	0.25	500	0.55	4	4

\* Total 45 µg antigens/trivalent dose.

Table 10: Experimental design (study # 2).

**Methods:** Blood samples were collected from the jugular vein from all animals once during the pre-dose phase, on days 3 and 17 of the dosing phase, and on day 13 of the recovery phase. The anticoagulant was sodium citrate for coagulation tests and potassium EDTA for hematology tests. Samples for clinical chemistry were collected without anticoagulant.

Endpoint	Methodology
Hematology	NR*
Clinical chemistry	NR
Coagulation	NR

\* NR = Not reported

Randomization procedure: Yes

Statistical analysis plan: Leven's test, ANOVA, and t-test were used.

The following parameters were evaluated: clinical signs (twice daily), irritation score (pre-dose phase and approximately 24 and 48 hours post-dose on days 1 and 15 of the dosing phase), body weights (at least once during the pre-dose phase, prior to dosing on the first day of the dosing phase, and weekly thereafter), food consumption (once daily, qualitatively during dosing and recovery phases), ophthalmoscopy (once during the pre-dose phase, before the dosing phase final phase sacrifice (all animals), and before the recovery phase final phase sacrifice [all recovery animals]), body temperature (once during the pre-dose phase and prior to dosing and approximately 2 and 4 hours post-dosing on days 1 and 15 of the dosing phase), heart rate, oxygen saturation, and respiration rate (once during the pre-dose phase, and on days 1, 14, and 15), hematology and clinical chemistry (pre-dose phase, on days 3 and 17 of the dosing phase, and on day 13 of the recovery phase), gross anatomy at termination, organ weights and histopathology on a selection of tissues. Blood samples for antibody-determination (once during the pre-dose phase, pre-dose on day 15, and on the day of the recovery phase sacrifice) were taken and analyzed (non-GLP) under the responsibility of the sponsor.

Parameters	Frequency of Testing
Cageside observation <sup>8</sup>	Once daily during the dosing and the recovery phases
Clinical observations <sup>9</sup>	Twice daily
Body weight	At least once during the pre-dose phase, prior to dosing on the first day of the dosing phase, and weekly thereafter
Food consumption	Once daily, qualitatively during dosing and recovery phases
Body temperature	Once during the pre-dose phase and prior to dosing and approximately 2 and 4 hours post-dose on days 1 and 15 of the dosing phase
Heart rate, oxygen saturation, and respiration rate	Once during the pre-dose phase, and on days 1, 14, and 15
Ophthalmologic exam	Once during the pre-dose phase, before the dosing phase final phase sacrifice (all animals), and before the recovery phase final phase sacrifice (all recovery animals)
Clinical chemistry*	Pre-dose phase, on days 3 and 17 of the dosing phase, and on day 13 of the recovery phase

<sup>8</sup> Cageside observations include mortality, morbidity, general health and signs of toxicity.

<sup>9</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Parameters	Frequency of Testing
Hematology*	Pre-dose phase, on days 3 and 17 of the dosing phase, and on day 13 of the recovery phase
Coagulation*	Pre-dose phase, on days 3 and 17 of the dosing phase, and on day 13 of the recovery phase
Immunological response	Once during the pre-dose phase, pre-dose on day 15, and on the day of the recovery phase sacrifice
Evaluation of site of inoculation (e.g., the dermal Draize scoring method)	Pre-dose phase and approximately 24 and 48 hours post-dose on days 1 and 15 of the dosing phase
Necropsy	SDs 17 and 29
Tissues for histopathology	SDs 17 and 29

\* Collected from the jugular vein.

Table 11: Parameters evaluated (study # 2).

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	! <sup>++</sup>	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum)	! <sup>+</sup>	
Brain	! <sup>++</sup>	
Cervix	! <sup>+</sup>	
Colon	!	
Duodenum	!	
Epididymides	! <sup>+</sup>	
Esophagus	!	
Eyes (optic nerve)	! <sup>+</sup>	
Fallopian tubes (oviduct)		X
Gall bladder	! <sup>*</sup>	
Lesions	!	
Harderian gland		X
Heart	! <sup>++</sup>	
Ileum	!	
Injection site(s)	! <sup>+</sup>	
Jejunum	!	
Kidneys	! <sup>++</sup>	
Lacrimal glands		X



Organ/Tissue	Collected	Not collected
Larynx	!	
Liver	!+*	
Lung (large bronchi)	!+	
Lymph nodes (axillary)	!+	
Lymph nodes (mandibular)	!+	
Lymph nodes (medial iliac)	!+	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	!+*	
Pancreas	!	
Peyer's patch	!+	
Pituitary gland	!	
Prostate	!+	
Rectum	!	
Salivary glands (mandibular, parotid, and sublingual)	!	
Sciatic nerve	!	
Seminal vesical	!+	
Skeletal muscle	!	
Skin/subcutis	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!+*	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters	!	
Uterus (w/ cervix)	!+	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		X

Table 12: Tissues collected (study # 2).

Table of Histology – Tissues labeled with a + sign were preserved and examined microscopically. All remaining tissues will be held (wet) for possible future examination.

## Results:

Morbidity and mortality: All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ )	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Sorbitol dehydrogenase (SDH) SD 3 M $\uparrow \geq 1.7$ G2	Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Total bile acids
B) HEPATOBILIARY	Total bilirubin SD 17 M $\downarrow \leq 0.3$ G2	Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides SD 29 F $\downarrow \leq 0.7$ G2	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase

C-reactive protein was not measured.

Table 13: Clinical chemistry results (study # 2).

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>10</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Neutrophil count SD 3 M $\uparrow \geq 2.5$ G2  Monocyte count SD 3 M $\uparrow \geq 2.1$ G2 SD 17 M $\uparrow \geq 1.7$ G2  Total Leukocytes (WBC) SD 3 M $\uparrow \geq 2.0$ G2 SD 29 F $\downarrow \leq 0.7$ G2	Basophils count Eosinophils count Lymphocyte count Macrophage Large Unstained Cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Table 14: Hematology results (study # 2).

**Systemic toxicity:**

There were no test article-related effects on clinical observations, body weight, body temperature, respiration rate, heart rate, oxygen saturation, ophthalmic examinations, organ weights, macroscopic, and microscopic findings were reported.

In both dose groups and sexes, body weight remained steady throughout the dosing phase. Mean body weight of males in group 2 was statistically different from group 1 on SD 13 of the recovery phase. This was considered an isolated, spurious finding.

<sup>10</sup> With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

Sorbitol dehydrogenase (SDH) levels were increased significantly in group 2 males on SD 3. Total bilirubin in group 2 males and triglyceride levels in group 2 females were reduced on SD's 17 and 29, respectively. Several other statistically significant or otherwise notable differences for clinical pathology test results were observed between control and treated animals. Some of the differences were considered incidental because they were extremely small, inconsistent over time and between sexes, similar to differences present before initiation of treatment, and/or not correlated with other findings.

Minimally to mildly higher white blood cell count, absolute neutrophil count, and absolute monocyte count for males and moderately higher fibrinogen, mildly higher globulin, and mildly lower albumin-to-globulin ratio for males and females were differences considered test article-related. These findings were observed after each dose (i.e., days 3 and 17 of the dosing phase), and exhibited reversibility following recovery. The leukocyte and fibrinogen effects were completely reversed, and the globulin and albumin-to-globulin ratio effects were at least partially reversed by SD 13 of the recovery phase. These effects were consistent with an inflammatory response. Minimally lower red blood cell count, hemoglobin, and hematocrit and minimally higher activated partial thromboplastin time for males were of uncertain relationship to test article administration. These minor differences were not considered toxicologically meaningful.

#### Organ Weight:

SEX	MALES (DOSING PHASE/RECOVERY PHASE)	MALES (DOSING PHASE/RECOVERY PHASE)	FEMALES (DOSING PHASE/RECOVERY PHASE)	FEMALES (DOS NG PHASE/RECOVERY PHASE)
GROUPS	1 (CONTROL)	2	1 (CONTROL)	2
NUMBER OF ANIMALS	4/4	4/4	4/4	4/4
BODY WEIGHT (terminal)	2626/2892	2663/2787	2579/2888	2605/2830
BRAIN	9.15/9.37	9.20/9.11	9.39/9.47	8.95/9.34
ADRENALS	0.26/0.32	0.24/0.29	0.26/0.26	0.32/0.36
EPIDIDYMIDES	ND	ND		
HEART	5.91/6.52	6.36/7.29	5.59/5.99	5.88/6.29
KIDNEYS	14.48/15.30	15.57/16.40	14.63/14.96	13.62/15.07
LIVER	72.34/77.19	71.87/83.13	70.21/69.95	70.58/69.74
SPLEEN	1.43/1.37	1.60/1.34	1.63/1.47	1.61/1.62
TESTES	3.25/4.67	2.54/3.74*		
THYROID and PARATHYROID	ND	ND	ND	ND
THYMUS	4.12/3.32	4.66/4.53	4.44/3.71	4.12/4.62*
OVARIES			0.28/0.23	0.27/0.22
UTERUS			ND	ND

Absolute weights are expressed as mean (grams). \*Different from controls at  $P \leq 0.05$ . ND = Not determined.

Table 15: Organs weight (study # 2).

Testes weight was reduced significantly in males at the recovery period.  
Significant increase in the thymus weight was reported in test-article treated females at the recovery period.

Gross Pathology:

Group	Findings (Dosing phase/Recovery phase)
1M	NF// NF
2M	Discolored IM left site (1/4); discolored IM right site (1/4)// NF
1F	Discolored IM left site (1/4); discolored IM right site (1/4)// cyst in the oviduct (1/4)
2F	NF// NF

NF = No findings

Table 16: Macroscopic findings (study # 2).

Microscopic finding in dosing phase animals are listed below:

Groups	Findings
1M	Alveolus macrophages infiltrate in lung (1/4); acute inflammation in lung (1/4); lymphocyte/macrophages infiltrate in kidney (2/4); tubule mineralization in kidney (2/4); lymphocyte/macrophages infiltrate in liver (1/4); lymphocyte/macrophages infiltrate in heart (1/4); erythrophagocytosis in mandibular lymph node [LN] (2/4); hemorrhage in left medial iliac LN (1/4); chronic active inflammation in intramuscular left (2/4) and right (4/4) sites; necrosis in intramuscular left site (1/4)
2M	Lymphocyte infiltrate in lung (1/4); lymphocyte/macrophages infiltrate in liver (3/4); erythrophagocytosis in mandibular lymph node [LN] (1/3); atrophy/degeneration in testis (3/4); hemorrhage in left medial iliac LN (1/4); acute inflammation in left medial iliac LN (1/4); edema in intramuscular left and right sites (1/4); chronic active inflammation in intramuscular left (3/4) and right (2/4) sites; necrosis in intramuscular left site (1/4); degeneration/necrosis in intramuscular right site (1/4)
1F	Lymphocyte infiltrate in lung (2/4); alveolus macrophages infiltrate in lung (1/4); acute inflammation in lung (2/4); lymphocyte/macrophages infiltrate in liver (4/4); erythrophagocytosis in mandibular lymph node [LN] (1/4); mineralization in ovary (4/4); hemorrhage and acute inflammation in left and right medial iliac LN (1/4); chronic active inflammation in intramuscular left (1/4) and right (3/4) sites
2F	Alveolus macrophages infiltrate in lung (1/4); acute inflammation in lung (2/4); tubule mineralization in kidney (1/4); lymphocyte/macrophages infiltrate in liver (2/4); mineralization in ovary (3/4); hemorrhage in left and right medial iliac LN (1/4); acute inflammation in left (2/4) and right (1/4) medial iliac LN; chronic

Groups	Findings
	active inflammation in intramuscular left (1/4) and right (4/4) sites

Microscopic finding in recovery phase animals are listed below:

Groups	Findings
1M	Acute inflammation in lung (2/4); lymphocyte/macrophages infiltrate in liver (2/4); hemorrhage in thymus (1/4); edema in mandibular lymph node [LN] (2/4); hemorrhage in mandibular LN (1/4); hemorrhage in right axillary LN (1/4); chronic active inflammation in intramuscular right site (1/4); fibrosis in intramuscular right site (1/4)
2M	Alveolus macrophages infiltrate in lung (3/4); acute inflammation in lung (2/4); hemorrhage in right axillary LN (1/4); chronic active inflammation in intramuscular right site (1/4)
1F	Lymphocyte infiltrate in lung (2/4); alveolus macrophages infiltrate in lung (1/4); acute inflammation in lung (3/4); tubule mineralization in kidney (1/4); lymphocyte/macrophages infiltrate in liver (1/4); sinusoids dilatation in mandibular LN (1/4); hemorrhage in mandibular LN (1/4); mineralization in ovary (2/4); hemorrhage and acute inflammation in right medial iliac LN (1/4); chronic active inflammation in intramuscular left (2/4) and right (3/4) sites; cyst in oviduct (1/4)
2F	Hemorrhage in lung (1/4); alveolus epithelium hyperplasia in lung (1/4); alveolus macrophages infiltrate in lung (2/4); acute inflammation in lung (2/4); lymphocyte/macrophages infiltrate in liver (1/4); chronic active inflammation in liver (1/4); mineralization in ovary (3/4); epithelium hyperplasia in ovary (1/4); acute inflammation in left medial iliac LN (1/4); chronic active inflammation in intramuscular left (1/4) and right (1/4) sites

Table 17: Microscopic findings (study # 2).

An extensive number of tissues were examined for histology. Findings relative to the kidney, liver, lung, heart, LN, and ovary appeared evenly distributed between treated and control groups of animals. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

### Body temperature

No test-article related changes in body temperature were reported.

Group	Males	Females
1Control	0	0
2	0	0

Table of occurrences for body temperature  $\geq 40^{\circ}$  C

Table 18: Body temperature (study # 2).

**Local toxicity:**

Draize scoring of the injection site revealed the following as presented in the table below<sup>11</sup>.

**Erythema/Edema**

Treatment group	Frequency of score to +3 days post-injection (day of injection is day 0). Erythema/Edema.									
	Test site A (n=8)					Test site B (n=8)				
		1	2	3	4		1	2	3	4
1 M		2/0	0/0	0/0	0/0		0/1	0/0	0/0	0/0
2 M		0/2	0/1	0/0	0/0		1/1	0/0	0/0	0/0
1 F		0/0	0/0	0/0	0/0		0/0	0/0	0/0	0/0
2 F		0/1	0/0	0/0	0/0		1/1	0/0	0/0	0/0

Test Site A = right longissimus dorsi. Test Site B = left longissimus dorsi. M = Males. F = Females.

Table 19: Draize scoring at the injection site (study # 2).

Minor dermal irritation, consisting of very slight or slight erythema or edema, was observed for up to 3 days after dosing in control and test article treated animals. No evidence of exacerbation was apparent with the inclusion of CpG 7909, in the formulation.

The microscopic findings were typical of those expected from intramuscular injections and were reduced at the recovery phase sacrifice.

**Serology:**

Sera collected from all animals at pre-dose had titers of anti-A/NC/20/99, anti-A/NY/55/04 and anti-B/Jiangsu/10/03 below limit of detection when tested by hemagglutination inhibition assays (HAI). Positive titers were detected for all three antigens on SD 15 in response to the first dose administration for each test article.

Thus, both vaccine formulations were considered immunogenic. An increase in the titers in the SD 29 samples, collected after the second administration, substantiated this finding.

<sup>11</sup> Draize, Dermal Toxicity, In: Association of Food and Drug Officials US Appraisal of the Safety of Chemicals and Food, Drugs and Cosmetics, pp 46-59, Texas State Dept of Health, Austin, 1959.

Table of antibody titers: anti-A/NC/20/99, anti-A/NY/55/04, and anti-B/Jiangsu/10/03 titers

Group	Dose	HAI Titer anti-A/NC/20/99 SD 15 (n=8)	HAI Titer anti-A/NC/20/99 SD 29 (n=4)	HAI Titer anti-A/NY/55/04 SD 15 (n=8)	HAI Titer anti-A/NY/55/04 SD 29 (n=4)	HAI Titer anti-B/Jiangsu/10/03 SD 15 (n=8)	HAI Titer anti-B/Jiangsu/10/03 SD 29 (n=4)
<u>Males</u> 1	Control MF59- adjuvanted influenza vaccine; Fluad®	113	761	905	2153	247	640
2 (n=8)	MF59- adjuvanted influenza + CpG 7909	147	1280	830	3044	269	1076
<u>Females</u> 1 (n=8)	Control MF59- adjuvanted influenza vaccine; Fluad®	104	761	640	2153	269	640
2 (n=8)	MF59- adjuvanted influenza + CpG 7909	135	1280	830	3044	381	761

Table 20: Serology results (study # 2).

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental
Injection sites findings ↑ Fibrinogen ↑ Globulin ↓ Albumin-to-globulin ratio	Clinical chemistry findings Hematology findings

#### Assessment:

In summary, as based on in-life findings and clinical and anatomic pathology, there were no adverse findings in male or female (b) (4) rabbits following treatment with MF59-adjuvanted influenza vaccine + CpG 7909.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

The increase in mean globulin values and the decrease in A/G ratio might be the result of an increase in immunoglobulin synthesis due to polyclonal activation of B lymphocytes by the adjuvant.

There were no clear treatment-related effects on clinical pathology parameters, although there were a number of statistically significant differences (data not



shown). Many of these differences were of a magnitude or nature that was not clinically significant or that remained within the normal range of values established for gender, laboratory or species.

Adverse gross or microscopic alteration that could be indicative of systemic or local toxicity was not observed.

There were no treatment-related effects on clinical observations, body weight, body temperature, respiration rate, heart rate, oxygen saturation, ophthalmic examinations, organ weights, macroscopic, and microscopic findings were reported.

Treatment-related toxicity was only observed at the sites of inoculation in terms of Draize scoring and gross and/or histopathology and was primarily attributable to inflammation due to the intended immune response to the vaccine. These findings were recoverable and were predominately mild to moderate.

Immunology performed in this study verified that an active dose was administered. No differences in immune responses were found between male and female animals.

**GLP study deviations or amendments:** No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

**Conclusions:** Based on nonclinical toxicity assessments of this study there are no significant safety issues to report. The delivery of an active dose of the product in the toxicology study was verified.

### **Study # 3:**

Title and study number: 4-week vaccine toxicity study with FLUAD® + IC31® vaccine by two intramuscular injections in (b) (4) rabbits including a 2-week recovery period. Study number 486688.

**Performing laboratory:** (b) (4)

**Study initiation date:** 10/26/2007

**Final Report date:** 09/15/2009

**Test article batch/lot:**

<u>Test article</u>	<u>Batch No.</u>	<u>Purity</u>	<u>Expiration Date</u>
Fluad® (180063/A)	079903	100%	07/31/2008
IC31 (180297/A)	IC31-h-P03207/01	100%	10/31/2008

**Animal species and strain:** SPF-quality Albino, (b) (4) rabbits

**Breeder/supplier:** (b) (4) .

**Number of animal per group and sex:** Four per sex per group

**Age:** 12-14 weeks

**Body weight range:** 2255-2780 grams

**Route and site of administration:** Intramuscular (IM) injection into the right and left hindlimbs (total injection volume: 1.0 mL per treatment day).

**Volume of injection:** Test article Fluad®; 0.50 mL for groups 1 and 2. Test article IC31; 0 and 0.5 mL for groups 1 and 2, respectively. Injection volume was 1.0 mL per treatment day, divided into 2 injections of 0.5 mL.

**Frequency of administration and study duration:** Test article was administered on study days 1 and 15. Study duration was 29 days.

**Dose:**

Fluad® (180063/A): 45 µg per dose, 15 µg HA from each of the three influenza strains A/Solomon Islands/3/2006 IVR-145, A/Wisconsin/67/2005 NYMCX-161-B, and B/Malaysia/2506/2004, combined with MF59 adjuvant (0.25 mL per dose).

IC31 (180297/A): The test substance (IC31) is an adjuvant containing 500 nmol peptide (KLK) and 20 nmol oligodeoxynucleotide (ODN1a) in PBS buffer. Total volume of adjuvant per dose is 0.5 mL. The adjuvant will be combined with antigens prior to dosing.

The dose volume of 1.0 mL Fluad® + IC31® containing 0.5 mL Fluad® and 0.5 mL IC31® was selected because it is the maximum proposed dose to be used in a human clinical trial.

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study (see appendix: certificate of analysis). Batch number IC31-h-P03207/01 was manufactured on (b) (4) and the expiration date listed for this batch was October, 2008. According to this information, IC31 is stable for 18 months. As for Fluad® (180063/A), only the expiration date of 07/31/2008 was included in this report. Expiration dates for both test articles exceeded the experimental completion date of December 3<sup>rd</sup>, 2007.

**Means of administration:** IM injection

**Report status:** Final

Animals were assigned to 2 different groups. Four animals per sex per group were treated with saline or test article on SD's 1 and 15. Four rabbits/sex/group of main and recovery sacrifices was euthanized on SD's 17 and 29, respectively. The details of the study design are listed in the following table:

## Experimental design

Group	Test Substance	Dose Volume (mL)	Number of Animals Treatment Phase (males/females)	Number of Animals Recovery Phase (males/females)
1	Fluad® (180063/A)	0.5 mL Fluad® + 0.5 mL Saline	4/4	4/4
2	IC31 (180297/A)	0.5 mL Fluad® + 0.5 mL IC31®	4/4	4/4

Table 21: Experimental design (study # 3).

Methods: Haematology, clinical chemistry, and coagulation parameters were determined using the following:

Endpoint	Methodology
Hematology	(b) (4)
Clinical chemistry	(b) (4)
Coagulation	(b) (4)

Randomization procedure: Yes

Statistical analysis plan: Dunnet-test, Steel-test, and Fisher-exact test were used.

The following parameters were evaluated: Clinical signs (at least once daily), skin irritation score (approximately 24 and 48 hours post-dose on days 1 and 15 of the dosing phase), body weights (weekly during pre-test, treatment and recovery period, and on SD 17), food consumption (twice weekly during pretest, treatment and recovery period, and on SD 17), ophthalmoscopy (once during pre-test and at the end of treatment and recovery phases), body temperature, heart rate, and respiratory rate (once during pre-test, prior to each dose and approximately 2 hours after dosing [days 1 and 15]), coagulation, haematology, and clinical chemistry (pre-test and on days 3, 17 and 29), gross anatomy at termination, organ weights and histopathology on a selection of tissues (days 17 and 29). Blood samples for antibody-determination (pre-test, prior to dosing on day 15, and prior to necropsy on days 17 and 29) were taken and analyzed (non-GLP) under the responsibility of the sponsor.

Parameters	Frequency of Testing
Cageside observation <sup>12</sup>	Twice daily
Clinical observations <sup>13</sup>	At least once daily
Body weight	Weekly during pre-test, treatment and recovery period, and on SD 17
Food consumption	Twice weekly during pretest, treatment and recovery period, and on SD 17

<sup>12</sup> Cageside observations include mortality, morbidity, general health and signs of toxicity.

<sup>13</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Parameters	Frequency of Testing
Body temperature	Once during pre-test, prior to each dose and approximately 2 hours after dosing [days 1 and 15]
Heart rate and respiration rate	Once during pre-test, prior to each dose and approximately 2 hours after dosing [days 1 and 15]
Ophthalmologic exam	Once during pre-test and at the end of treatment and recovery phases
Clinical chemistry*	Pre-test and on days 3, 17 and 29
Hematology*	Pre-test and on days 3, 17 and 29
Coagulation*	Pre-test and on days 3, 17 and 29
Immunological response	Pre-test, prior to dosing on day 15, and prior to necropsy on days 17 and 29
Evaluation of site of inoculation (e.g., the dermal Draize scoring method)	Approximately 24 and 48 hours post-dose on days 1 and 15 of the dosing phase
Necropsy	SDs 17 and 29
Tissues for histopathology	SDs 17 and 29

\* Collected from the ear artery.

Table 22: Parameters evaluated (study # 3).

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone (femur)	!+	
Bone marrow (sternum)	!	
Brain (cerebellum, mid-brain, cortex)	!*+	
Caecum	!	
Colon	!	
Duodenum	!	
Epididymides	!+	
Esophagus	!	
Eyes (optic nerve)	!+	
Fallopian tubes (oviduct)		X
Gall bladder	!	
Gross lesions	!+	
Harderian gland		X
Heart	!*+	

Organ/Tissue	Collected	Not collected
Inguinal gland	!	
Ileum	!	
Injection site(s)	!+	
Jejunum	!	
Kidneys	!*+	
Lacrimal glands		X
Larynx	!	
Liver	!*+	
Lung	!+	
Lymph nodes (iliac)	!+	
Lymph nodes (cervical)	!+	
Lymph nodes (mesenteric)	!+	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	!*+	
Pancreas	!	
Peyer's patch	!+	
Pituitary gland	!	
Prostate	!+	
Rectum	!	
Salivary glands (mandibular, parotid)	!	
Sciatic nerve	!	
Seminal vesical		X
Skeletal muscle	!	
Skin (abdominal)	!	
Spinal cord (cervical, lumbar, midthoracic)	!	
Spleen	!*+	
Stomach	!	
Testes	!*+	
Thymus	!*+	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters		X
Uterus (horns and cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if		X

Organ/Tissue applicable)	Collected	Not collected

Table 23: Tissues collected (study # 3).

Table of Histology – Tissues were fixed in proper fixation solution and stored. Tissues labeled with + were examined microscopically. Bone marrow cytology was not performed as there were no indications for a toxic effect on bone marrow.

## Results:

Morbidity and mortality: All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Total bile acids Sorbitol dehydrogenase Alanine aminotransferase (ALT or SGPT)
B) HEPATOBILIARY		Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides SD 17 F $\downarrow \leq 0.7$ G2  Creatine kinase SD 29 M $\uparrow \geq 1.9$ G2 SD 29 F $\uparrow \geq 1.6$ G2	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Lactate dehydrogenase (LDH)

C-reactive protein was not measured.

Table 24: Clinical chemistry results (study # 3).

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>14</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes %RBC
WHITE BLOOD CELLS	<p>Heterophils %WBC Pre-test F <math>\downarrow \leq 0.7</math> G2 SD 3 F <math>\downarrow \leq 0.7</math> G2 SD 29 F <math>\downarrow \leq 0.6</math> G2</p> <p>Monocyte %WBC SD 29 M <math>\downarrow \leq 0.6</math> G2 Pre-test F <math>\downarrow \leq 0.7</math> G2 SD 3 F <math>\downarrow \leq 0.5</math> G2 SD 17 F <math>\downarrow \leq 0.5</math> G2 SD 29 F <math>\downarrow \leq 0.6</math> G2</p> <p>Eosinophils %WBC Pre-test M <math>\downarrow \leq 0.5</math> G2 SD 17 M <math>\downarrow \leq 0.5</math> G2 SD 29 M <math>\downarrow \leq 0.1</math> G2 Pre-test F <math>\downarrow \leq 0.5</math> G2 SD 17 F <math>\downarrow \leq 0.6</math> G2 SD 29 F <math>\downarrow \leq 0.6</math> G2</p> <p>Basophils %WBC SD 3 F <math>\downarrow \leq 0.5</math> G2 SD 29 F <math>\downarrow \leq 0.6</math> G2</p>	<p>lymphocyte count Macrophage Large Unstained Cells (LUC)</p>
CLOTTING POTENTIAL		<p>Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen</p>
OTHERS		Bone marrow cytology

Table 25: Hematology results (study # 3).

<sup>14</sup> With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.



**Systemic toxicity:**

No test article-related effects were reported on clinical observations, body weight, food consumption, body temperature, respiration rate, heart rate, ophthalmic examinations, organ weights, macroscopic, and microscopic findings.

Triglyceride levels in group 2 females were reduced on SD 17. The levels of creatine kinase in males and females were increased in group 2 on SD 29. Fibrinogen levels increased (compared to pretest levels) following dosing, and by the end of recovery levels were slightly below pretest values. The results of this study were consistent with those observed in previous studies provided by the sponsor. Lower glucose concentrations in group 2 females (when compared to group 1) were reported on SD 17. This isolated observation was considered of no toxicological relevance because it was not correlated to any histopathological findings.

Heterophil levels were decreased in group 2 females on SD's 3 and 29. This decrease was not considered toxicologically significant because it was also reported at pre-test. Monocyte levels were decreased in group 2 males on SD 29 and females at pre-test and at SD's 3, 17, and 29. Eosinophil levels were decreased in group 2 males and females at pre-test and at SD's 17 and 29. Reduction in basophil levels was reported at SD's 3 and 29 in females. Since these reductions were either reported at pre-test and/or in one sex, they were not considered toxicologically significant.

## Organ Weight:

SEX	MALES (DOS NG PHASE/RECOVERY PHASE)	MALES (DOSING PHASE/RECOVERY PHASE)	FEMALES (DOSING PHASE/RECOVERY PHASE)	FEMALES (DOS NG PHASE/RECOVERY PHASE)
GROUPS	1 (CONTROL)	2	1 (CONTROL)	2
NUMBER OF ANIMALS	4/4	4/4	4/4	4/4
BODY WEIGHT (terminal)	2809/3049	2766/2755	2906/3157	3032/2956
BRAIN	9.7/10.4	9.7/9.6*	10.0/9.7	9.7/9.7
ADRENALS	0.18/0.14	0.15/0.15	0.16/0.19	0.15/0.16
EPIDIDYMIDES	ND	ND		
HEART	6.8/7.58	6.3/6.56	6.74/6.99	7.24/6.39
KIDNEYS	15.8/19.0	15.3/16.00	17.27/16.65	19.65/14.23
LIVER	76.8/85.6	67.0/78.9	83.5/77.1	95.8/70.6
SPLEEN	1.06/1.14	1.12/0.83*	1.42/1.11	1.89/1.11
TESTES	2.08/3.21	2.12/3.24		
THYROID and PARATHYROID	ND	ND	ND	ND
THYMUS	4.08/3.74	3.65/3.47	4.19/3.40	4.46/3.50
OVARIES			0.25/0.30	0.29/0.26
UTERUS			ND	ND

Absolute weights are expressed as mean (grams). \*Different from controls at  $P \leq 0.05$ . ND = Not determined.

Table 26: Organs wieght (study # 3).

Males brain and spleen weights were reduced (8% and 27%, respectively) significantly at the recovery period. Kidneys weight was reduced by 16% in males at the recovery phase. Because this reduction was not reported in their weight when expressed relative to body weights, they were considered to be caused by the relative low terminal body weight of this group of animals. One group 2 female and one group 1 male reported with higher mean liver weight. This was consistent with the enlarged livers noted for these animals at necropsy. This was not considered treatment-related but due to a necropsy artifact because the histopathological examination revealed terminal congestion as the underlying cause.

## Gross Pathology:

Group	Findings (Dosing phase//Recovery phase)
1M	Enlarged liver (1/4); enlarged iliac lymph node (4/4)// agenesis in testes (1/4); agenesis in epididymides (1/4); enlarged iliac lymph node (1/4)
2M	Prostate reduced in size (1/4); discoloration of cervical lymph node (1/4); enlarged iliac lymph node (4/4); discoloration at injection sites (2/4)// testes reduced in size (1/4); epididymides reduced in size (1/4)
1F	Cyst in the kidneys and the oviduct (1/4); enlarged iliac lymph node (4/4)// enlarged iliac lymph node (4/4)
2F	Focus/foci in lungs (1/4); enlarge liver (2/4); enlarged iliac lymph node (4/4)// NF

NF = No findings

Table 27: Macroscopic findings (study # 3).

Enlarged iliac lymph nodes were reported in all animals of groups 1 and 2 males and females on SD 17. This enlargement was either reported in one animal or was not reported in group 2 males and females at SD 29 (recovery animals). Non-treatment related livers enlargement were reported in one group 1 male and two group 2 females.

Incidental findings (focus/foci in the lungs, cyst(s) in kidneys and oviducts, unilateral agenesis of testes and epididymides, discolouration of the cervical lymph nodes) were also reported. These findings were not considered toxicologically significant because they are occasionally seen among rabbits used in these types of study and/or the absence of correlated microscopic findings. Reduced size of male reproductive organs (testes, epididymides, prostate) is related to immaturity of the animals and is considered to be unrelated to treatment.

Microscopic finding in terminal phase animals are listed below:

Groups	Findings
1M	Limbal keratitis in eyes (1/4); intramuscular terminal hemorrhage in heart ventricles (1/4); dermal hemorrhage in left (2/4) and right (1/4) injection sites; panniculus focal macrophage infiltration in left injection site (1/4); intermuscular connective tissue macrophages in right injection site (1/4); deep dermal diffuse macrophage infiltration in right injection site (1/4); deep muscle inflammatory cell infiltration in right injection site (2/4); focal mononuclear cell infiltration in liver (1/4); terminal congestion in liver (1/4); pneumonitis in lung (1/4); sinus erythrophagia in iliac LN (2/4); medullary plasmacytic proliferation in iliac LN (4/4); juvenile prostate (2/4) and testes (4/4); extracapsular growth in spleen (1/4); single tubule germinal epithelial absence in testes (1/4); atrophy in thymus (2/4)
2M	Limbal keratitis in eyes (1/4); dermal hemorrhage in left and right injection sites (2/4); deep dermal diffuse macrophage infiltration in left injection site (1/4); deep dermal acute inflammation in left injection site (1/4); deep muscle focal degeneration in right injection site (1/4); deep dermal diffuse macrophage infiltration in right injection site (2/4); panniculus focal macrophage infiltration in right injection site (1/4); deep muscle inflammatory cell infiltration in right injection site (2/4); deep muscle acute inflammatory infiltration in right injection site (1/4); dilated cortical tubules in kidneys (1/4); cortical tubule luminal mineralization in kidneys (1/4); sinus erythrophagia in cervical and iliac LN (1/4); medullary plasmacytic proliferation in cervical (1/4) and iliac (3/4) LN; parasitic granuloma in mesenteric LN (1/4); juvenile prostate (3/4) and testes (4/4); single tubule germinal epithelial absence in testes (1/4); atrophy in thymus (2/4)
1F	Conjunctival lymphoid hyperplasia in eyes (1/4); intramuscular terminal hemorrhage in heart ventricles (1/4); dermal hemorrhage in left injection site (2/4); deep muscle focal degeneration in left and right injection sites (1/4); deep dermal diffuse macrophage infiltration in left (2/4) and right (3/4) injection sites; deep muscle hemorrhage in left injection site (1/4); deep muscle inflammatory cell infiltration in left and right injection sites (2/4); deep muscle acute inflammatory infiltration in right injection site (1/4); basophilic tubules in kidneys (1/4); superficial cortical cysts in kidneys (1/4); cortical tubule luminal mineralization in kidneys (2/4); focal mononuclear cell infiltration in liver (1/4); sinus erythrophagia in iliac LN (2/4); medullary plasmacytic proliferation in iliac LN (4/4); tertiary (3/4) and secondary (1/4) follicles predominate in ovaries; atrophy in thymus (1/4); petechial hemorrhage in thymus (1/4); cyst dilatation in oviducts (1/4)
2F	Intermuscular connective tissue macrophages in left (2/4) and right (4/4) injection sites; deep dermal diffuse macrophage infiltration in left (2/4) and right (1/4) injection sites; deep muscle inflammatory cell

Groups	Findings
	inflammation in left injection site (1/4); basophilic tubules in kidneys (1/4); straight tubule dilatation in kidneys (1/4); terminal congestion in liver (1/4); sinus erythrophagia in iliac LN (1/4); medullary polymorphonuclear leucocyte infiltration in iliac LN (1/4); medullary plasmacytic proliferation in iliac LN (2/4); tertiary follicles predominate in ovaries (4/4); atrophy in thymus (1/4)

Microscopic finding in recovery phase animals are listed below:

Groups	Findings
1M	Juvenile epididymides (1/4); abnormal sperm forms (1/4); intramuscular terminal hemorrhage in heart ventricles (3/4); basophilic tubules in kidneys (1/4); cortical tubule luminal mineralization in kidneys (2/4); focal mononuclear cell infiltration in liver (1/4); juvenile testes (2/4); single tubule germinal epithelial absence in testes (1/4); focal mononuclear cell infiltration in testes (1/4); atrophy in thymus (2/4); petechial hemorrhage in thymus (1/4)
2M	Juvenile epididymides (1/4); abnormal sperm forms (1/4); retinal outer nuclear degeneration in the eye (1/4); intramuscular terminal hemorrhage in heart ventricles (2/4); intermuscular acute inflammation in left injection sites (1/4); deep muscle focal degeneration in left injection site (1/4); deep dermal diffuse macrophage infiltration in right injection site (1/4); deep muscle acute inflammatory infiltration in right injection site (1/4); dilated cortical tubules in kidneys (2/4); basophilic tubules in kidneys (2/4); cortical tubule luminal mineralization in kidneys (3/4); focal mononuclear cell infiltration in liver (1/4); juvenile testes (1/4); single tubule germinal epithelial absence in testes (1/4); spermatid giant cells in testes (2/4); little germinal epithelial maturity in testes (1/4); petechial hemorrhage in thymus (1/4)
1F	Retinal outer nuclear degeneration in eyes (1/4); deep muscle focal degeneration in left injection site (1/4); deep dermal acute inflammation in right injection site (1/4); cortical tubule luminal mineralization in kidneys (1/4); focal mononuclear cell infiltration in liver (1/4); sinus erythrophagia in iliac LN (1/4); medullary plasmacytic proliferation in iliac LN (1/4); tertiary follicles predominate in ovaries (4/4); sinus erythrophagia in peyer's patches (jejunum) (1/4); atrophy in thymus (1/4)
2F	Intermuscular terminal hemorrhage in heart ventricles (1/4); deep muscle inflammatory cell inflammation in right injection site (1/4); basophilic tubules in kidneys (1/4); dilated cortical tubules in kidneys (1/4); cortical tubule luminal mineralization in kidneys (2/4); focal mononuclear cell infiltration in liver (1/4); pneumonitis in lungs (1/4); tertiary follicles predominate in ovaries (4/4)

Table 28: Microscopic findings (study # 3).

An extensive number of tissues were examined for histology. Findings relative to the eyes, kidney, liver, lung, heart, iliac LN, injection site observations, spleen, thymus, prostate, testes, and ovary appeared evenly distributed between treated and control groups of animals. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

### Body temperature

No test-article related changes in body temperature were reported.

Group	Males	Females
1Control	0	0
2	0	0

Table of occurrences for body temperature  $\geq 40^{\circ}\text{C}$

Table 29: Body temperature (study # 3).

### Local toxicity:

#### Erythema/Edema

It has been reported in the materials and methods section that skin irritation will be tested for erythema and edema; however, no results were reported in the results section.

#### Serology:

Sera collected from all animals at pre-dose had titers of anti-A/Solomon Island/3/2006 (BMISOLOMON-E01) below limits of detection when tested by hemagglutination inhibition assays (HAI). Positive titers were detected in group 1 for this antigen on SD 15 in response to the first dose administration and two days after the second dose (SD 17) administration. The immunogenic response in group 2 was developed more slowly when compared to group 1. Highest titer levels were detected in recovery animals (SD29) of groups 1 and 2 (males and females). Thus, both vaccine formulations were considered immunogenic. An increase in the titers in the SD 29 samples, collected after the second administration, substantiated this finding.

Table of antibody titer: Anti-A/Solomon Island/3/2006 (BMISOLOMON-E01) titer [Influenza A (H1N1) strain]

Group	Dose	HAI Titer anti- A/Solomon Island/3/2006 (BMISOLOMON-E01)			
		Pre-Test (n=8)	Pre-dose SD 15 (n=8)	SD 17 (n=4)	SD 29 (n=4)
<u>Males</u> 1	Fluad® (180063/A)	<10	207.5	320	640

Group	Dose	HAI Titer anti- A/Solomon Island/3/2006 (BMISOLOMON-E01)			
		Pre-Test (n=8)	Pre-dose SD 15 (n=8)	SD 17 (n=4)	SD 29 (n=4)
2	IC31 (180297/A)	<10	80	113	640
<u>Females</u> 1	Fluad® (180063/A)	<10	207.5	190.3	640
2	IC31 (180297/A)	<10	54*	57	640

\*n=7, as one animal reported with <10. SD = Study day.

Table 30: Serology results (study # 3).

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental
Injection sites findings Iliac LN enlargement ↑ Fibrinogen	Clinical chemistry findings Hematology findings Creatine kinase

#### Assessment:

There were no treatment-related effects on clinical observations, body weight, body temperature, respiration rate, heart rate, oxygen saturation, ophthalmic examinations, organ weights, macroscopic, and microscopic findings were reported.

The enlargement in iliac LNs noted at the macroscopic level in both treatment groups frequently correlated with an increased population of large lymphocytic cells in the medulla, many of which were morphologically identified as plasma cells. These changes were (largely) resolved by the end of the recovery period. Since the iliac lymph nodes are the main draining nodes at the injection site, the observed response in the iliac lymph nodes is considered to be a normal physiological response to the vaccination.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

There were no clear treatment-related effects on clinical pathology parameters, although there were a number of statistically significant differences (data not shown). Many of these differences were of a magnitude or nature that was not clinically significant or that remained within the normal range of values established for gender, laboratory, or species.

The elevation in creatine kinase levels was not considered test article-related because this elevation was either observed during pretest and/or could be related to local tissue damage caused by the intramuscular injection of the test substances.

Adverse gross or microscopic alteration that could be indicative of systemic or local toxicity was not observed.

Treatment-related toxicity was only observed at the sites of inoculation in terms of gross and/or histopathology and was primarily attributable to inflammation due to the intended immune response to the vaccine. These findings were recoverable.

Immunology performed in this study verified that an active dose was administered. No differences were found between female and male animals.

**GLP study deviations or amendments:** No significant deviations or amendments were recorded that influenced the quality, integrity or interpretation of the results.

**Conclusions:** Based on nonclinical toxicity assessments of this study there are no significant safety issues to report. The delivery of an active dose of the product in the toxicology study was verified.

#### **Study # 4:**

Title and study number: 4-week vaccine toxicity study with Fludac®, Fludac High B, and Fludac High H3+IC31® influenza vaccine formulations by three intramuscular injections in (b) (4) rabbits followed by a 2-week recovery period.  
Study number 488182.

**Performing laboratory:** (b) (4)

**Study initiation date:** 05 May 2008

**Final Report date:** April, 29<sup>th</sup> 2009

**Test article batch/lot:** Fludac: 079703; Fludac High B: FLUDIFFB08; Fludac High H3: FLUDIFFA08

**Animal species and strain:** (b) (4) rabbits

**Breeder/supplier:** (b) (4)

**Number of animal per group and sex:** 8

**Age:** Approximately 12-13 weeks

**Body weight range:** Between 1.8 and 2.8 kg

**Route and site of administration:** Intramuscular, day 1: right hind limb, day 15: left hind limb, day 29: right hind limb (at the same position as day 1 injection).

**Volume of injection:** Group 1, 2, 3: 0.5 mL, group 4: 1.0 mL

**Frequency of administration and study duration:** Three intramuscular injections on days 1, 15 and 29. Necropsy was performed on days 31 and 43.



**Dose:** Group 1: 0.5 mL/animal saline

Group 2: A single 0.5 mL dose of Fluad contained 45 mcg antigens: 15 mcg hemagglutinin (HA) from each of A/H3N2, A/H1N1 and B influenza strains, adjuvanted with MF59 (0.25 mL).

Group 3: A single 0.5 mL dose of Fluad High B contained 60 mcg antigens: 15 mcg HA from the A/H3N2 and A/H1N1 influenza strains and 30 mcg HA from the B strain adjuvanted with MF59 (0.25 mL).

Group 4: A single 1.0 mL dose of Fluad High H3+IC31 contained 60 mcg antigens: 15 mcg HA from the B strain and the A/H1N1 influenza strains and 30 mcg HA from the A/H3N2 strain, adjuvanted with MF59 (0.25 mL) and combined with the second adjuvant IC31 (0.5mL).

**Stability:** Expiration date: Fluad: 31 July 2008; Fluad High B: 01 January 2009; Fluad High H3: 01 January 2009

Dates of administration: Day 1: 13<sup>th</sup> and 14<sup>th</sup> May 2008  
 Day 15: 27<sup>th</sup>, and 28<sup>th</sup> May 2008  
 Day 29: 10<sup>th</sup>, and 11<sup>th</sup> June 2008

**Means of administration:** Intramuscular

**Report status:** Final

**Experimental design:**

Group	Treatment	Number of animals (#/sex/group)	Number of animals (#/sex/group)
		Treatment phase day 31 (2 days after the last injection)	Recovery phase day 43 (14 days after the last injection)
1. saline	saline	4	4
2. Fluad	45 mcg per dose, 15 mcg HA from each of the three influenza strains A/Solomon Islands/3/2006 IVR-145 (H1N1), A/Wisconsin/67/2005 NYMCX-161-B, and B/Malasia/2506/2004 (H3N2) combined with MF59 adjuvant (0.25 mL per dose)	4	4
3. Fluad High B	60 mcg per dose, 15 mcg HA from the A/H3N2 and A/H1N1 influenza strains and 30 mcg HA from the B strain combined with MF59 adjuvant (0.25 mL per dose)	4	4
4. Fluad	60 mcg per dose, 15 mcg HA	4	4

Group	Treatment	Number of animals (#/sex/group)	Number of animals (#/sex/group)
		Treatment phase day 31 (2 days after the last injection)	Recovery phase day 43 (14 days after the last injection)
High H3 + IC31	from the B strain and the A/H1N1 influenza strains and 30 mcg HA from the A/H3N2 strain combined with MF59 adjuvant (0.25 mL per dose) and combined with the second adjuvant IC31® (0.5mL)		

Table 31: Experimental design (study # 4).

Methods: Blood samples for antibody-determination were taken (during pretest, predose on days 15 and 29 and on day 43 for recovery animals) and analyzed (non-GLP) under the responsibility of the performing laboratory (b) (4)

**Statistical analysis plan:** The following statistical methods were used to analyze the data: If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex. The Steel-test (many-to-one rank test) was applied instead of the Dunnett-test if the data could not be assumed to follow a normal distribution. The Fisher-exact test was applied to frequency data. All tests were two-sided and in all cases  $p < 0.05$  was accepted as the lowest level of significance.

Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables. Test statistics were calculated on the basis of exact values for means and pooled variances. Individual values, means and standard deviations may have been rounded off before printing.

The following parameters have been evaluated:

Parameters	Frequency of Testing
Cageside observation <sup>15</sup>	Twice daily
Clinical observations <sup>16</sup>	Daily
Body weight	Weekly

<sup>15</sup> Cageside observations include mortality and viability .

<sup>16</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Parameters	Frequency of Testing
Food consumption	Twice weekly
Heart rate, respiratory rate and rectal body temperature	Once during pretest, prior to dosing and approximately 2 hours after dosing on days 1, 15 and 29
Ophthalmologic exam	Once during pretest, at the end of the treatment phase and recovery phase
Clinical chemistry*	Once during pretest, on days 8, 17, 31 and on day 43 for the recovery animals
Hematology*	Once during pretest, on days 8, 17, 31 and on day 43 for the recovery animals
Coagulation	Once during pretest, on days 8, 17, 31 and on day 43 for the recovery animals
Immunological response	During pretest, predose on days 15 and 29 and on day 43 for recovery animals
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Approximately 24 and 48 hours after dosing
Necropsy	Days 31 and 43
Tissues for histopathology	Days 31 and 43

\*Vena jugulars.

Table 32: Parameters evaluated (study # 4).

**Postmortem procedures:** The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	!*	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		X
Gall bladder	!	

Organ/Tissue	Collected	Not collected
Gross lesions (if any)	!	
Harderian gland (if applicable)		X
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		X
Larynx	!	
Liver	!*	
Lung (main-stem; bronchi)	!	
Lymph nodes (iliac, cervical, popliteal)	!	
Lymph nodes (mandibular)		X
Lymph nodes (mesenteric)	!	
Mammary glands		X
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)	!	
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicles	!	
Skeletal muscle		
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach (squamous and glandular)	!	
Testes	!*	
Thymus	!*	

Organ/Tissue	Collected	Not collected
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters		X
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		X

Table 33: Tissues collected (study # 4).

Table of Histology – Tissues examined (all dose groups were examined)

Results:

**Morbidity and mortality:** All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP ( <b>G</b> ), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ ))	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR  B) HEPATOBILIARY		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids Lactate dehydrogenase (LDH)
		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin: <b>Not done</b>
ACUTE PHASE REACTANTS		C-reactive protein: <b>Not done</b> Fibrinogen
KIDNEY FUNCTION		Creatinine

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ ))	NOT OF NOTE
		Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	LDH: Males: Day 8: 1.6x increased in group 4 (142U/L)	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase: <b>Not done</b> Total protein Creatine kinase Fasting triglycerides

Table 34: Clinical chemistry results (study # 4).

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	<p><b>Male:</b>  <b>Lymphocytes %WBC:</b>            Pretest: 1.7x increased in group 4 (58.1)</p> <p><b>Monocytes:</b>            Pretest: 1.7x decreased in group 4 (2.1%)            Day 8: 1.9x decreased in group 2 (1.6%)            Day 31: 2.7x decreased in group 2 (0.6%), in group 4 (0.0%)</p> <p><b>Eosinohils %WBC:</b>            Pretest: reduced in all treatment groups (1.7x group 2 (1.1%); 3.2x in group 3 (0.6%); 2.1x in group 4 (0.9%)            Day 8: reduced in all treatment groups (2x group 2 (0.9%); 3.2x in group 3 (0.6%); 3.2x in group 4 (0.6%)            Day 17: reduced in all treatment groups (4x group 2 (0.4%); 3.2x in group 3 (0.5%); in group 4 (0%)            Day 31: 3.3x decreased in group 4 (0.4%)            Day 43: 1.8x increased in group 2 (2.3%)</p> <p><b>Basophils %WBC:</b>            Day 17: 1.6x decreased in group 4 (6%)            Day 31: 1.3x decreased in group 4 (4%)</p> <p><b>Female:</b>  <b>Heterophils %WBC:</b>            Pretest: 1.6x decreased in group 4 (29.4)  <b>Monocytes:</b>            Pretest: 1.9x decreased in group</p>	Basophils, Eosinophils count Lymphocyte count Macrophage/Monocyte count Neutrophil count Total Leukocytes (WBC) Large Unstained Cells (LUC)

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
	<p>2 (1.3%) Day 8: 2.6x decreased in group 4 (1.0%) Day 43: 6.7x decreased in group 2 (0.3%)</p> <p><b>Basophils %WBC:</b> Pretest: 2.2x decreased in group 2 (5.3) Day 17: reduced in all treatment groups (2x group 2 (5.9%); 1.5x in group 3 (7.9%); 3.2x in group 4 (06%) Day 31: reduced in all treatment groups (2.4x group 2 (5.1%); 1.5x in group 3 (8.1%); 2.2x in group 4 (5.5%) Day 43: 1.6x decreased in group 4 (7.8%)</p> <p><b>Eosinohils %WBC:</b> Day 8: decreased 10x in group2 (0.1%); 1.7x in group 4 (0.6%) Day 17: 2.6x decreased in group 4 (4.6%) Day 31: reduced in all treatment groups (2.2x in group 2 (0.6%); 4.3x in group 3 (0.3%); 3.3x in group 4 (0.4%)</p>	
CLOTTING POTENTIAL	<p><b>Male:</b> <b>Fibrinogen (g/L):</b> Day 17: increased in all treatment groups (1.7x in group 2 (5.84; 1.8x in group 3 (6.26); 1.6x in group 4 (5.49) Day 31: increased in all treatment groups (1.6x in group 2 (5.04); 1.6x in group 3 (4.87); 1.4x in group 4 (4.37)</p> <p><b>Female:</b> <b>Fibrinogen (g/L):</b> Day 17: increased in all treatment groups (2.3x in group 2 (5.01; 1.9x in group 3 (4.22); 1.8x in group 4 (3.92) Day 31: increased in all treatment groups (1.9x in group 2 (4.42); 1.4x in group 3 (3.18); 1.5x in group 4 (3.52)</p>	<p>Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume</p>



HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
OTHERS		Bone marrow cytology

Table 35: Hematology results (study # 4).

Increased levels of fibrinogen were observed in all treatment groups on days 17 and 31, after the recovery phase they returned to control values.

Changes in white blood cell parameters were observed, no absolute numbers have been provided. The percentage of eosinophils was reduced in all male treatment groups on days 8 and 16 as well as pretest; the percentage of eosinophils was also reduced in all female treatment groups on day 31 and groups 2 and 4 on day 17. Moreover, basophils were reduced in all female treatment groups on days 17 and 31. After the recovery phase on day 43 the values were not reduced in comparison to the control values except for the percentage of basophils of the female group 4.

#### **Systemic toxicity:**

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, clinical chemistry, gross anatomy or organ weight were found.

Increased levels of fibrinogen were observed in all treatment groups on days 17 and 31, after the recovery phase they returned to control values. This observation is most likely a reflection of the overall inflammation after the vaccination.

Changes in white blood cell parameters were observed, no absolute numbers have been provided. The percentage of eosinophils was reduced in all male treatment groups on days 8 and 16 as well as pretest; the percentage of eosinophils was also reduced in all female treatment groups on day 31 and groups 2 and 4 on day 17. Moreover, basophils were reduced in all female treatment groups on days 17 and 31. After the recovery phase on day 43 the values were not reduced in comparison to the control values anymore except for the % of basophils of the female group 4.

**Organ Weight:**

Day 31

SEX		MALES	MALES	MALES	MALES	FEMALES	FEMALES	FEMALES	FEMALES
GROUPS		1	2	3	4	1	2	3	4
NUMBER OF ANIMALS		4	4	4	4	4	4	4	4
BODY WEIGHT (gram) <sup>a</sup>		2633	2742	2812	2780	3071	2881	2888	2992
BRAIN									
Absolute Weight <sup>a</sup>	gram	9.8	8.5	9.7	9.7	9.8	9.5	9.4	9.6
Per Body Weight <sup>a</sup>	%	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.3
ADRENALS									
Absolute Weight <sup>a</sup>	gram	0.147	0.183	0.192	0.173	0.170	0.189	0.216*	0.202
Per Body Weight <sup>a</sup>	%	0.006	0.007	0.007	0.006	0.006	0.007	0.008*	0.007
HEART									
Absolute Weight <sup>a</sup>	gram	6.04	7.01	6.43	6.22	7.11	6.19	6.17	6.63
Per Body Weight <sup>a</sup>	%	0.23	0.26	0.23	0.22	0.23	0.22	0.21	0.22
KIDNEYS									
Absolute Weight <sup>a</sup>	gram	13.92	14.63	15.63	15.08	15.91	16.25	15.63	15.42
Per Body Weight <sup>a</sup>	%	0.53	0.53	0.56	0.54	0.52	0.57	0.54	0.52
LIVER									
Absolute Weight <sup>a</sup>	gram	65.0	70.6	74.4	81.2	80.2	73.8	71.0	95.8
Per Body Weight <sup>a</sup>	%	2.5	2.6	2.6	2.9	2.6	2.5	2.4	3.2
SPLEEN									
Absolute Weight <sup>a</sup>	gram	0.147	0.183	0.192	0.173	1.28	1.36	1.63	1.25
Per Body Weight <sup>a</sup>	%	0.03	0.04	0.05	0.04	0.04	0.05	0.06	0.04
TESTES									
Absolute Weight <sup>a</sup>	gram	2.86	2.99	3.13	3.02				
Per Body Weight <sup>a</sup>	%	0.11	0.11	0.11	0.11				
THYMUS									
Absolute Weight <sup>a</sup>	gram	3.16	3.37	2.86	3.98	3.02	3.52	3.09	3.26
Per Body Weight <sup>a</sup>	%	0.12	0.12	0.10	0.14	0.10	0.12	0.11	0.11
OVARIES									
Absolute Weight <sup>a</sup>	gram					0.269	0.303	0.269	0.368
Per Body Weight <sup>a</sup>	%					0.009	0.011	0.009	0.013

Absolute weights are expressed as mean (grams) ± standard deviation (sd). \*Different from controls at P≤0.05; \*\*Different from controls at P≤0.01.

Table 36: Organs weight at study day 31 (study # 4).

## Day 43

SEX		MALES	MALES	MALES	MALES	FEMALES	FEMALES	FEMALES	FEMALES
GROUPS		<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
NUMBER OF ANIMALS		4	4	4	4	4	4	4	4
BODY WEIGHT (gram) <sup>a</sup>		2789	2898	2873	2748	2935	2890	2839	3010
BRAIN									
Absolute Weight <sup>a</sup>	gram	10.0	9.8	9.9	10.2	9.6	9.9	10.1	9.7
Per Body Weight <sup>a</sup>	%	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.3
ADRENALS									
Absolute Weight <sup>a</sup>	gram	0.158	0.248	0.197	0.174	0.208	0.181	0.179	0.192
Per Body Weight <sup>a</sup>	%	0.006	0.009	0.007	0.006	0.007	0.006	0.006	0.007
HEART									
Absolute Weight <sup>a</sup>	gram	6.78	6.97	6.83	7.02	6.82	6.67	6.77	6.54
Per Body Weight <sup>a</sup>	%	0.24	0.24	0.24	0.25	0.23	0.23	0.24	0.22
KIDNEYS									
Absolute Weight <sup>a</sup>	gram	15.53	16.17	16.65	16.03	16.32	15.50	15.05	15.86
Per Body Weight <sup>a</sup>	%	0.55	0.56	0.58	0.58	0.56	0.54	0.53	0.53
LIVER									
Absolute Weight <sup>a</sup>	gram	75.4	67.5	78.2	65.8	76.0	73.3	69.1	70.7
Per Body Weight <sup>a</sup>	%	2.7	2.3	2.7	2.4	2.6	2.5	2.4	2.3
SPLEEN									
Absolute Weight <sup>a</sup>	gram	1.09	0.87	0.99	1.24	1.15	1.29	1.20	1.38
Per Body Weight <sup>a</sup>	%	0.04	0.03	0.03	0.05	0.04	0.04	0.04	0.05
TESTES									
Absolute Weight <sup>a</sup>	gram	3.92	3.57	4.61	3.86				
Per Body Weight <sup>a</sup>	%	0.14	0.12	0.16	0.14				
THYMUS									
Absolute Weight <sup>a</sup>	gram	3.41	3.11	3.91	3.66	2.99	3.29	3.05	3.21
Per Body Weight <sup>a</sup>	%	0.12	0.11	0.14	0.13	0.10	0.11	0.11	0.11
OVARIES									
Absolute Weight <sup>a</sup>	gram					0.244	0.266	0.283	0.240
Per Body Weight <sup>a</sup>	%					0.008	0.009	0.010	0.008

Absolute weights are expressed as mean (grams)  $\pm$  standard deviation (sd). \*Different from controls at  $P \leq 0.05$ ; \*\*Different from controls at  $P \leq 0.01$ .

Table 37: Organs weight at study day 43 (study # 4).

The absolute and relative organ weight of the adrenals was statistically significantly higher in the female treatment group 3 compared to the control group on day 31.

**Pathology:****Macroscopic findings:**

Day 31

Group	Findings
3M	Angenesis in the epididymides (3M: 1/4 animals)
2M, 3M, 4M, 2F, 3F, 4F	Enlarged iliac lymph nodes (2M: 4/4 animals, 3M: 4/4 animals, 4M: 4/4 animals, 2F: 3/4 animals, 3F: 3/4 animals, 4F: 1/4 animals)
4M	Discoloration in the iliac lymph nodes (4M: 1/4 animals)
4F	Enlarged ovary (4F: 1/4 animals)
2F	Enlarged popliteal lymph node (2F: 2/4 animals)
4M	Alopecia of the skin (4M: 1/4 animals)
4M	Testes reduced in size (4M: 1/4 animals)
3M	Angenesis in the epididymides (3M: 1/4 animals)

Table 38: Macroscopic findings at study day 31 (study # 4).

Day 43

Group	Findings
1M	Discoloration in the cervical lymph nodes (1M: 1/4 animals)
2M, 4M	Reduced in size epididymides (2M: 3/4 animals, 4M: 3/4 animals)
3M, 4F	Enlarged iliac lymph nodes (3M: 1/4 animals, 4F: 1/4 animals)
1M, 3F	Right site discoloration at the subcutis on the injection site (1M: 1/4 animals, 3F: 1/4 animals)
1M	Left site discoloration at the subcutis on the injection site (1M: 1/4 animals)
1M, 1F	Foci in the lung (1M: 1/4 animals, 1F: 1/4 animals)
2F, 3F	Nodules in the oviducts (2F: 1/4 animals, 3F: 1/4 animals)
1F, 3F	Discoloration in the mesenteric lymph nodes (1F: 1/4 animals, 3F: 1/4 animals)
4M	Small prostate (4M: 1/4 animals)
4M	Small seminal vesicles (4M: 1/4 animals)
1M	Small spleen (1M: 1/4 animals)
2M, 3M, 4M	Small testis (2M: 3/4 animals, 3M: 1/4 animals, 4M: 3/4 animals)
2M, 3M, 4M, 1F, 2F, 3F, 4F	Foci in the thymus (2M: 1/4 animals, 3M: 2/4 animals, 4M: 3/4 animals, 1F: 1/4 animals, 2F: 2/4 animals, 3F: 1/4 animals, 4F: 1/4 animals)

Table 39: Macroscopic findings at study day 43 (study # 4).

**Microscopic findings:**

Grade 1: minimal

Grade 2: slight  
 Grade 3: moderate  
 Grade 4: marked  
 Grade 5: sever

Day 31:

Groups	Findings
1M, 2M, 3M, 4M	Few spermatozoa present in the epididymides (minimal) (1M: 3/4 animals, 2M: 3/3 animals, 3M: 2/4 animals, 1/3 animals)
3F	Inflammatory cell infiltration in the lacrimal gland (3F: 1/4 animals)
2F	Synovial inflammatory cell infiltration in the femur joint (minimal) (2F: 1/4 animals)
1M, 3M, 4M	Myocardial inflammation (1M: 2/4 animals, 3M: 1/4 animals, 4M: 1/4 animals)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Inflammation of the dermis/subcutis on right injection site (minimal to moderate) (1M: 1/4 animals, 2M: 1/4 animals, 3M: 4/4 animals, 4M: 2/4 animals, 2F: 3/4 animals, 3F: 4/4 animals, 4F: 4/4 animals)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Inflammation of the interstitial muscle on right injection site (minimal to slight) (1M: 1/4 animals, 2M: 2/4 animals, 3M: 2/4 animals, 4M: 1/4 animals, 2F: 2/4 animals, 3F: 4/4 animals, 4F: 2/4 animals)
4M, 4F	Inflammation of the dermis/subcutis on left injection site (minimal) (4M: 1/4 animals, 4F: 1/4 animals)
4M, 4F	Inflammation of the interstitial muscle on left injection site (minimal to slight) (4M: 1/4 animals, 4F: 1/4 animals)
3M	Interstitial inflammatory cell infiltration in the kidneys (minimal) (3M: 1/4 animals)
3M, 3F, 4F	Tubular dilatation in the kidney (minimal) (3M: 1/4 animals, 4M: 1/4 animals, 2F: 1/4 animals)
1F	Tubular mineralization in the kidney (minimal) (1F: 1/4 animals)
3M, 1F, 2F, 3F	Parenchymal inflammatory cell infiltration in the liver (minimal) (3M: 1/4 animals, 1F: 1/4 animals, 2F: 1/4 animals, 3F: 1/4 animals)
1M, 3M, 1F, 2F, 3F	Periportal inflammatory cell infiltration in the liver (minimal to moderate) (1M: 2/4 animals, 3M: 1/4 animals, 2F: 3/4 animals, 3F: 4/4 animals, 4F: 1/4 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Alveolitis (minimal to slight) (1M: 2/4 animals, 2M: 1/4 animals, 3M: 2/4 animals, 4M: 2/4 animals, 1F: 1/4 animals, 2F: 3/4 animals, 3F: 3/4 animals, 4F: 1/4 animals)
1M, 2M, 4F	Alveolar macrophage aggregates (minimal) (1M: 2/4 animals, 2M: 1/4 animals, 4F: 1/4 animals)

Groups	Findings
2M, 3M, 4M, 2F, 3F, 4F	Medulla mixed inflammatory cell infiltration in the iliac lymph node (minimal to moderate) (2M: 3/4 animals, 3M: 4/4 animals, 4M: 2/4 animals, , 2F: 4/4 animals, 3F: 4/4 animals, 4F: 1/4 animals)
2M, 3M, 4M, 2F, 3F, 4F	Germinal center development in the iliac lymph node (minimal to slight) (2M: 4/4 animals, 3M: 4/4 animals, 4M: 4/4 animals, 2F: 4/4 animals, 3F: 4/4 animals, 4F: 2/4 animals)
3M	Macrophage aggregates (minimal) in the mesenteric lymph node (3M: 2/4 animals)
2M, 3M, 4M, 2F, 3F, 4F	Germinal center development in the popliteal lymph node (minimal to moderate) (2M: 3/4 animals, 3M: 3/4 animals, 4M: 3/4 animals, 2F: 4/4 animals, 3F: 4/4 animals, 4F: 4/4 animals)
3M, 2F, 3F, 4F	Medulla mixed inflammatory cell infiltration in the popliteal lymph node (minimal to moderate) (3M: 2/4 animals, , 2F: 1/4 animals, 3F: 3/4 animals, 4F: 1/4 animals)
4M	Pigmented macrophage aggregates in the Peyers patches (4M: 1/4 animals)
4M	Foreign body microgranuloma in the Peyers patches (4M: 1/4 animals)
1M, 2M, 3M, 4M,	Hypospermatogenesis in the testis (minimal to marked) (1M: 2/4 animals, 2M: 3/4 animals, 3M: 2/4 animals, 4M: 1/4 animals)
1M, 2M, 3M, 4M,	Immaturity in the testis (minimal to marked) (1M: 1/4 animals, 2M: 2/4 animals, 3M: 2/4 animals, 4M: 1/4 animals)
1M, 2M, 3M, 4M,	Few spermatozoa present in the epididymides (1M: 1/4 animals, 2M: 2/4 animals, 3M: 1/4 animals, 4M: 2/4 animals)

Table 40: Microscopic findings at study day 31 (study # 4).

## Day 43:

Groups	Findings
1M, 2M, 3M, 4M,	Few spermatozoa present in the epididymides (1M: 1/4 animals, 2M: 2/4 animals, 3M: 1/4 animals, 4M: 2/4 animals)
2M	Interstitial cell nodule in the epididymides (2M: 1/4 animals)
1F	Choroid inflammatory cell infiltration (minimal) (1F: 1/4 animals)
1M	Synovial inflammatory cell infiltration in the femur joint (minimal to slight ) (1M: 2/4 animals)
2F	Myocardial inflammation (minimal) (2F: 1/4 animals)
3F	Endocardial inflammation (minimal) (3F: 1/4 animals)
2M, 4M, 3F, 4F	Inflammation of the dermis/subcutis on right injection site (minimal to moderate) (2M: 2/4 animals, 4M: 1/4 animals, 3F: 1/4 animals, 4F: 1/4 animals)
4F	Inflammation of the interstitial muscle on right injection site

Groups	Findings
	(minimal to slight) (4F: 1/4 animals)
4M, 4F	Muscle interstitial foamy macrophages on the right injection site (minimal) (4M: 1/4 animals, 4F: 1/4 animals)
1M, 2M, 4M, 3F	Interstitial inflammatory cell infiltration in the kidneys (minimal) (1M: 1/4 animals, 2M: 1/4 animals, 3M: 1/4 animals, 3F: 1/4 animals)
3F	Pelvic dilatation in the kidney (minimal) (3F: 1/4 animals)
4M, 1F, 2F, 3F, 4F	Tubular dilatation in the kidney (minimal to slight) (4M: 1/4 animals, 1F: 2/4 animals, 2F: 1/4 animals, 3F: 1/4 animals, 4F: 1/4 animals)
4M, 3F, 4F	Tubular degeneration in the kidney (minimal) (4M: 1/4 animals, 3F: 1/4 animals, 4F: 2/4 animals)
1M, 1F, 4F	Tubular mineralization in the kidney (minimal to slight) (1M: 1/4 animals, 1F: 2/4 animals, 4F: 1/4 animals)
1M, 3M, 4M, 1F, 2F, 4F	Parenchymal inflammatory cell infiltration in the liver (minimal) (1M: 4/4 animals, 3M: 1/4 animals, 4M: 1/4 animals, 1F: 1/4 animals, 2F: 1/4 animals, 4F: 1/4 animals)
1M, 3M, 4M, 1F, 2F, 3F	Periportal inflammatory cell infiltration in the liver (minimal to slight) (1M: 1/4 animals, 3M: 1/4 animals, 4M: 3/4 animals, 1F: 2/4 animals, 2F: 2/4 animals, 3F: 3/4 animals)
4M, 3F, 4F	Alveolar macrophage aggregates (minimal) (4M: 1/4 animals, 3F: 1/4 animals, 4F: 1/4 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Alveolitis (minimal to slight) (1M: 3/4 animals, 2M: 1/4 animals, 3M: 1/4 animals, 4M: 3/4 animals, 1F: 3/4 animals, 2F: 4/4 animals, 3F: 3/4 animals, 4F: 3/4 animals)
1M	Agonal congestion in the cervical lymph node (1M: 1/4 animals)
2M, 3M, 4M, 2F, 3F, 4F	Germinal center development in the iliac lymph node (minimal to slight) (2M: 4/4 animals, 3M: 4/4 animals, 4M: 3/4 animals, 2F: 4/4 animals, 3F: 3/4 animals, 4F: 3/4 animals)
4M, 2F, 3F	Medulla mixed inflammatory cell infiltration in the iliac lymph node (minimal to moderate) (4M: 1/4 animals, , 2F: 1/4 animals, 3F: 1/4 animals)
3M	Macrophage aggregates (minimal) in the mesenteric lymph node (3M: 1/4 animals)
2F, 4F	Agonal congestion in the mesenteric lymph nodes (minimal) (2F: 1/4 animals, 4F: 1/4 animals)
2M, 3M, 4M, 2F, 3F, 4F	Germinal center development in the popliteal lymph node (minimal to moderate) (2M: 2/4 animals, 3M: 2/4 animals, 2F: 2/4 animals, 3F: 3/4 animals, 4F: 2/4 animals)
1F	Parovarian cyst (1F: 1/4 animals)
4F	Pigmented macrophage aggregates in the Peyers patches (minimal) (4F: 1/4 animals)
2F	Squamous metaplasia in the prostate (minimal) (2F: 1/4

Groups	Findings
	animals)
1M, 2M, 3M, 4M,	Hypospermatogenesis in the testis (minimal to marked) (1M: 1/4 animals, 2M: 1/4 animals, 3M: 2/4 animals, 4M: 1/4 animals)
1M, 3M	Spermatid giant cells (minimal to slight) (3M: 1/4 animals, 4M: 1/4 animals)
2M, 3M, 4M, 1F, 2F, 3F, 4F	Agonal congestion in the thymus (2M: 1/4 animals, 3M: 2/4 animals, 4M: 3/4 animals, 1F: 1/4 animals, 2F: 2/4 animals, 3F: 1/4 animals, 4F: 1/4 animals)

Table 41: Microscopic findings at study day 43 (study # 4).

Small testis and hypospermatogenesis were observed, probably because of immaturity of the animals.

Histologically, germinal center development in the popliteal and iliac lymph node as well as mixed inflammatory cell infiltration in the medulla of the popliteal and iliac lymph node of minimal to moderate intensity was observed in the vaccinated groups on days 31 and 43. Furthermore, increased incidence of inflammation of the dermis/subcutis and muscle interstitium was observed at the injection site in all vaccinated groups. These findings were especially present on the right injection site which was treated twice and strongly improved after the recovery phase.

#### Body temperature

Group	Males	Females
1 (control)	0	0
2	0	0
3	0	0
4	0	0

Table of occurrences for body temperature  $\geq 40^{\circ}\text{C}$ 

Table 42: Body temperature (study # 4).

Treatment with Flud®<sup>®</sup>, Flud High B and Flud High H3+IC31®<sup>®</sup> did not result in treatment related changes in rectal body temperature.

#### Local toxicity:

Enlarged iliac lymph nodes were observed in all groups that received a vaccine on day 31 (2M: 4/4 animals, 3M: 4/4 animals, 4M: 4/4 animals, 2F: 3/4 animals, 3F: 3/4 animals, 4F: 1/4 animals), but not in the control group. After the recovery phase, enlarged iliac lymph nodes were only observed in two animals (3M: 1/4 animals, 4F: 1/4 animals).

Histologically, germinal center development in the popliteal and iliac lymph node as well as mixed inflammatory cell infiltration in the medulla of the popliteal and



iliac lymph node of minimal to moderate intensity was observed in the vaccinated groups on days 31 and 43. Furthermore, increased incidence of inflammation of the dermis/subcutis and muscle interstitium was observed at the injection site in all vaccinated groups. These findings were especially present on the right injection site which was treated twice and strongly improved after the recovery phase.

The sponsor stated that Draize score evaluation was performed 24h and 48h after the injection, but no data could be found in the submission.

### Serology:

The hemagglutination inhibition assay (HAI) was used in order to determine the titer of neutralizing antibodies in the rabbit sera once pretest and on days 15, 29 and 43.

No. of Animals	Treatment	Dosing Days	Sera to be analyzed for antibodies (days)	Strain and number of samples
8M/8F	Control	0, 15, 29	Pre-dose, 29, 43	H3N2 and B (56x2)
8M/8F	Fluad	0, 15, 29	Pre-dose, 15, 29, 43	H3N2 and B (56x2)
8M/8F	Fluad High B	0, 15, 29	Pre-dose, 15, 29, 43	B (56)
8M/8F	Fluad High H3+IC31	0, 15, 29	Pre-dose, 15, 29, 43	H3N2 (56)

**Total number of samples: 336**

Group	Mean of HAI titer pretest anti-B/Malaysia/2505/04	Mean of HAI titer day 15 anti-B/Malaysia/2505/04	Mean of HAI titer day 29 anti-B/Malaysia/2505/04	Mean of HAI titer day 43 anti-B/Malaysia/2505/04	Mean of HAI titer pretest anti-A/Wisconsin/67/05 (H3N2)	Mean of HAI titer day 15 anti-A/Wisconsin/67/05 (H3N2)	Mean of HAI titer day 29 anti-A/Wisconsin/67/05 (H3N2)	Mean of HAI titer day 43 anti-A/Wisconsin/67/05 (H3N2)
1	< 10	ND	ND	< 10	< 10	ND	< 10	< 10
2	< 10	225	910	960	< 10	2060	3200	4480
3	< 10	205	740	900	< 10	1460	2720	ND
4	< 10	45	435	ND	< 10	1295	3160	5920

ND: Not determined

Table 43: Serology results (study # 4).

Analysis of serum samples for neutralizing antibodies against the influenza A (H3N2) strain and influenza B strain revealed an immunogenic response in all males and females in all groups on day 15 (14 days after the first dose), which was significantly higher on day 29 (14 days after the second dose) and increased slightly more up to day 43 (after recovery).

Table of the range of HA1 titers for males and females.

	Range HAI titer Anti-B/malaysia/2506/04			Range HAI titer Anti-A/Wisconsin/67/05		
	Day 15	Day 29	Day 43	Day 15	Day 29	Day 43
Group 2 males	120-320	480-1280	640-960	960-2560	1280-3840	2560-3840
Group 3 males	120-320	480-1920	640-960	960-2560	1280-3840	NA
Group 4 males	15-40	240-640	NA	640-2560	1280-7680	1280-3840
Group 2 females	120-320	<10-3200	640-1280	1920-2560	2560-10240	3840-10240
Group 3 females	80-320	480-960	640-1280	960-2560	1280-7680	NA
Group 4 females	20-120	240-960	NA	640-2560	2560-3840	3840-15360

NA=not analyzed.

Table 44: Range of HA1 titers for males and females (study # 4).

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental
Inflammation of the dermis/subcutis on injection site ↑ Fibrinogen Germinal center development in the popliteal and iliac lymph node Medulla mixed inflammatory cell infiltration in the popliteal and iliac lymph node	Small testis Hypospermatogenesis and immaturity in the testis

#### Assessment:

The A/H1N1 S-OIV+/- MF59 formulation will be produced using the same manufacturing process as for Fluvirin, a Novartis seasonal trivalent influenza vaccine licensed in USA (License No. 1450, BLA No. 103837). For more details see the toxicology review for the original IND 14073 submissions.

This toxicology study 488182 has been submitted as 10<sup>th</sup> amendment to IND 14073 on August 24<sup>th</sup> 2009. Three intramuscular injections of trivalent influenza vaccine in combination with MF59 (Fluad, Fluad High B and Fluad High H3+IC31) are given to (b) (4) rabbits. In the proposed clinical trial 100% or 75% of the MF59 adjuvant amount used in Fluad will be used. The antigen dose in the proposed clinical trial will be 7.5- 30 µg of A/H1N1 S-OIV vaccine. For the toxicology study a trivalent vaccine containing 15 µg of A/H1N1 vaccine in combination with a 15 to 30 µg of a B strain and H3N2 strain has been used. The vaccine for the proposed clinical trial will be prepared using the Fluvirin-platform (egg-based). Group 4 in this toxicology study includes a combination of MF59 as well as IC31 as adjuvant. Only MF59, but not IC31 will be used in the proposed clinical study, therefore changes which are only seen in

group 4, but not in group 2 or 3 (Fluad and Fluad High B) are not specifically reported in this review.

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, clinical chemistry, gross anatomy or organ weight were reported.

Enlarged iliac lymph nodes were observed in all groups that received a vaccine on day 31 (2M: 4/4 animals, 3M: 4/4 animals, 4M: 4/4 animals, 2F: 3/4 animals, 3F: 3/4 animals, 4F: 1/4 animals), but not in the control group. After the recovery phase, enlarged iliac lymph nodes were only observed in two animals (3M: 1/4 animals, 4F: 1/4 animals). This enlargement might be related to the immune response due to vaccination.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Histologically, germinal center development in the popliteal and iliac lymph node as well as mixed inflammatory cell infiltration in the medulla of the popliteal and iliac lymph node of minimal to moderate intensity was observed in the vaccinated groups on days 31 and 43. Furthermore, increased incidence of inflammation of the dermis/subcutis and muscle interstitium was observed at the injection site in all vaccinated groups. These findings were especially present on the right injection site which was treated twice and strongly improved after the recovery phase.

No data for Draize score evaluation (performed 24h and 48h after the injection), were found in this submission, the sponsor was asked to submit these data if available.

Increased levels of fibrinogen were observed in all treatment groups on day 17 and 31. After the recovery phase fibrinogen levels were returned to control values. This observation is most likely a reflection of the overall transient inflammation after the vaccination.

Changes in white blood cell parameters were observed, no absolute numbers have been provided. The percentage of eosinophils was reduced in all male treatment groups on day 8 and 16 as well as pretest. The percentage of eosinophils was also reduced in all female treatment groups on day 31 and groups 2 and 4 on day 17. Moreover, basophils were reduced in all female treatment groups on days 17 and 31. After the recovery phase on day 43 the values were not reduced in comparison to the control values anymore except for the % of basophils of the female group 4.

**GLP study deviations or amendments:** No significant deviations or amendments were recorded that influenced the quality, integrity or interpretation of the results.

**Conclusions:** Based on nonclinical toxicity assessments of this study there are no significant safety issues to report.

#### **Study # 5:**

Title and study number: 8-month intramuscular toxicity study of Biocine® HIV gp120 antigen and Biocine® HIV Env 2-3 antigen in rabbits. Study number 2670-100.

**Performing laboratory:** (b) (4)

**Study initiation date:** 09/09/1992

**Final Report date:** 04/30/1997

**Test article batch/lot:**

Test article	Lot No.	Purity	Expiration Date
Biocine® HIV gp120 antigen	MGC016	NR*	NR
Biocine® HIV Env 2-3 antigen	MGC014	NR	NR
Biocine® MF59-0	MHC489	NR	NR
Biocine® MF59-100	MGB151	NR	NR
0.9% Sodium Chloride Injection, USP	J1S555	NR	06/1994

\*NR = Not reported.

**Animal species and strain:** Male and female (b) (4) rabbits

**Breeder/supplier:** (b) (4)

**Number of animal per group and sex:** Six per sex per group

**Age:** Approximately 4 months

**Body weight range:** 2267-2804 grams for males and 2127-2663 grams for females

**Route and site of administration:** Intramuscular (IM) injection into the hindlimb

**Volume of injection:** 0.5 mL

**Frequency of administration and study duration:** Test article was administered on study days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, and 232. The injection sites were alternated between left and right hindlegs on each successive dosing interval. Study duration was 247 days.

**Dose:** Varied "see experimental design table".

**Stability:** No stability data were submitted with this study.

**Means of administration:** IM injection

**Report status:** Final

## Experimental design

Group	Adjuvant Material	Dose Level*	Antigen Material	Dose Level*	Dose volume (mL)	Number of Animals (#/sex/group)	Number of Animals (#/sex/group)
						Treatment phase	Recovery phase
1	Saline	0	Saline	0	0.5	6	6
2	MF59-0	0	Saline	0	0.5	6	6
3	MF59-0	0	HIV gp120	50	0.5	6	6
4	MF59-100	100	Saline	0	0.5	6	6
5	MF59-100	100	HIV gp120	50	0.5	6	6
6	MF59-100	100	HIV Env 2-3	100	0.5	6	6

\*Dose level given in µg/animal/dose.

Table 45: Experimental design (study # 5).

## Methods:

Hematology, clinical chemistry, and coagulation parameters were determined using the following:

Endpoint	Methodology
Hematology	(b) (4)
Clinical chemistry	(b) (4)
Coagulation	(b) (4)
Urinalysis	(b) (4)

Randomization procedure: Yes

Statistical analysis plan: Yes

The following parameters were evaluated: cage side observations (twice daily), detailed observations (once daily), irritation score (approximately 24 hours post-dose and once daily for 1 week post injection), body weights (day 1 and weekly thereafter), food consumption (not determined), ophthalmoscopy (pre-dose phase and on days 107 [males]/106 [females] and 233 [males]/232 [females]), body temperature (day -1; pre-dose on days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, and 232; 2 days post-dose on days 3, 24, 45, 66, 87, 108, 129, 150, 171, 192, and 213; prior to terminal sacrifice on day 233; and once weekly during the non-dose weeks), hematology, coagulation, and clinical chemistry (days -8 and -1 and on days 3, 21, 24, 42, 45, 63, 66, 84, 87, 105, 108, 126, 129, 147, 150, 168, 171, 189, 192, 210, 213, 233, and 247 [recovery animals]), gross anatomy at termination, organ weights and histopathology on a selection of tissues, urinalysis (days 233 and 247). Serology (not determined).

Parameters	Frequency of Testing
Cageside observation <sup>17</sup>	Twice daily
Clinical observations <sup>18</sup>	Once daily

<sup>17</sup> Cageside observations include mortality, morbidity, general health and signs of toxicity.

Parameters	Frequency of Testing
Body weight	Day 1 and weekly thereafter
Food consumption	Not determined
Body temperature	Day -1; pre-dose on days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, and 232; 2 days post-dose on days 3, 24, 45, 66, 87, 108, 129, 150, 171, 192, and 213; prior to terminal sacrifice on day 233; and once weekly during the non-dose weeks
Ophthalmologic exam	Pre-dose phase and on days 107 [males]/106 [females] and 233 [males]/232 [females]
Clinical chemistry*	Days -8 and -1 and on days 3, 21, 24, 42, 45, 63, 66, 84, 87, 105, 108, 126, 129, 147, 150, 168, 171, 189, 192, 210, 213, 233, and 247 [recovery animals]
Hematology*	Days -8 and -1 and on days 3, 21, 24, 42, 45, 63, 66, 84, 87, 105, 108, 126, 129, 147, 150, 168, 171, 189, 192, 210, 213, 233, and 247 [recovery animals]
Coagulation*	Days -8 and -1 and on days 3, 21, 24, 42, 45, 63, 66, 84, 87, 105, 108, 126, 129, 147, 150, 168, 171, 189, 192, 210, 213, 233, and 247 [recovery animals]
Immunological response	Not determined
Evaluation of site of inoculation (e.g., the dermal Draize scoring method)	Approximately 24 hours post-dose and once daily for 1 week post injection
Necropsy	Days 233 and 247
Tissues for histopathology	Days 233 and 247

\*Collected from the medial ear artery.

Table 46: Parameters evaluated (study # 5).

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta		X
Bone (femur)	!	
Bone marrow (femur)	!	

<sup>18</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Organ/Tissue	Collected	Not collected
Brain	!*	
Cecum	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		X
Gall bladder	!	
Gross Lesions	!	
Harderian gland		X
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		X
Larynx		X
Liver	!*	
Lung (with bronchi)	!	
Lymph nodes (cervical)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Ovaries	!*	
Pancreas	!	
Peyer's patch		X
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve		X
Seminal vesical		X
Skeletal muscle (psoas)	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters		X

Organ/Tissue	Collected	Not collected
Uterus	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		X

Table 47: Tissues collected (study # 5).

Table of Histology – Tissues listed above were collected from all animals and examined microscopically.

**Results:**

Morbidity and mortality: All animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ )	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Lactate dehydrogenase (LDH) SD 3 M $\downarrow \leq 0.6$ G2 SD 45 M $\uparrow \geq 1.7$ G5 SD 105 M $\downarrow \leq 0.7$ G2 SD 105 M $\downarrow \leq 0.7$ G4 SD 105 M $\downarrow \leq 0.7$ G6 SD 108 M $\uparrow \geq 1.7$ G6 SD 147 M $\downarrow \leq 0.7$ G2 SD 150 M $\uparrow \geq 3.9$ G6 SD 171 M $\uparrow \geq 1.7$ G6 SD 192 M $\uparrow \geq 1.8$ G4 SD 192 M $\uparrow \geq 1.7$ G5 SD 192 M $\uparrow \geq 3.6$ G6 SD 210 M $\uparrow \geq 1.9$ G6 SD 213 M $\uparrow \geq 2.0$ G5 SD 213 M $\uparrow \geq 3.8$ G6 SD 233 M $\uparrow \geq 5.5$ G2 SD 233 M $\uparrow \geq 4.3$ G3 SD 247 M $\uparrow \geq 2.2$ G6  SD 42 F $\downarrow \leq 0.7$ G2	



CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
	SD 42 F $\downarrow \leq 0.7$ G3 SD 42 F $\downarrow \leq 0.7$ G4 SD 42 F $\downarrow \leq 0.7$ G5 SD 66 F $\uparrow \geq 1.7$ G5 SD 105 F $\downarrow \leq 0.7$ G4 SD 108 F $\uparrow \geq 1.8$ G6 SD 129 F $\uparrow \geq 1.7$ G2 SD 129 F $\uparrow \geq 1.7$ G5 SD 129 F $\uparrow \geq 2.2$ G6 SD 147 F $\uparrow \geq 2.1$ G6 SD 150 F $\uparrow \geq 1.9$ G4 SD 150 F $\uparrow \geq 1.9$ G6 SD 168 F $\uparrow \geq 1.8$ G6 SD 189 F $\uparrow \geq 2.1$ G6 SD 192 F $\uparrow \geq 1.9$ G4 SD 192 F $\uparrow \geq 2.3$ G6 SD 210 F $\uparrow \geq 2.0$ G6 SD 213 F $\uparrow \geq 1.7$ G4 SD 213 F $\uparrow \geq 1.8$ G6 SD 231 F $\uparrow \geq 1.7$ G6 SD 233 F $\uparrow \geq 9.4$ G2 SD 233 F $\uparrow \geq 7.1$ G3 SD 247 F $\uparrow \geq 1.8$ G6  Aspartate aminotransferase (AST or SGOT) SD 3 M $\downarrow \leq 0.7$ G6 SD 21 M $\uparrow \geq 1.7$ G4 SD 24 M $\uparrow \geq 1.8$ G3 SD 24 M $\uparrow \geq 2.7$ G4 SD 24 M $\downarrow \leq 0.7$ G5 SD 42 M $\uparrow \geq 1.9$ G4 SD 45 M $\uparrow \geq 2.9$ G4 SD 63 M $\uparrow \geq 2.0$ G4 SD 65 M $\uparrow \geq 2.3$ G4 SD 87 M $\uparrow \geq 1.8$ G4 SD 129 M $\downarrow \leq 0.6$ G5 SD 129 M $\downarrow \leq 0.7$ G6 SD 147 M $\uparrow \geq 1.9$ G4	

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
	SD 150 M $\uparrow \geq 1.8$ G4 SD 210 M $\uparrow \geq 1.7$ G4 SD 233 M $\uparrow \geq 3.4$ G4  SD 21 F $\uparrow \geq 2.2$ G4 SD 66 F $\uparrow \geq 1.9$ G4 SD 147 F $\uparrow \geq 1.8$ G3 SD 150 F $\uparrow \geq 1.7$ G4 SD 168 F $\downarrow \leq 0.7$ G6 SD 192 F $\uparrow \geq 2.4$ G4 SD 233 F $\uparrow \geq 1.7$ G4  Alanine aminotransferase (ALT or SGPT) SD 24 M $\uparrow \geq 2.1$ G4 SD 42 M $\uparrow \geq 1.7$ G4 SD 45 M $\uparrow \geq 2.4$ G4 SD 63 M $\uparrow \geq 2.1$ G4 SD 66 M $\uparrow \geq 2.5$ G4 SD 84 M $\uparrow \geq 1.8$ G4 SD 87 M $\uparrow \geq 2.0$ G4 SD 105 M $\uparrow \geq 1.7$ G4 SD 108 M $\uparrow \geq 1.9$ G4 SD 108 M $\downarrow \leq 0.7$ G5 SD 126 M $\uparrow \geq 1.8$ G4 SD 129 M $\uparrow \geq 2.4$ G4 SD 129 M $\downarrow \leq 0.7$ G5 SD 150 M $\uparrow \geq 2.1$ G4 SD 192 M $\uparrow \geq 2.3$ G4 SD 210 M $\uparrow \geq 1.7$ G4 SD 213 M $\uparrow \geq 2.2$ G4 SD 231 M $\uparrow \geq 1.7$ G4 SD 233 M $\uparrow \geq 2.7$ G4  SD 108 F $\downarrow \leq 0.7$ G5 SD 108 F $\downarrow \leq 0.6$ G6 SD 192 F $\downarrow \leq 0.7$ G6	
LIVER FUNCTION: A) HEPATOCELLULAR		Glutamate dehydrogenase Total bile acids

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ )	NOT OF NOTE
B) HEPATOBILIARY	Alkaline phosphatase (ALK) SD 3 M $\downarrow \leq 0.5$ G4 SD 3 M $\downarrow \leq 0.5$ G5 SD 3 M $\downarrow \leq 0.7$ G6 SD 24 M $\downarrow \leq 0.6$ G5 SD 24 M $\downarrow \leq 0.6$ G6 SD 45 M $\downarrow \leq 0.5$ G5 SD 45 M $\downarrow \leq 0.7$ G6 SD 66 M $\downarrow \leq 0.6$ G5 SD 87 M $\downarrow \leq 0.6$ G5 SD 129 M $\downarrow \leq 0.6$ G5 SD 129 M $\downarrow \leq 0.7$ G6 SD 150 M $\downarrow \leq 0.6$ G5 SD 171 M $\downarrow \leq 0.6$ G5 SD 192 M $\downarrow \leq 0.7$ G5  SD 3 F $\downarrow \leq 0.7$ G5 SD 45 F $\downarrow \leq 0.7$ G5 SD 66 F $\downarrow \leq 0.7$ G5 SD 150 F $\downarrow \leq 0.7$ G5 SD 192 F $\downarrow \leq 0.7$ G5 SD 231 F $\uparrow \geq 1.8$ G4	Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides SD 3 M $\uparrow \geq 1.7$ G5 SD 24 M $\uparrow \geq 2.3$ G4 SD 45 M $\uparrow \geq 2.2$ G4 SD 45 M $\uparrow \geq 2.0$ G5 SD 63 M $\downarrow \leq 0.7$ G5 SD 66 M $\uparrow \geq 2.5$ G4 SD 66 M $\uparrow \geq 1.9$ G5 SD 87 M $\uparrow \geq 2.8$ G4 SD 66 M $\uparrow \geq 2.2$ G5 SD 66 M $\uparrow \geq 1.8$ G6 SD 108 M $\uparrow \geq 2.7$ G4 SD 108 M $\uparrow \geq 2.1$ G5 SD 108 M $\uparrow \geq 1.8$ G6	Albumin (A) Cholinesterase Total protein

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
	SD 129 M $\uparrow \geq 3.1$ G4 SD 129 M $\uparrow \geq 1.9$ G5 SD 150 M $\uparrow \geq 3.4$ G4 SD 150 M $\uparrow \geq 3.1$ G5 SD 150 M $\uparrow \geq 2.5$ G6 SD 171 M $\uparrow \geq 3.9$ G4 SD 171 M $\uparrow \geq 3.4$ G5 SD 171 M $\uparrow \geq 2.4$ G6 SD 192 M $\uparrow \geq 3.1$ G4 SD 192 M $\uparrow \geq 2.1$ G5 SD 192 M $\uparrow \geq 1.9$ G6 SD 213 M $\uparrow \geq 2.8$ G4 SD 213 M $\uparrow \geq 2.3$ G5 SD 213 M $\uparrow \geq 1.9$ G6  SD 3 F $\uparrow \geq 1.7$ G4 SD 3 F $\uparrow \geq 2.7$ G5 SD 45 F $\uparrow \geq 2.1$ G5 SD 45 F $\uparrow \geq 1.9$ G6 SD 66 F $\uparrow \geq 2.4$ G5 SD 87 F $\uparrow \geq 2.5$ G5 SD 87 F $\uparrow \geq 1.8$ G6 SD 129 F $\uparrow \geq 1.7$ G4 SD 129 F $\uparrow \geq 2.6$ G5 SD 150 F $\uparrow \geq 2.7$ G5 SD 150 F $\uparrow \geq 1.9$ G6 SD 171 F $\uparrow \geq 2.3$ G5 SD 192 F $\uparrow \geq 2.4$ G5 SD 210 F $\downarrow \leq 0.7$ G4 SD 210 F $\downarrow \leq 0.7$ G6 SD 213 F $\uparrow \geq 2.1$ G5  Creatine kinase (CK) SD 42 M $\uparrow \geq 1.7$ G2 SD 45 M $\downarrow \leq 0.7$ G2 SD 66 M $\uparrow \geq 1.7$ G6 SD 87 M $\uparrow \geq 1.9$ G5 SD 87 M $\uparrow \geq 1.9$ G6 SD 108 M $\downarrow \leq 0.6$ G3	

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
	SD 108 M $\downarrow \leq 0.4$ G4 SD 108 M $\uparrow \geq 1.7$ G6 SD 129 M $\uparrow \geq 3.9$ G2 SD 129 M $\uparrow \geq 1.8$ G3 SD 129 M $\uparrow \geq 2.6$ G5 SD 129 M $\uparrow \geq 2.5$ G6 SD 150 M $\uparrow \geq 13.2$ G2 SD 150 M $\uparrow \geq 6.5$ G3 SD 150 M $\uparrow \geq 3.9$ G5 SD 150 M $\uparrow \geq 16.6$ G6 SD 171 M $\uparrow \geq 7.1$ G2 SD 171 M $\uparrow \geq 7.9$ G3 SD 171 M $\uparrow \geq 2.8$ G5 SD 171 M $\uparrow \geq 3.8$ G6 SD 192 M $\uparrow \geq 5.1$ G2 SD 192 M $\uparrow \geq 7.2$ G3 SD 192 M $\uparrow \geq 2.4$ G4 SD 192 M $\uparrow \geq 3.3$ G5 SD 192 M $\uparrow \geq 13.2$ G6 SD 213 M $\uparrow \geq 6.8$ G2 SD 213 M $\uparrow \geq 6.7$ G3 SD 213 M $\uparrow \geq 2.6$ G4 SD 213 M $\uparrow \geq 7.0$ G5 SD 213 M $\uparrow \geq 17.8$ G6 SD 233 M $\uparrow \geq 22.1$ G2 SD 233 M $\uparrow \geq 16.8$ G3 SD 233 M $\downarrow \leq 0.6$ G4 SD 233 M $\downarrow \leq 0.7$ G5 SD 247 M $\uparrow \geq 2.1$ G6  SD 105 F $\downarrow \leq 0.4$ G2 SD 105 F $\downarrow \leq 0.4$ G3 SD 105 F $\downarrow \leq 0.4$ G4 SD 105 F $\downarrow \leq 0.4$ G5 SD 105 F $\downarrow \leq 0.5$ G6 SD 108 F $\uparrow \geq 2.0$ G2 SD 108 F $\downarrow \leq 0.5$ G5 SD 129 F $\uparrow \geq 5.1$ G2 SD 129 F $\uparrow \geq 4.2$ G3	

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
	SD 129 F $\uparrow \geq 2.1$ G6 SD 150 F $\uparrow \geq 13.1$ G2 SD 150 F $\uparrow \geq 10.7$ G3 SD 150 F $\uparrow \geq 4.8$ G4 SD 150 F $\uparrow \geq 3.5$ G6 SD 171 F $\uparrow \geq 6.0$ G2 SD 171 F $\uparrow \geq 5.1$ G3 SD 171 F $\uparrow \geq 1.7$ G4 SD 171 F $\uparrow \geq 1.7$ G5 SD 171 F $\uparrow \geq 2.3$ G6 SD 192 F $\uparrow \geq 7.1$ G2 SD 192 F $\uparrow \geq 5.3$ G3 SD 192 F $\uparrow \geq 3.8$ G4 SD 192 F $\uparrow \geq 2.2$ G5 SD 192 F $\uparrow \geq 3.6$ G6 SD 210 F $\uparrow \geq 1.7$ G6 SD 213 F $\uparrow \geq 11.1$ G2 SD 213 F $\uparrow \geq 12.4$ G3 SD 213 F $\uparrow \geq 3.4$ G4 SD 213 F $\uparrow \geq 2.7$ G5 SD 213 F $\uparrow \geq 2.9$ G6 SD 231 F $\uparrow \geq 2.0$ G3 SD 233 F $\uparrow \geq 19.8$ G2 SD 233 F $\uparrow \geq 11.1$ G3 SD 233 F $\downarrow \leq 0.6$ G5 SD 247 F $\downarrow \leq 0.6$ G2 SD 247 F $\downarrow \leq 0.7$ G3 SD 247 F $\downarrow \leq 0.6$ G5  Total cholesterol SD 150 M $\uparrow \geq 1.7$ G4 SD 150 M $\uparrow \geq 1.7$ G5  SD 213 F $\uparrow \geq 1.7$ G5  A/G Ratio SD 108 M $\downarrow \leq 0.7$ G5 SD 108 M $\downarrow \leq 0.7$ G6 SD 213 M $\downarrow \leq 0.7$ G5 SD 213 M $\downarrow \leq 0.7$ G6	

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 0.7$ ))	NOT OF NOTE
	SD 150 F $\downarrow \leq 0.7$ G5 SD 171 F $\downarrow \leq 0.7$ G5 SD 192 F $\downarrow \leq 0.7$ G5	

Table 48: Clinical chemistry results (study # 5).

In males, lactate dehydrogenase levels were decreased in group 2 on study days 3, 105, and 147. In males, lactate dehydrogenase levels were decreased in groups 4 and 6 on study day 105. However, a trend of increase in lactate dehydrogenase levels were reported in general after study day 108 in groups 4, 5, and 6. This increase was also reported in group 5 on study day 45 and in groups 2 and 3 on study day 233. In females, lactate dehydrogenase levels were decreased in groups 2, 3, 4, and 5 on study day 42. This decrease was also reported in group 4 on study day 105. However, in general a trend of increase in lactate dehydrogenase levels were reported after study day 108 in groups 4, 5, and 6. This increase was also reported in group 5 on study day 66 and in groups 2 and 3 on study day 233. It is worth noting that the frequency of increase was the highest in group 6.

In males, aspartate aminotransferase levels were decreased in group 5 on study days 24 and 129 and in group 6 on study days 3 and 129. An increase in aspartate aminotransferase levels were reported in group 3 on study day 24. The highest frequency of increase was reported in group 4 (study days 21, 24, 42, 45, 63, 65, 87, 147, 150, 210, and 233). In females, decrease in aspartate aminotransferase levels was reported in group 6 on study day 168. The highest frequency of increase was reported in group 4 (study days 21, 66, 147, 150, 192, and 233).

In males, alanine aminotransferase levels were decreased in group 5 on study days 108 and 129. The highest frequency of increase was reported in group 4 (study days 24, 42, 45, 63, 66, 84, 87, 105, 108, 126, 129, 150, 192, 210, 213, 231, and 233). In females, decreases in aspartate aminotransferase levels were reported in group 5 on study day 108 and in group 6 on study days 108 and 192.

In males, alkaline phosphatase (ALK) levels were decreased in groups 4, 5, and 6 on study day 3. Alkaline phosphatase (ALK) levels were decreased in group 6 on study days 24, 45, and 129. The highest frequency of decrease was reported in group 5 (study days 24, 45, 66, 87, 129, 150, 192, 171, and 192). In females, decreases in alkaline phosphatase (ALK) levels were reported in group 5 on

study days 3, 45, 66, 150, and 192 and in group 4 on study day 231.

In males, significant increases in triglyceride levels were reported in groups 4 (starting on day 24), 5 (starting on day 3), and 6 (starting on day 66). Triglyceride levels were decreased in group 5 on study day 63. In females, significant increases in triglyceride levels were reported in groups 4 (starting on day 3), 5 (starting on day 3), and 6 (starting on day 45). Triglyceride levels were decreased in groups 4 and 6 on study day 210.

In males, significant increases in creatine kinase levels were reported in groups 2 (starting on study day 42), 3 (starting on study day 129), 4 (starting on study day 192), 5 (starting on study day 87), 6 (starting on study day 66). Creatine kinase levels were decreased in groups 2 (study day 45), 3 (study day 108), 4 (study days 108 and 233), and 5 (study day 233). In females, significant increases in creatine kinase levels were reported in groups 2 (starting on study day 108), 3 (starting on study day 129), 4 (starting on study day 150), 5 (starting on study day 171), 6 (starting on study day 129). Creatine kinase levels were decreased in groups 2, 3, 4, 5, and 6 (study day 105), 5 (study day 108, 233, and 247), 2 (study day 247), and 3 (study day 247).

Total cholesterol levels were increased in groups 4 and 5 males on study day 150 and in group 5 females on study day 213.

Differences associated with inflammation included significant increases in globulin in groups 4, 5, and 6 (occasionally groups 2 and 3), with compensatory significant, but mild, decreases in albumin. Inconsistent, mild increases in total protein levels were reported in all treated groups.

A decrease in the A/G ratio was reported in groups 5 and 6 males on study days 108 and 213. A decrease in the A/G ratio was reported in group 5 females on study days 150, 171, and 192.



HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
RED BLOOD CELLS	Reticulocytes SD 108 M $\downarrow \leq 0.6$ G5 SD 247 M $\downarrow \leq 0.6$ G4 SD 247 M $\downarrow \leq 0.7$ G6  SD 24 F $\uparrow \geq 2.1$ G3 SD 84 F $\downarrow \leq 0.7$ G5 SD 233 F $\downarrow \leq 0.6$ G6	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
WHITE BLOOD CELLS	Lymphocyte count SD 108 M $\downarrow \leq 0.7$ G3 SD 108 M $\downarrow \leq 0.7$ G5 SD 108 M $\downarrow \leq 0.7$ G6 SD 126 M $\downarrow \leq 0.7$ G3 SD 147 M $\downarrow \leq 0.7$ G6 SD 192 M $\downarrow \leq 0.7$ G3 SD 233 M $\downarrow \leq 0.7$ G4 SD 233 M $\downarrow \leq 0.7$ G6  WBC SD 84 M $\downarrow \leq 0.6$ G3 SD 108 M $\downarrow \leq 0.7$ G3 SD 126 M $\downarrow \leq 0.7$ G3 SD 147 M $\downarrow \leq 0.7$ G6  Segmented Neutrophils SD 42 M $\uparrow \geq 2.1$ G5 SD 42 M $\uparrow \geq 1.6$ G6 SD 45 M $\uparrow \geq 1.6$ G5 SD 63 M $\downarrow \leq 0.5$ G3 SD 63 M $\downarrow \leq 0.6$ G4 SD 63 M $\downarrow \leq 0.7$ G5 SD 63 M $\downarrow \leq 0.6$ G6 SD 66 M $\uparrow \geq 1.6$ G5 SD 84 M $\downarrow \leq 0.3$ G2 SD 84 M $\downarrow \leq 0.2$ G3 SD 84 M $\downarrow \leq 0.4$ G4	Eosinophils count Macrophage Large Unstained Cells (LUC)

<sup>19</sup> With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
	SD 84 M $\downarrow \leq 0.5$ G5 SD 84 M $\downarrow \leq 0.5$ G6 SD 87 M $\uparrow \geq 1.6$ G5 SD 126 M $\downarrow \leq 0.6$ G3 SD 126 M $\downarrow \leq 0.6$ G4 SD 126 M $\downarrow \leq 0.7$ G5 SD 126 M $\downarrow \leq 0.5$ G6 SD 129 M $\uparrow \geq 2.1$ G4 SD 129 M $\uparrow \geq 2.8$ G5 SD 129 M $\uparrow \geq 1.8$ G6 SD 168 M $\downarrow \leq 0.6$ G3 SD 168 M $\downarrow \leq 0.6$ G4 SD 168 M $\downarrow \leq 0.7$ G6 SD 171 M $\uparrow \geq 1.9$ G4 SD 171 M $\uparrow \geq 2.1$ G5 SD 171 M $\uparrow \geq 1.7$ G6 SD 189 M $\downarrow \leq 0.5$ G3 SD 189 M $\downarrow \leq 0.6$ G6 SD 192 M $\uparrow \geq 2.4$ G5 SD 213 M $\uparrow \geq 3.2$ G4 SD 213 M $\uparrow \geq 2.6$ G5 SD 213 M $\uparrow \geq 2.9$ G6 SD 231 M $\downarrow \leq 0.5$ G6 SD 233 M $\uparrow \geq 1.8$ G4 SD 233 M $\uparrow \geq 2.2$ G5 SD 247 M $\uparrow \geq 2.1$ G5  SD 21 F $\downarrow \leq 0.5$ G2 SD 21 F $\downarrow \leq 0.7$ G3 SD 21 F $\downarrow \leq 0.7$ G4 SD 21 F $\downarrow \leq 0.7$ G5 SD 24 F $\downarrow \leq 0.6$ G2 SD 42 F $\uparrow \geq 1.7$ G6 SD 45 F $\uparrow \geq 2.4$ G5 SD 63 F $\downarrow \leq 0.6$ G2 SD 66 F $\uparrow \geq 1.9$ G5 SD 66 F $\uparrow \geq 2.0$ G6 SD 87 F $\uparrow \geq 1.8$ G5 SD 87 F $\uparrow \geq 1.8$ G6	

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
	SD 108 F $\uparrow \geq 1.8$ G5 SD 150 F $\uparrow \geq 1.9$ G5 SD 171 F $\uparrow \geq 2.1$ G5 SD 189 F $\downarrow \leq 0.5$ G2 SD 189 F $\downarrow \leq 0.5$ G4 SD 189 F $\downarrow \leq 0.5$ G6 SD 210 F $\downarrow \leq 0.6$ G4 SD 233 F $\uparrow \geq 3.2$ G4 SD 233 F $\uparrow \geq 2.4$ G5 SD 233 F $\uparrow \geq 2.8$ G6 SD 247 F $\downarrow \leq 0.5$ G2  Basophils count SD 84 M $\downarrow \leq 0.3$ G3 SD 84 M $\downarrow \leq 0.3$ G6 SD 87 M $\downarrow \leq 0.5$ G6 SD 147 M $\downarrow \leq 0.3$ G2 SD 150 M $\downarrow \leq 0.3$ G3 SD 150 M $\downarrow \leq 0.5$ G4 SD 150 M $\downarrow \leq 0.5$ G6 SD 168 M $\uparrow \geq 2.5$ G5 SD 171 M $\downarrow \leq 0.3$ G2 SD 171 M $\downarrow \leq 0.3$ G3 SD 171 M $\downarrow \leq 0.3$ G4 SD 171 M $\downarrow \leq 0.7$ G5 SD 171 M $\downarrow \leq 0.7$ G6 SD 189 M $\downarrow \leq 0.3$ G2  SD 3 F $\downarrow \leq 0.3$ G2 SD 3 F $\downarrow \leq 0.3$ G3 SD 3 F $\downarrow \leq 0.3$ G4 SD 21 F $\downarrow \leq 0.3$ G2 SD 42 F $\downarrow \leq 0.3$ G3 SD 45 F $\downarrow \leq 0.6$ G2 SD 45 F $\downarrow \leq 0.6$ G3 SD 45 F $\downarrow \leq 0.6$ G6 SD 63 F $\downarrow \leq 0.4$ G2 SD 63 F $\downarrow \leq 0.4$ G3 SD 63 F $\downarrow \leq 0.4$ G4	

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
	SD 63 F $\downarrow \leq 0.6$ G5 SD 63 F $\downarrow \leq 0.6$ G6 SD 66 F $\downarrow \leq 0.5$ G3 SD 66 F $\downarrow \leq 0.5$ G6 SD 87 F $\downarrow \leq 0.3$ G3 SD 105 F $\downarrow \leq 0.5$ G4 SD 105 F $\downarrow \leq 0.5$ G6 SD 108 F $\downarrow \leq 0.5$ G2 SD 168 F $\downarrow \leq 0.5$ G5 SD 171 F $\downarrow \leq 0.5$ G2 SD 171 F $\downarrow \leq 0.3$ G3 SD 171 F $\downarrow \leq 0.5$ G5 SD 171 F $\downarrow \leq 0.5$ G6 SD 189 F $\downarrow \leq 0.5$ G3 SD 189 F $\downarrow \leq 0.5$ G4 SD 189 F $\downarrow \leq 0.5$ G5 SD 192 F $\downarrow \leq 0.5$ G2 SD 192 F $\downarrow \leq 0.3$ G3 SD 192 F $\downarrow \leq 0.5$ G4 SD 192 F $\downarrow \leq 0.5$ G5 SD 192 F $\downarrow \leq 0.5$ G6 SD 213 F $\downarrow \leq 0.3$ G2 SD 213 F $\downarrow \leq 0.3$ G3 SD 213 F $\downarrow \leq 0.5$ G4 SD 213 F $\downarrow \leq 0.3$ G5 SD 231 F $\downarrow \leq 0.5$ G2 SD 233 F $\downarrow \leq 0.5$ G2 SD 233 F $\downarrow \leq 0.5$ G3 SD 247 F $\downarrow \leq 0.5$ G2  Monocyte count SD 105 F $\downarrow \leq 0.3$ G3 SD 105 F $\downarrow \leq 0.3$ G6 SD 108 F $\downarrow \leq 0.6$ G6 SD 129 F $\downarrow \leq 0.3$ G2 SD 129 F $\uparrow \geq 2.0$ G4 SD 129 F $\uparrow \geq 2.0$ G6 SD 150 F $\uparrow \geq 2.5$ G4 SD 150 F $\uparrow \geq 2.0$ G5	

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
	SD 150 F $\uparrow \geq 2.0$ G6	

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
CLOTTING POTENTIAL	<p>Platelet count</p> <p>SD 66 M <math>\downarrow \leq 0.7</math> G5</p> <p>SD 66 M <math>\downarrow \leq 0.7</math> G6</p> <p>SD 87 M <math>\downarrow \leq 0.7</math> G5</p> <p>SD 87 M <math>\downarrow \leq 0.7</math> G6</p> <p>SD 108 M <math>\downarrow \leq 0.6</math> G5</p> <p>SD 233 M <math>\downarrow \leq 0.7</math> G4</p> <p>SD 233 M <math>\downarrow \leq 0.5</math> G5</p> <p>SD 233 M <math>\downarrow \leq 0.7</math> G6</p> <p>Fibrinogen</p> <p>SD 3 M <math>\uparrow \geq 2.1</math> G4</p> <p>SD 3 M <math>\uparrow \geq 2.5</math> G5</p> <p>SD 3 M <math>\uparrow \geq 2.1</math> G6</p> <p>SD 24 M <math>\uparrow \geq 2.8</math> G4</p> <p>SD 24 M <math>\uparrow \geq 3.4</math> G5</p> <p>SD 24 M <math>\uparrow \geq 2.9</math> G6</p> <p>SD 45 M <math>\uparrow \geq 1.8</math> G3</p> <p>SD 45 M <math>\uparrow \geq 3.1</math> G4</p> <p>SD 45 M <math>\uparrow \geq 4.3</math> G5</p> <p>SD 45 M <math>\uparrow \geq 3.6</math> G6</p> <p>SD 66 M <math>\uparrow \geq 1.8</math> G3</p> <p>SD 66 M <math>\uparrow \geq 3.0</math> G4</p> <p>SD 66 M <math>\uparrow \geq 3.9</math> G5</p> <p>SD 66 M <math>\uparrow \geq 3.2</math> G6</p> <p>SD 87 M <math>\uparrow \geq 1.8</math> G2</p> <p>SD 87 M <math>\uparrow \geq 1.7</math> G3</p> <p>SD 87 M <math>\uparrow \geq 3.6</math> G4</p> <p>SD 87 M <math>\uparrow \geq 4.3</math> G5</p> <p>SD 87 M <math>\uparrow \geq 3.5</math> G6</p> <p>SD 108 M <math>\uparrow \geq 2.8</math> G4</p> <p>SD 108 M <math>\uparrow \geq 3.2</math> G5</p> <p>SD 108 M <math>\uparrow \geq 2.5</math> G6</p> <p>SD 129 M <math>\uparrow \geq 1.7</math> G2</p> <p>SD 129 M <math>\uparrow \geq 3.2</math> G4</p> <p>SD 129 M <math>\uparrow \geq 3.4</math> G5</p> <p>SD 129 M <math>\uparrow \geq 2.8</math> G6</p> <p>SD 150 M <math>\uparrow \geq 1.8</math> G2</p> <p>SD 150 M <math>\uparrow \geq 3.5</math> G4</p>	<p>Activated partial-thromboplastin time clotting time</p> <p>Prothrombin time</p> <p>Mean platelet volume</p>

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
	SD 150 M $\uparrow \geq 3.7$ G5 SD 150 M $\uparrow \geq 3.3$ G6 SD 171 M $\uparrow \geq 3.2$ G4 SD 171 M $\uparrow \geq 3.6$ G5 SD 171 M $\uparrow \geq 3.1$ G6 SD 192 M $\uparrow \geq 2.9$ G4 SD 192 M $\uparrow \geq 2.8$ G5 SD 192 M $\uparrow \geq 2.5$ G6 SD 213 M $\uparrow \geq 3.1$ G4 SD 213 M $\uparrow \geq 2.8$ G5 SD 213 M $\uparrow \geq 2.9$ G6 SD 233 M $\uparrow \geq 2.4$ G4 SD 233 M $\uparrow \geq 2.3$ G5 SD 233 M $\uparrow \geq 2.2$ G6  SD 3 F $\uparrow \geq 2.9$ G4 SD 3 F $\uparrow \geq 3.5$ G5 SD 3 F $\uparrow \geq 2.8$ G6 SD 24 F $\uparrow \geq 2.7$ G4 SD 24 F $\uparrow \geq 3.3$ G5 SD 24 F $\uparrow \geq 2.6$ G6 SD 45 F $\uparrow \geq 2.7$ G4 SD 45 F $\uparrow \geq 3.6$ G5 SD 45 F $\uparrow \geq 3.5$ G6 SD 66 F $\uparrow \geq 1.7$ G2 SD 66 F $\uparrow \geq 3.0$ G4 SD 66 F $\uparrow \geq 4.3$ G5 SD 66 F $\uparrow \geq 3.3$ G6 SD 87 F $\uparrow \geq 2.9$ G4 SD 87 F $\uparrow \geq 3.9$ G5 SD 87 F $\uparrow \geq 3.2$ G6 SD 108 F $\uparrow \geq 2.8$ G4 SD 108 F $\uparrow \geq 3.5$ G5 SD 108 F $\uparrow \geq 2.8$ G6 SD 129 F $\uparrow \geq 1.8$ G2 SD 129 F $\uparrow \geq 3.4$ G4 SD 129 F $\uparrow \geq 3.8$ G5 SD 129 F $\uparrow \geq 3.1$ G6 SD 150 F $\uparrow \geq 1.8$ G2	

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5 <sup>19</sup> , ie, $\geq 1.6$ or $\leq 0.7$	NOT OF NOTE
	SD 150 F $\uparrow \geq 3.3$ G4 SD 150 F $\uparrow \geq 4.2$ G5 SD 150 F $\uparrow \geq 3.3$ G6 SD 171 F $\uparrow \geq 1.9$ G2 SD 171 F $\uparrow \geq 3.2$ G4 SD 171 F $\uparrow \geq 3.9$ G5 SD 171 F $\uparrow \geq 3.5$ G6 SD 192 F $\uparrow \geq 3.0$ G4 SD 192 F $\uparrow \geq 3.5$ G5 SD 192 F $\uparrow \geq 2.8$ G6 SD 213 F $\uparrow \geq 3.3$ G4 SD 213 F $\uparrow \geq 3.5$ G5 SD 213 F $\uparrow \geq 2.7$ G6 SD 233 F $\uparrow \geq 2.7$ G4 SD 233 F $\uparrow \geq 2.9$ G5 SD 233 F $\uparrow \geq 2.6$ G6	
OTHERS		Bone marrow cytology

Table 49: Hematology results (study # 5).

Reticulocyte levels were decreased in groups 4, 5, and 6 males on study days 247, 108, and 247, respectively. Reticulocyte levels were decreased in groups 5 and 6 females on study days 84 and 233, respectively. Reticulocyte levels were increased in group 3 females on study day 24.

Lymphocyte levels were decreased in groups 3, 5, and 6 males on study day 108. Lymphocyte levels were decreased in group 3 males on study days 126 and 192. Lymphocyte levels were decreased in group 6 males on study days 147 and 233. Lymphocyte levels were decreased in group 4 males on study day 233.

WBC levels were decreased in group 3 males on study days 84, 108, and 126. WBC levels were decreased in group 6 males on study day 147.

In males, segmented neutrophils levels were increased in groups 4 (study days 129, 171, 213, and 233), 5 (study days 42, 45, 66, 87, 129, 171, 192, 213, 233, and 247) and 6 (study days 42, 129, 171, and 213). In males, segmented neutrophil levels were decreased in groups 2 (study day 84), 3 (study days 63, 84, 126, 168, and 189), 4 (study days 63, 84, 126, and 168), 5 (study days 63, 84, and 126) and 6 (study days 63, 84, 126, 168, 189, and 231). In females, segmented neutrophil levels were decreased in groups 2 (study days 21, 24, 63,



189, and 247), 3 (study day 21), 4 (study days 21, 189, and 210), 5 (study day 21), and 6 (study day 189). In females, segmented neutrophil levels were increased in groups 4 (study day 233), 5 (study days 45, 66, 87, 108, 150, 171, and 233), and 6 (study days 42, 66, 87, and 233).

In males, sharp decrease in basophil levels were reported in groups 2 (starting at study day 147), 3 (starting at study day 84), 4 (starting at study day 150), and 6 (starting at study day 84). In males, increases in basophil levels in group 5 at study day 168 were reported. In females, sharp decrease in basophil levels were reported in groups 2 (starting at study day 3), 3 (starting at study day 3), 4 (starting at study day 3), 5 (starting at study day 63), and 6 (starting at study day 45).

In females, monocyte levels were decreased in groups 2 (study day 129), 3 (study day 105), and 6 (study days 105 and 108). In females, monocyte levels were increased in groups 4 (study days 129 and 150), 5 (study day 150), and 6 (study days 129 and 150).

In males, platelet count levels were decreased in groups 4 (study day 233), 5 (study days 66, 87, 108, and 233), and 6 (study days 66, 87 and 233).

In males, sharp increase in fibrinogen levels were reported in groups 2 (starting at study day 87), 3 (starting at study day 45), 4 (starting at study day 3), 5 (starting at study day 3), and 6 (starting at study day 3). These increases were continued up to study day 233. In females, sharp increase in fibrinogen levels were reported in groups 2 (starting at study day 66), 4 (starting at study day 3), 5 (starting at study day 3), and 6 (starting at study day 3). These increases were continued up to study day 233. Significant decreases in prothrombin time were reported in groups 4, 5, and 6 (frequently in groups 2 and 3) at the 2-day-postinjection intervals. These decreases were paralleled the elevation in fibrinogen.

**Systemic toxicity:**

There were no test article-related effects on clinical observations, body weight, ophthalmic examinations, urinalysis, and macroscopic findings were reported.

In group 5 males, occasional significant differences in body weight were reported.

**Organ Weight: (TERMINAL PHASE/RECOVERY PHASE)**

GROUPS	MALES 1 (CONTROL)	MALES 2	MALES 3	MALES 4	MALES 5	MALES 6
NUMBER OF ANIMALS	3/3	3/3	3/3	3/3	6/6	3/3
BODY WEIGHT (terminal)	3315/3395	3671/3277	3529/3174	3368/3331	3241/3207	3295/3202
BRAIN	12.4/9.3	10.0/9.8	10.0/9.5	9.6/9.6	9.2/9.3	10.0/9.9
ADRENALS	0.44/0.48	0.41/0.52	0.47/0.49	0.54/0.49	0.61/0.60	0.56/0.53
HEART	7.4/7.7	7.9/7.5	7.6/7.5	8.6/7.8	6.6/7.0	6.8/7.7
KIDNEYS	16.1/17.9	17.3/17.3	17.9/17.3	17.5/18.1	16.9/16.4	15.8/17.0
LIVER/gall bladder	72/65	75/58	80/66	75/72	63/55	70/61
SPLEEN	1.1/1.1	1.6/0.9	1.1/1.0	1.2/1.1	1.1/1.3	1.7/1.0
TESTES/EPIDID	9.1/9.6	9.8/9.3	8.6/8.7	9.3/8.6	8.0/8.7	8.7/9.5
THYROID and PARATHYROID	ND	ND	ND	ND	ND	ND
THYMUS	3.06/3.82	3.78/4.58	4.25/3.29	4.41/5.14	4.61/4.38	4.00/3.22
OVARIES						
UTERUS						

Absolute weights are expressed as mean (grams). ND = Not determined.

Table 50: Males' organ weight (study # 5).

**Organ Weight: (TERMINAL PHASE/RECOVERY PHASE)**

GROUPS	FEMALES 1 (CONTROL)	FEMALES 2	FEMALES 3	FEMALES 4	FEMALES 5	FEMALES 6
NUMBER OF ANIMALS	3/3	3/3	3/3	3/3	6/6	3/3
BODY WEIGHT (terminal)	3610/3603	3527/3571	3595/3390	3710/3513	3285/3372	3564/3606
BRAIN	9.1/9.5	9.5/9.3	9.2/9.4	10.0/9.9	9.6/9.7	10.0/10.0
ADRENALS	0.39/0.37	0.35/0.40	0.34/0.58	0.37/0.39	0.51/0.42	0.48/0.52
HEART	8.1/7.2	8.7/7.4	7.7/8.6	8.0/7.4	8.1/6.9	7.5/7.5
KIDNEYS	15.0/15.2	18.1/16.2	13.4/17.0	17.7/15.6	14.5/14.2	16.4/16.2
LIVER/gall bladder	59/64	72/59	65/67	68/61	54/57	69/65
SPLEEN	1.6/1.2	1.3/1.5	1.1/1.3	2.1/1.8	2.4/1.3	2.5/1.5
TESTES/EPIDID						
THYROID and PARATHYROID	ND	ND	ND	ND	ND	ND
THYMUS	4.30/5.75	3.65/4.45	4.97/3.66	5.46/3.60	2.57/3.43	3.51/4.60
OVARIES	0.36/0.47	0.48/0.36	0.34/0.54	0.39/0.72	0.59/0.33	0.43/0.41
UTERUS	ND	ND	ND	ND	ND	ND

Absolute weights are expressed as mean (grams). ND = Not determined.

Table 51: Females' organ weight (study # 5).

At terminal sacrifice, brain weight was reduced 19%, 19%, 23%, 26%, and 19% in groups 2, 3, 4, 5, and 6 males, respectively. At terminal sacrifice, adrenal weight was increased 23%, 39%, and 27% in groups 4, 5, and 6 males, respectively. At recovery sacrifice, adrenal weight was increased 25% and 10% in groups 5 and 6 males, respectively. At terminal sacrifice, heart weight was increased 16% in group 4 males. At terminal sacrifice, spleen weight was increased 45% and 55% in groups 5 and 6 males, respectively. At recovery

sacrifice, spleen weight was increased 18% in group 5 males. At terminal sacrifice, thymus weight was increased 24%, 39%, 44%, 51%, and 31% in groups 2, 3, 4, 5, and 6 males, respectively. At recovery sacrifice, thymus weight was increased 20%, 35%, and 15% in groups 2, 4, and 5 males, respectively. At recovery sacrifice, thymus weight was decreased 14% and 16% in groups 3 and 6 males, respectively.

At terminal sacrifice, adrenal weight was increased 31% and 23% in groups 5 and 6 females, respectively. At recovery sacrifice, adrenal weight was increased 57%, 14, and 41% in groups 3, 5, and 6 females, respectively. At recovery sacrifice, heart weight was increased 19% in group 3 females. At terminal sacrifice, kidney weight was increased 21% and 18% in group 2 and 4 females, respectively. At recovery sacrifice, kidney weight was increased 12% in group 3 females. At terminal sacrifice, liver weight was increased 22%, 10%, 15%, and 17% in group 2, 3, 4, and 6 females, respectively. At terminal sacrifice, spleen weight was decreased 19% and 31% in groups 2 and 3 females, respectively. At terminal sacrifice, spleen weight was increased 31%, 50%, and 56% in groups 4, 5, and 6 females, respectively. At recovery sacrifice, spleen weight was increased 25%, 50%, and 25% in groups 2, 4, and 6 females, respectively. At terminal sacrifice, thymus weight was decreased 15%, 40%, and 18% in groups 2, 5, and 6 females, respectively. At terminal sacrifice, thymus weight was increased 16% and 27% in groups 3 and 4 females, respectively.

#### Gross Pathology:

Group	Findings (Terminal phase/Recovery phase)
1M	NF// NF
2M	NF// NF
3M	NF// NF
4M	NF// NF
5M	NF// enlarged cortex in adrenal (1/3)
6M	Dark area in pancreas (1/3)// NF

M = Males. NF = No findings.

Group	Findings (Terminal phase/Recovery phase)
1F	NF// NF
2F	Dark area in ovary (1/3)// NF
3F	NF// dark area in ovary (1/3)
4F	Dark area in ovary (1/3); dark area in injection site (2/3)// enlarged ovary (1/3); dark area in pancreas (1/3)
5F	Dark area in ovary (2/3); dark area in injection site (2/3); dark area in pancreas (2/3)// NF
6F	Dark area in ovary (1/3); dark area in injection site (2/3)// NF

F = Females. NF = No findings

Table 52: Macroscopic findings in males and females (study # 5).

Enlarged cortex in adrenal glands was reported in one group 5 males at recovery sacrifice. Dark area in pancreas was reported in one group 6 males at terminal sacrifice.

Dark area in ovary was reported in one groups 2, 4, 5, and 6 females at terminal sacrifice. Dark area in ovary was reported in one group 3 females at recovery sacrifice. Dark area in injection site was reported in two groups 4, 5, and 6 females at terminal sacrifice. Dark area in pancreas was reported in two group 5 females at terminal sacrifice. Enlarged ovary (1/3) and dark area in pancreas (1/3) was reported in group 4 females at recovery sacrifice.

Microscopic finding in males (terminal sacrifice) are listed below:

Groups	Findings
1M	Hemorrhage in adrenal (2/3); focal mineralization in lung (1/3); periportal chronic inflammation in liver (1/3); renal tubule mineralization in kidney (1/3); calculus in urinary bladder (1/3)  Injection site: Muscle focal chronic inflammation (1/3); subcutaneous foreign body [hair] (1/3)
2M	Periportal chronic inflammation in liver (1/3); renal tubule mineralization in kidney (1/3); degeneration (1/3) and acute inflammation (1/3) in skeletal muscle  Injection site: Muscle necrosis (1/3); muscle acute/subacute inflammation (1/3); subcutaneous acute/subacute inflammation (2/3)
3M	Hemorrhage in adrenal (1/3); germinal center activity in spleen (1/3); periportal chronic inflammation in liver (3/3); medulla microcalculi in kidney (1/3)  Injection site: Subcutaneous chronic inflammation (1/3)
4M	Cystic follicle in thyroid (1/3); germinal center activity in spleen (1/3); periportal chronic inflammation in liver (3/3)  Injection site: Subcutaneous hemorrhage (1/3); muscle acute/subacute inflammation (1/3); subcutaneous acute/subacute inflammation (2/3)
5M	Hemorrhage in adrenal (1/3); chronic inflammation in lung (1/3); germinal center activity in spleen (2/3); periportal chronic inflammation in liver (2/3); medulla microcalculi in kidney (2/3); ulcer in rectum (1/3);

Groups	Findings
	sperm granuloma in epididymis (1/3)  Injection site: Muscle acute/subacute inflammation (1/3); subcutaneous acute/subacute inflammation (3/3); dermis acute/subacute inflammation (1/3); muscle focal chronic inflammation (1/3)
6M	Hemorrhage in adrenal (1/3); germinal center activity in spleen (2/3); periportal chronic inflammation in liver (2/3); medulla microcalculi in kidney (1/3)  Injection site: Subcutaneous hemorrhage (1/3); muscle acute/subacute inflammation (1/3); subcutaneous acute/subacute inflammation (1/3); subcutaneous edema (1/3)

Microscopic finding in females (terminal sacrifice) are listed below:

Groups	Findings
1F	Focal chronic inflammation in lung (1/3); periportal chronic inflammation in liver (2/3); medulla microcalculi in kidneys (1/3); focal mineralization in ovary (1/3); hemorrhage in thymus (2/3)  Injection site: NF
2F	Periportal chronic inflammation in liver (3/3); renal tubule mineralization in kidneys (1/3); hemorrhage in thymus (1/3);  Injection site: Subcutaneous hemorrhage (1/3); muscle hemorrhage (1/3); subcutaneous acute/subacute inflammation (2/3)
3F	Cystic follicle in thyroid (2/3); periportal chronic inflammation in liver (3/3); medulla microcalculi in kidneys (3/3)  Injection site: Subcutaneous edema (1/3); subcutaneous acute/subacute inflammation (1/3)
4F	Heterophilic perivascular infiltrate in lung (1/3); periportal chronic inflammation in liver (2/3); medulla microcalculi in kidneys (1/3)  Injection site: Muscle acute/subacute inflammation (1/3); subcutaneous acute/subacute inflammation (1/3); dermis acute/subacute inflammation (1/3)
5F	Germinal center activity in spleen (3/3); periportal chronic inflammation in liver (2/3); renal tubule mineralization in kidneys (1/3); medulla

Groups	Findings
	microcalculi in kidneys (1/3)  Injection site: Muscle necrosis (3/3); subcutaneous edema (1/3); subcutaneous hemorrhage (2/3); muscle acute/subacute inflammation (2/3); subcutaneous acute/subacute inflammation (2/3); dermis acute/subacute inflammation (1/3)
6F	Hemorrhage in adrenal (1/3); focal chronic inflammation in lung (1/3); focal mineralization in lung (1/3); germinal center activity in spleen (3/3); periportal chronic inflammation in liver (2/3); hemorrhage in thymus (1/3)  Injection site: Muscle necrosis (3/3); subcutaneous hemorrhage (2/3); subcutaneous edema (2/3); muscle acute/subacute inflammation (3/3); subcutaneous acute/subacute inflammation (3/3); dermis acute/subacute inflammation (1/3); muscle focal chronic inflammation (1/3)

NF = No findings.

Table 53: Microscopic findings in males and females (study # 5).

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls. However, injection site findings in groups 4, 5, and 6 were higher than control groups (1, 2, and 3).

### Body temperature

Males Groups	Days 1-45	Days 50-99	Days 106-150	Days 155-204	Days 211-246
1Control	2	5	7	3	4
2	5	8	9	3	7
3	3	3	5	3	6
4	3	5	8	2	7
5	3	7	5	2	6
6	2	4	8	2	7

Table of occurrences for body temperature  $\geq 40^{\circ}$  C.

Females Groups	Days 1-45	Days 50-99	Days 106-150	Days 155-204	Days 211-246
1Control	3	7	6	4	7
2	3	8	8	7	6
3	5	6	8	8	8
4	6	8	8	8	7

Females Groups	Days 1-45	Days 50-99	Days 106-150	Days 155-204	Days 211-246
5	3	8	8	7	7
6	4	8	8	9	7

Table of occurrences for body temperature  $\geq 40^{\circ}\text{C}$ .

Table 54: Body temperature results in males and females (study # 5).

Temperature equal to or above  $40^{\circ}\text{C}$  was reported in all groups including the control group. However, the incidence number in the control group was lower than the treated groups. No differences in the number of incidences between animals treated with MF59 only (groups 4) and animals treated with HIV gp120 or HIV Env2-3 with or without MF59 (groups 5 and 6) were reported.

### Local toxicity:

Draize scoring of the injection site revealed the following as presented in the table below<sup>20</sup>.

#### Erythema/Edema

Treatment group	Frequency of score to +3 days post-injection (day of injection is day 0). Erythema/Edema.									
	Males (n=6)					Females (n=6)				
	0	1	2	3	4	0	1	2	3	4
1	0/0	0/0	0/0	0/0	0/0	0/0	2/0	0/1	0/0	0/0
2	0/0	0/0	0/0	0/0	0/0	0/0	3/2	1/0	0/0	0/0
3	0/0	0/0	0/0	0/0	0/0	0/0	3/1	0/0	0/0	0/0
4	0/0	0/1	0/0	0/0	0/0	0/0	4/3	0/0	0/0	0/0
5	0/0	2/2	0/0	0/0	0/0	0/0	9/1	2/3	0/0	0/0
6	0/0	0/1	0/0	0/0	0/0	0/0	9/1	0/0	0/0	0/0

Table 55: Draize scoring at the injection site (study # 5).

In males, minor dermal irritation, consisting of very slight erythema or edema, was reported in groups 4, 5, and 6. In females, dermal irritation, consisting of very slight or slight erythema or edema, was reported in all groups including the control group. However, the number of these incidences was higher in groups 4, 5, and 6.

### Serology:

Not determined.

<sup>20</sup> Draize, Dermal Toxicity, In: Association of Food and Drug Officials US Appraisal of the Safety of Chemicals and Food, Drugs and Cosmetics, pp 46-59, Texas State Dept of Health, Austin, 1959.

Adjuvant MF59 related effects are listed in the table below:

Test article related effects	Effects considered incidental
Injection sites findings ↑ LDH in M&F ↑ AST in M&F ↑ ALT in M ↑ Triglycerides in M&F ↓ Basophils M&F ↑ Fibrinogen in M&F ↑ Adrenal weight in M&F ↑ Thymus weight in M	↓ Neutrophils M&F ↑ Creatine Kinase in M&F ↑ Body temperature

#### Assessment:

This study was not requested by the toxicology team to support this BLA. The test article used in this study is not related to the product that the sponsor is seeking licensure in this BLA. Group 4, treated with the adjuvant MF59 only, is the only group which might be related to this BLA. Findings related to groups 5 and 6 will be ignored in this assessment as it is related to a different test article.

There were no test article-related effects on clinical observations, body weight, ophthalmic examinations, urinalysis, and macroscopic findings were reported.

Dermal irritation was higher in females than males. The increase in dermal irritation in females was higher in group 4 and might be related to test article treatment.

Lactate dehydrogenase (LDH) elevation might indicate cell damage by increased membrane permeability to frank cell lysis. The inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD<sup>+</sup> is catalyzed by LDH. Pyruvate is the final product of glycolysis and it is converted to lactate, when oxygen is absent or in short supply, by LDH. LDH performs the reverse reaction during the Cori cycle in the liver. LDH may be used as a tumor marker because many cancers can raise LDH levels. Also, measuring LDH levels can be helpful in monitoring treatment for cancer. LDH levels could be raised by heart failure, hypothyroidism, anemia, and lung or liver disease. <sup>[1]</sup> LDH can be measured as a surrogate for tissue breakdown, e.g. hemolysis, because tissue breakdown releases LDH. Elevated LDH could be indication of other disorders like meningitis, encephalitis, acute pancreatitis, and HIV. It can also be used as a marker of myocardial infarction. Levels of LDH peak at 3-4 days and remain elevated for up to 10 days following a myocardial infarction.

The hepatocellular leakage enzymes (AST and ALT) are useful in detecting injury to liver parenchymal cells. Generally, increased serum activity represents



enzyme leakage from cells through damaged cell membranes. AST is useful as an indicator of liver and/or muscle injury in large and small animals.

A triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived from glycerol and three fatty acids.<sup>[2]</sup> As a blood lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so. High levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of stroke<sup>[3]</sup> and heart disease.<sup>[4]</sup> However, the relative negative impact of raised levels of triglycerides compared to that of LDL:HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level.

Basophils play a role in both parasitic infections and allergies. Basopenia has been reported in association with autoimmune urticaria.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Adrenal glands are responsible for releasing hormones in response to stress through the synthesis of corticosteroids such as cortisol and catecholamines such as adrenaline (epinephrine) and noradrenaline. They also produce androgens in their innermost cortical layer. The adrenal glands affect kidney function through the secretion of aldosterone.

The increase in thymus weight might be related to the immune responses due to test article-treatment.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. The decrease of neutrophil levels was not consistent throughout the study, thus, considered incidental.

The increase in creatine kinase activity values for the test article-treated groups might be a reflection of minimal muscle degeneration subsequent to the inflammatory response to intramuscular injection of the vaccine.

Since the increase in body temperature was also reported in the control group, it is considered incidental.

**Conclusions:** Based on nonclinical toxicity assessments of this study there are no significant safety issues, related to the adjuvant MF59, to report.

## Genotoxicology studies:

**Study # 6:** Title and study number: Micronucleus Cytogenetic Assay in Mice.  
Study number G96AQ61.122.

### Introduction

The *in vitro* micronucleus assay is a mutagenic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments i.e. micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes which are unable to migrate with the rest of the chromosomes during the anaphase of cell division. The purpose of the micronucleus assay is to detect those agents which modify chromosome structure and segregation in such a way as to lead to induction of micronuclei in interphase cells.

**Performing laboratory:** (b) (4)

**Study initiation date:** May 2, 1996

**Final Report date:** September 9, 1996

**Test article, batch/lot:** MF59C.1, MECPK002

**Animal species and strain:** (b) (4) mice

**Breeder/supplier:** (b) (4)

**Number of animal per group and sex:** Micronucleus assay: 6 groups; 5 males/5 females per group; positive control group has 5 males/5 females; total animals = 130

**Age:** 6 – 8 weeks

**Body weight range:** Pilot: (males) 29.0 – 33.9 g (females) 26.5 – 29.0 g;  
micronucleus assay: (males) 28.6 – 36.6 g (females) 22.8 – 29.9 g

**Route and site of administration:** IP (intraperitoneal); abdomen

**Volume of injection:** 20 mL/kg

**Frequency of administration and study duration:** Single dose; bone marrow cells read at 24, 48, and 72 hours post dose

**Dose:** Pilot assay: 1, 10, 100, 1000 mg/kg (males); males and females 5000 mg/kg; micronucleus assay: males and females 1250, 2500, or 5000 mg/kg of body weight

**Means of administration:** Needle/syringe

**Report status:** Final

### Experimental design

In a pilot assay, male mice were dosed with 1, 10, 100, or 1000 mg test article/kg body weight and male and female mice were dosed with 5000 mg/kg. In the absence of mortality in the pilot assay and in consultation with the sponsor, the high dose for the micronucleus test was set at 5000 mg/kg.

Treatment	Number of mice/sex/group	Number of mice/sex dosed for bone marrow collection		
		24 hrs	48 hrs	72 hrs
Vehicle control (0.9% saline)	15/15	5/5	5/5	5/5
Low dose (1250 mg/kg)	15/15	5/5	5/5	5/5
Mid dose (2500 mg/kg)	15/15	5/5	5/5	5/5
High dose (5000 mg/kg)	15/15	5/5	5/5	5/5
Positive control (CP 60 mg/kg)*	5/5	5/5		

\*CP = cyclophosphamide

Table 56: Micronucleus assay experimental design (study # 6).

### Sample preparation

Immediately following sacrifice, the femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing fetal bovine serum. The bone marrow cells were transferred to a capped centrifuge tube containing approximately 1 ml fetal bovine serum. The bone marrow cells were pelleted by centrifugation at approximately 100 x g for five minutes and the supernatant was drawn off, leaving a small amount of serum with the remaining cell pellet. The cells were resuspended by aspiration with a capillary pipet and a small drop of bone marrow suspension was spread onto a clean glass slide. Two to four slides were prepared from each mouse. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted.

**Validity of assay:** The mean incidence of micro-nucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control. The incidence of micro-nucleated polychromatic erythrocytes in the positive control group must be significantly increased relative to the vehicle control group ( $p \leq 0.05$ )

### Methods:

Parameters	Frequency of Testing
Cageside observation	NC
Clinical observations	Recorded day of dosing (24, 48, and 72 hrs)
Body weight	Prior to dosing; SD 1 and SD 3 post-dose
Food consumption	NC
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry	NC
Hematology	NC
Bone marrow	SD 1, 2, and 3 (24, 48, and 72 hrs post dose)
Immunological response	NC

Parameters	Frequency of Testing
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	NC
Necropsy	NC
Histopathology	NC

NC = Not collected

Table 57: Tissues collected (study # 6).

#### Results:

Morbidity and mortality: All animals survived to their scheduled termination.

#### Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in body weight (gain).

#### Assessment:

Neither test article related findings with regard to clinical observations nor any changes in body weights were reported. The following parameters were not collected: clinical chemistry, hematology, serology, body temperatures, coagulation, gross pathology, or histopathology. The micronucleus assay was a valid assay based on the criteria set forth in the protocol. The assay used the appropriate positive and negative controls. There was no genotoxicity found in this assay.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity or interpretation of the results.

**Study #7:** Title and study number: AMES assay. Study number G96AQ61.502.

**Performing laboratory:** (b) (4)

**Study initiation date:** May 5, 1996

**Final Report date:** June 4, 1996

**Test article batch/lot:** MECPK002

**Strain/species/cell line:** (b) (4)

**Dose:** Preliminary test: 0.067, 0.1, 0.33, 0.67, 1.0, 3.3, 6.7, 10.0, 33.0, and 50.0 mg/plate; definitive test: 100, 333, 1000, 3333, and 5000 µg/plate

**Report status:** Final

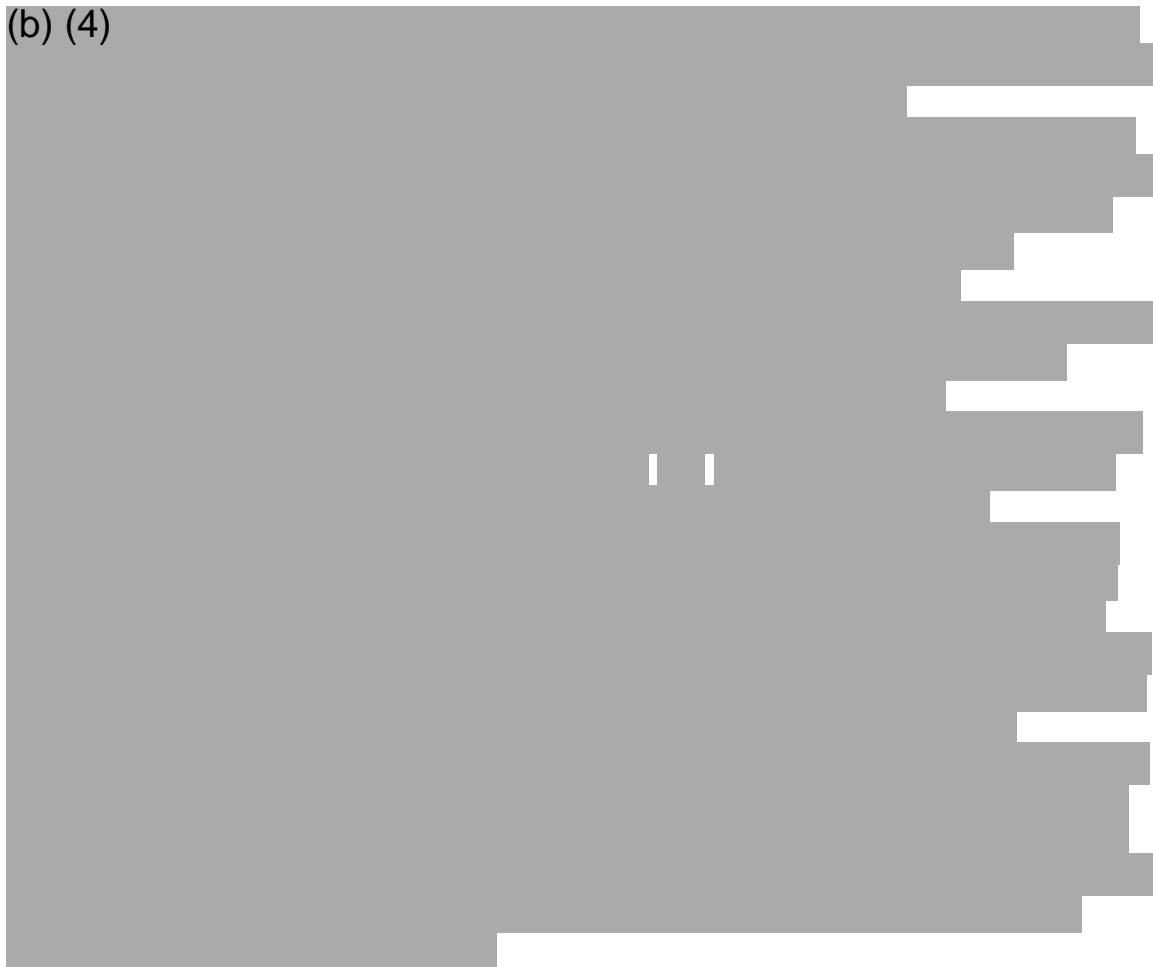
#### Methods:

Doses used in definitive study: 100, 333, 1000, 3333, and 5000 µg/plate

Basis of dose selection: In the preliminary toxicity assay, the test article was inadvertently measured by volume rather than by weight. As a result, the maximum dose tested was 50000 µg per plate assuming a test article density of 1 gm/mL; this dose was achieved using a 50 µL plating aliquot of the neat test

article. Neither precipitate nor appreciable toxicity was observed at this dose. Based on the findings of the preliminary toxicity assay, the maximum dose in the definitive mutagenicity assay was 5000 µg per plate.

(b) (4)



**Evaluation criteria for study validity:**

The following criteria must be met for the mutagenicity assay to be considered valid. All (b) (4) tester strain cultures must demonstrate the presence of the (b) (4) gene. Cultures of tester strains (b) (4) must demonstrate the presence of the (b) (4). All (b) (4) cultures must demonstrate the deletion in the (b) (4) gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): (b) (4)

To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to (b) (4). The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic

dose levels up to 5000 ug/plate are required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) A reduction in the background lawn. For a result to be positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester starting with a minimum of two increasing concentrations of test article.

**Results:**

Study validity: All criteria for a valid study were met as described in the protocol. No significant increase in revertant colony numbers was observed at the concentrations up to 5000 ug/plate. DMF 138584

Conclusions: The results indicate that MF59C.1 did not cause a positive response with any of the tester strains in the presence and absence of Aroclor-induced rat liver S9 in the bacterial reverse mutation assay.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity or interpretation of the results.

**Reproduction toxicity studies:**

**Study # 8:** Title and study number: A reproductive and developmental toxicity study with four intramuscular injections in (b) (4) rabbits. Study number: AB09779.

**Key study findings:** No significant findings were reported.

**Study no.:** AB09779

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 04/11/2013

**Date of study completion:** 03/03/2014

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, and % purity:**

<u>Test article</u>	<u>Batch/Lot #'s</u>	<u>Purity %</u>	<u>Expiration Date</u>
Fluad®	128802	100	July 2013
0.9% NaCl	NR*	NR	NR

\*NR = Not reported.

**Animal species and strain:** (b) (4) rabbit, (b) (4)

**Breeder/supplier: (b) (4)**

**Number of animal per group and sex:** 110 virgin females (25 Cesarean and 30 littering females per group). 20 males used for mating only (not allocated to the study).

**Age:** Females: 19 to 21 weeks old (i.e. 16 to 18 weeks old at first vaccination).

Males used for littering sub-group females: 20 to 22 weeks old.

Males used for Cesarean sub-group females: 22 to 24 weeks old.

**Body weight range:** Females, at mating:

- Gestation sub-groups: 3.2 to 4.6 kg
- Lactation sub-groups: 3.0 to 4.5 kg.

Males used for littering sub-group females, 4 days before mating: 2.8 to 3.9 kg.

Males used for Cesarean sub-group females at mating: 3.0 to 4.0 kg.

**Route and site of administration:** Intramuscular injections into the dorsal lumbar muscle.

**Volume of injection:** 0.5 mL per animal per dose.

**Frequency of administration and study duration:** 21 days (M-21) and 7 days (M-7) before mating, then on days 7 and 20 of gestation. Study duration was 83 days.

**Dose:** A 0.5 mL dose of Fluad<sup>®</sup> contained nominally 15 µg of hemagglutinin (HA) from each of the 3 influenza strains (listed below) as recommended by the WHO for the formulation of the 2012/2013 influenza virus vaccine (northern hemisphere), for a total of 45 µg of HA antigen:

- A/California/7/2009, NYMC X-181 (H1N1)
- A/Victoria/361/2011, IVR-165 (H3N2)
- B/Hube-Wujiagang/158/2009, BX-39 (a B/Wisconsin/1/2010-like-virus)

**Stability**

Stability summary for Fluad<sup>®</sup> was provided on page 36 and page 50.

**Methods:****Study design:**

Rabbits were treated at 21 days (M-21) and 7 days (M-7) before mating, then on days 7 and 20 of gestation. Animals were assigned to 2 different sub-groups (30 for littering and 25 for caesarean) and were dosed with 0.5 mL per animal per dose. The details of the study design are listed in the following table:

Group	Treatment	Dosage Volume (mL/rabbit)	Antigen Content <sup>1</sup> (µg)	Adjuvant Content (mL)	Number of Animals	
					Littering	Cesarean
1	Control	0.5	0	0	30	25
2	Fluad <sup>®</sup> Vaccine	0.5	45	0.25	30	25

<sup>1</sup> Each 0.5 mL dose of vaccine presented in a pre-filled syringe contained 15 µg of hemagglutinin (HA) antigen from each of three influenza strains for a total of 45 µg of antigen.

The vaccine was adjuvanted with MF59 (0.25 mL per 0.5 mL dose).

Table 58: Study design (study # 8).

#### Parameters and endpoints evaluated:

The following parameters were evaluated: Clinical signs (daily), mortality (twice daily), injection site observations (before each treatment, 24, and 48 hours post-dose), body weight for littering sub-group (on day 0 [mating day -21], on day 14 [mating day -7], on days 0, 6, 9, 13, 16, 20, 24, 27 and 29 of gestation and when necessary on day 34 of presumed gestation, and on days 4, 7, 11, 14, 17, 21 and 28 of lactation), body weight for Cesarean sub-group (on day 0 [mating day -21], on day 14 [mating day -7], and on days 0, 6, 9, 13, 16, 20, 24, 27 and 29 of gestation), food consumption (daily from the day of arrival to day 29 of gestation for the Cesarean sub-groups and to day 29 of lactation for the littering sub-groups).

#### Pregnancy and parturition (littering sub-group)

From day 30 *post-coitum*, each female was observed 4 times a day for the onset and duration of parturition.

#### Blood collection for immunogenicity assays (for does)

Littering sub-group:

- on day 0 (mating day -21) and on day 14 (mating day -7) of the study
- on days 7 and 20 of gestation
- on day 29 of lactation.

Cesarean sub-group:

- on day 0 (mating day -21) and on day 14 (mating day -7) of the study
- on days 7, 20 and 29 of gestation.

#### Blood sampling of the fetuses and kits

- Kits from the littering sub-group: on PND 29.
- Fetuses from the Cesarean sub-group: on day 29 of gestation.

#### Litter data – littering sub-group only

For each litter, the following was recorded:

- number of kits born (live and dead)
- external abnormalities of the kits
- number of kits alive on PND 4, 7, 11, 14, 21 and 28
- weight of kits alive on PND 4, 7, 11, 14, 21 and 28
- physical development of the offspring, as assessed by the intra-litter onset and duration of incisor eruption, fur growth and eye opening on PND 4 then from PND 7
- behavioral and functional tests in all kits as follows:
  - surface righting reflex on PND 11
  - auditory reflex from PND 14
  - pupil reflex on PND 22.

#### Necropsy schedule



- Cesarean sub-groups: on day 29 *post-coitum*.
- Littering sub-groups: on day 29 of lactation (with litter).

#### Tissue retention

The vagina, uterus and ovaries of all females were fixed in 10 % formalin. No histopathological examination was performed.

#### Organ weights

The ovaries from all surviving females (excluding females not pregnant, with total litter death, died delivering and with no defined day of mating after 10 days of copulation (failed to mate)) from both groups were weighed paired.

#### Cesarean examinations – Cesarean sub-group only

For each female, the ovaries and uterus were removed and examined. The placentae were also examined. The following data were recorded on day 29 *post-coitum*:

- Pregnancy status
- Number of corpora lutea
- Gravid uterus weight
- Number and distribution of intrauterine implantations classified as:
  - live fetuses
  - dead fetuses
  - early resorptions
  - late resorption
- Individual fetal weights
- Fetal sex.

#### Fetal examinations - Cesarean sub-group only

Each fetus was examined for external defects and euthanized by oral intubation of sodium pentobarbitone (b) (4) Late resorptions were examined externally (where possible), preserved in Harrison's fixative but not examined further. All fetuses were examined visceraally including the heart and great blood vessels, using a binocular microscope and sexed at the time of Cesarean section. The head of approximately half of the fetuses in each litter was removed and placed in Harrison's fluid for subsequent examination by serial sectioning. The eviscerated fetal carcasses were fixed and processed for skeletal examination. The skeletal examination was performed following maceration of the soft tissues with aqueous potassium hydroxide, staining of the skeleton with Alizarin red then passage into glycerol.

#### Necropsy of kits-littering sub-group

All kits were given a macroscopic examination (including thoracic, abdominal and pelvic viscera) for structural or pathological changes following euthanasia in association with terminal blood sampling.

Abnormal organs or tissues were sampled and preserved in 10 % formalin or other appropriate fixative but were not examined further. The kits (including decedents) were sexed by internal inspection.

Randomization: Yes

Statistical methods: Yes

Results:

Mortality/clinical signs:

No test article-related effects on mortality or clinical observations were reported.

Injection site observations

No test article-related observations at the injection sites were reported. Some local reactions, including hematoma (grade 1 or 2), very slight to slight edema, were reported after some injections in both groups. Neither the incidence nor the duration of these reactions in the treated group was remarkably different from those in the control group.

Food consumption and body weight:

No test article-related effects on body weight or food consumption were reported. A slightly but statistically significantly lower mean body weight gain in the treated group compared with the control between days 13 and 16 of gestation in the lactation treated sub-group was considered incidental since there was no similar finding in the Cesarean sub-group and the mean value remained within the historical control range.

Slight but statistically significant lower mean food consumption during days 4 and 11 of lactation in comparison with the concurrent control group was due to above average consumption in the control compared with the historical control data.

Mating performance and fertility:

No test article-related effects on mating performance and fertility parameters were reported.

GROUP TREATMENT	1 0 mcg	2 45 mcg
NUMBER OF FEMALES:		
Paired	55	55
Failed to mate	3	0
Inseminated	52	55
Pregnant	48	55
Not pregnant	4	0
With viable fetuses at Cesarean section	21	25
Pregnant females allowed to litter	27	30

GROUP TREATMENT	1 0 mcg	2 45 mcg
Died delivering	1	0
Total litter death <i>post-partum</i>	1	0
Reared pups to termination	25	30
Pre-coital interval-days		
MEAN	1.50	1.13
S.D.	1.08	0.39
N	52	55
Copulation index (%)	95	100
Fertility index (%)	92	100

Table 59: Summary of cohabitation data and maternal performance [littering and Cesarean sub-groups] (study # 8).

#### Gravid uterus weight – Cesarean sub-group

There was no treatment-related effect on gravid uterus weight in comparison with the control group.

Group		1	2
NET BODY WT. CHANGE	MEAN S.D. N	477 d 294 21	446 188 25
GRAVID UTERINE WT.	MEAN S.D. N	538 d 101 21	544 139 25
NET WEIGHT CHANGE MINUS UTERINE WT.	MEAN S.D. N	-61 d 261 21	-98 194 25

Statistical key: d=Anova/Dunnetttest

Net body wt. Change = terminal body wt. Minus day 0 body weight

Net weight change = net body wt. Change minus uterine weight

Table 60: Summary of gravid uterus weight and net body weight change (study # 8).

#### Cesarean data-Pregnancy incidence

At the terminal Cesarean examinations, there were 21 and 25 pregnant females in the control and the treated groups, respectively. All pregnant females had viable fetuses.

#### Pre-implantation data

No treatment-related effects on the pre-implantation data (mean numbers of corpora lutea and implantations and the corresponding percentage pre-implantation loss) were reported.

Group	N	Group 1 (Control 0µg)	Group 2 (45µg)
Pregnant	N	21	25
Dams with no viable fetuses	N	0	0
Dams with viable fetuses	N	21	25
Corpora Lutea	Total	214	273
No. per animal	Mean	10.2d	10.9
	S.D.	2.6	3.0
Implantation Sites	Total	187	229
No. per animal	Mean	8.9d	9.2
	S.D.	2.1	2.7
Preimplantation Loss	Total	27	44
No. per animal	Mean	1.3d	1.8
	S.D.	1.3	1.8
% per animal	Total	11.5k	16.1
	Mean	10.4	17.8
Live Fetuses	Total	179	212
No. per animal	Mean	8.5d	8.5
	S.D.	1.9	2.6
Males	Total	85	106
	Mean%	48.0k	51.5
	S.D.	23.5	18.8
Females	Total	94	106
	Mean%	52.0k	48.5
	S.D.	23.5	18.8

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test

Table 61: Summary of Cesarean section data (study # 8).

#### Post-implantation data

No treatment-related effects on the percentage embryo-fetal survival were reported. The mean live litter size was comparable in the treated and control groups. No dead fetuses were reported in control or test article-treated groups.

Group		Group 1 (Control 0µg)	Group 2 (45µg)
Postimplantation loss	Total	8	17
No. per animal	Mean	0.4d	0.7
	S.D.	0.6	0.9
%implants per animal	Mean%	4.0k	7.1
	S.D.	6.0	9.1
Dead fetuses	Total	0	0
No. per animal	Mean	0.0k	0.0
	S.D.	0.0	0.0

Group		Group 1 (Control 0µg)	Group 2 (45µg)
% of implants per animal	Mean% S.D.	0.0k 0.0	0.0 0.0
Early resorptions No. per animal	Total Mean S.D.	5 0.2k 0.4	8 0.3 0.6
% of implants per animal	Mean% S.D.	2.5k 4.7	3.5 6.9
Late resorptions No. per animal	Total Mean S.D.	3 0.1k 0.4	9 0.4 0.8
% of implants per animal	Mean% S.D.	1.5k 3.7	3.6 7.8
Fetal body weight (g)	Mean S.D. N	42.7d 4.9 21	43.2 5.4 25
Male fetuses	Mean S.D.	43.6d 5.5	43.4 5.8
Females fetuses	Mean S.D.	42.5d 5.1	42.3 4.4

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test  
Table 62: Summary of Cesarean section data (study # 8).

### Fetal data

In the treated and control groups, mean fetal weight and fetal sex ratio were comparable.

Group	Mean	Group 1 (Control 0µg)	Group 2 (45µg)
Fetal body weight (g)	Mean S.D. N	42.7d 4.9 21	43.2 5.4 25
Male fetuses	Mean S.D.	43.6d 5.5	43.4 5.8
Females fetuses	Mean S.D.	42.5d 5.1	42.3 4.4

Statistical key: d=Anova/Dunnett test.  
Table 63: Summary of fetal data (study # 8).

### External observations

No test article-related external malformations were reported.

Group Number	Female Fetus number number	Malformation(s) <sup>#</sup>
1	6836 10	Lumbar vertebrae: multiple abnormalities, scoliosis
	6839 4	Lumbar and thoracic vertebrae: multiple abnormalities, scoliosis
	6848 6	Sternebrae: fused 3 <sup>rd</sup> to 5 <sup>th</sup>
	6849 8	Testis: malpositioned, left cranially, adhesion with kidney
	6855 3	Testis and epididymis: absent, left
	6868 1	Heart and great vessels: multiple abnormalities
2		Palatine: split
	6874 3	Sternebrae: fused 1 <sup>st</sup> to 5 <sup>th</sup>
		Carotid: narrowed, left

<sup>#</sup>: including external, visceral and skeletal examinations.

Table 64: Summary of malformations-individual description (study # 8).

### Visceral observations

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Litters evaluated	N	21	25
Fetuses evaluated	N	179	212
Live	N	179	212
Dead	N	0	0
Heart			
Litter incidence	N	0	1
Fetal incidence	N	0	1
M Heart: Multiple abnormalities			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0	1
	%	0.0	4.0
Carotid artery			
Litter incidence	N	15	20
Fetal incidence	N	48	58
V common carotid trunk: Absent			
Fetal incidence	N	48	58
	%	26.8	27.4
Litter incidence	N	15f	20
	%	71.4	80.0
M carotid: Narrowed			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0f	1
	%	0.0	4.0

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Great vessels			
Litter incidence	N	0	1
Fetal incidence	N	0	1
M great vessels: Multiple abnormalities			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0	1
	%	0.0	4.0
Lung			
Litter incidence	N	1	3
Fetal incidence	N	1	3
V lung: Azygos lobe absent			
Fetal incidence	N	1	3
	%	0.6	1.4
Litter incidence	N	1f	3
	%	4.8	12.0
Liver			
Litter incidence	N	0	1
Fetal incidence	N	0	1
V liver: Cyst			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0	1
	%	0.0	4.0
Kidney			
Litter incidence	N	1	0
Fetal incidence	N	1	0
A dilated renal pelvis: Slight			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
Gonad			
Litter incidence	N	1	0
Fetal incidence	N	1	0
M epididymis: Absent			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Testis			
Litter incidence	N	2	0
Fetal incidence	N	2	0
M testis: Malpositioned			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
M testis: Absent			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
Ovary			
Litter incidence	N	1	3
Fetal incidence	N	1	5
A ovary: Cyst			
Fetal incidence	N	1	5
	%	0.6	2.4
Litter incidence	N	1f	3
	%	4.8	12.0
Litters evaluated	N	21	24
Fetuses evaluated	N	83	99
Live	N	83	99
Dead	N	0	0
Total fetal visceral observations	N	0	0

Statistical key: f=Chi2/Fisher Exact test

Observation code: M-Malformation, V-Variation, A-Anomaly

Table 65: Summary of fetal visceral observations (study # 8).

Two fetuses with visceral malformations in each of the control and treated groups were reported. Multiple changes associated with the heart and of the great vessels were reported in one group 2 fetus. This fetus also had cyst on the liver. A narrowed carotid artery was reported in the second malformed fetus in this group. These findings were considered incidental because they were sporadic.

In two group 1 fetuses, malpositioned testis (female no. 6849) and an absent testis and epididymis (female no. 6855) were reported.

In group 2, five fetuses had a single ovarian cyst (3 fetuses from female no. 6868 and one fetus in each of female nos. 6870 and 6883). In group 1, one



fetus only (female no. 6837) had a single ovarian cyst. Dilated renal pelvis was reported in one group 1 fetus (female no. 6848).

### Skeletal observations

Skeletal malformations were reported in three and one fetuses in groups 1 and 2, respectively.

Split palatine was reported in group 2. Because this finding was also reported in the historical control data, it was considered to be incidental. Fused sternbrae was also reported in this fetus together with one from the control group. The two other fetuses in the control group had multiple abnormalities in the lumbar and/or thoracic vertebrae associated with scoliosis (female nos. 6836 and 6839).

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Litters evaluated	N	21	25
Fetuses evaluated	N	179	212
Live	N	179	212
Dead	N	0	0
Pectorial girdle			
Litter incidence	N	0	1
Fetal incidence	N	0	1
A scapula: Misshapen			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0	1
	%	0.0	4.0
Paws			
Litter incidence	N	5	6
Fetal incidence	N	6	11
V phalanx: Unossified, middle			
Fetal incidence	N	3	10
	%	1.7	4.7
Litter incidence	N	3f	6
	%	14.3	24.0
A metacarpal: Unossified, 1 <sup>st</sup> digit			
Fetal incidence	N	4	2
	%	2.2	0.9
Litter incidence	N	4f	2
	%	19.0	8.0
A phalanx: Incomplete ossification, hindpaw			
Fetal incidence	N	0	1
	%	0.0	0.5

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Litter incidence	N	0	1
	%	0.0	4.0
A phalanx: Unossified, hindpaw			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0f	1
	%	0.0	4.0
A tarsal bone: Unossified			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
Sternebra			
Litter incidence	N	12	14
Fetal incidence	N	18	37
V Sternebra: 5 <sup>th</sup> unossified			
Fetal incidence	N	7	20
	%	3.9	9.4
Litter incidence	N	6f	10
	%	28.6	40.0
V Sternebra: 6 <sup>th</sup> unossified			
Fetal incidence	N	4	6
	%	2.2	2.8
Litter incidence	N	3f	5
	%	14.3	20.0
A Sternebra: Asymmetric			
Fetal incidence	N	2	1
	%	1.1	0.5
Litter incidence	N	2f	1
	%	9.5	4.0
A Sternebra: Minor fusion			
Fetal incidence	N	3	3
	%	1.7	1.4
Litter incidence	N	2f	2
	%	9.5	8.0
A Sternebra: Incomplete ossification of 6 <sup>th</sup>			
Fetal incidence	N	1	11
	%	0.6	5.2
Litter incidence	N	1f	5
	%	4.8	20.0
A Sternebra: Bipartite ossification			
Fetal incidence	N	0	1
	%	0.0	0.5
Litter incidence	N	0f	1

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
A Sternebra: Fused	%	0.0	4.0
Fetal incidence	N	1	1
	%	0.6	0.5
Litter incidence	N	1f	1
	%	4.8	4.0
Rib			
Litter incidence	N	21	24
Fetal incidence	N	108	126
A Rib: Short			
Fetal incidence	N	14	27
	%	7.8	12.7
Litter incidence	N	9f	15
	%	42.9	60.0
A Rib: Supernumerary lumbar			
Fetal incidence	N	38	40
	%	21.2	18.9
Litter incidence	N	16	18
	%	76.2	72.0
A Rib: Branched			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
A Rib: Number of full ribs = 12/12			
Fetal incidence	N	82	84
	%	45.8	39.6
Litter incidence	N	20f	22
	%	95.2	88.0
A Rib: Number of full ribs = 12/13			
Fetal incidence	N	12	15
	%	6.7	7.1
Litter incidence	N	9f	11
	%	42.9	44.0
A Rib: Detached			
Fetal incidence	N	3	1
	%	1.7	0.5
	N	3f	1
Litter incidence	%	14.3	4.0
A Rib: Cervical			
Fetal incidence	N	0	1
	%	0.0	0.5
	N	0f	1
Litter incidence	%	0.0	4.0

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Cervical Vertebra			
Litter incidence	N	1	0
Fetal incidence	N	1	0
A vertebra, cervical: Incomplete ossification of centrum			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
Thoracic Vertebra			
Litter incidence	N	20	22
Fetal incidence	N	79	84
V vertebra, thoracic: Number = 12			
Fetal incidence	N	79	84
	%	44.1	39.6
Litter incidence	N	20f	22
	%	95.2	88.0
Lumbar vertebra			
Litter incidence	N	17	19
Fetal incidence	N	37	38
V vertebra, lumbar: Number = 6			
Fetal incidence	N	35	35
	%	19.6	16.5
Litter incidence	N	16f	17
	%	76.2	68.0
V vertebra, lumbar: Number = 8			
Fetal incidence	N	1	3
	%	0.6	1.4
Litter incidence	N	1f	3
	%	4.8	12.0
V vertebra, lumbar: Multiple abnormalities			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
Caudal vertebra			
Litter incidence	N	1	2
Fetal incidence	N	1	2
A vertebra, caudal: Bipartite ossification			
Fetal incidence	N	0	1

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Litter incidence	%	0.0	0.5
	N	0f	1
	%	0.0	4.0
A vertebra, caudal: Malpositioned			
Fetal incidence	N	1	1
	%	0.6	0.5
Litter incidence	N	1f	1
	%	4.8	4.0
Pelvis			
Litter incidence	N	3	7
Fetal incidence	N	3	8
A pelvic girdle: Malpositioned			
Fetal incidence	N	3	6
	%	1.7	2.8
Litter incidence	N	3f	5
	%	14.3	20.0
A pelvis: Incomplete ossification of pubis			
Fetal incidence	N	0	2
	%	0.0	0.9
Litter incidence	N	0f	2
	%	0.0	8.0
General			
Litter incidence	N	1	0
Fetal incidence	N	1	0
M vertebra: Multiple abnormalities			
Fetal incidence	N	1	0
	%	0.6	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
Litters evaluated	N	21	25
Fetuses evaluated	N	96	113
Live	N	96	113
Dead	N	0	0
Skull			
Litter incidence	N	0	1
Fetal incidence	N	0	1
A fontanelle: Small			
Fetal incidence	N	0	1
	%	0.0	0.9

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Litter incidence	N	0	1
	%	0.0	4.0
Cranium			
Litter incidence	N	1	2
Fetal incidence	N	2	2
A parietal: Unossified area			
Fetal incidence	N	0	1
	%	0.0	0.9
Litter incidence	N	0	1
	%	0.0	4.0
A cranium: Sutural bone			
Fetal incidence	N	1	0
	%	1.0	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
A parietal: Unossified line			
Fetal incidence	N	1	0
	%	1.0	0.0
Litter incidence	N	1f	0
	%	4.8	0.0
A Interparietal: Unossified line			
Fetal incidence		0	1
		0.0	0.9
Litter incidence		0f	1
		0.0	4.0
Mandibular			
Litter incidence	N	0	2
Fetal incidence	N	0	2
V hyoid: Incomplete ossification			
Fetal incidence	N	0	1
	%	0.0	0.9
Litter incidence	N	0f	1
	%	0.0	4.0
M palatine: Split			
Fetal incidence	N	0	1
	%	0.0	0.9
Litter incidence	N	0f	1
	%	0.0	4.0
A hyoid: Unossified			
Fetal incidence	N	0	1
	%	0.0	0.9

Group	N	Group 1 (Control 0µg)	Group 2 (Control 45µg)
Litter incidence	N	0f	1
	%	0.0	4.0

Statistical key: f=Chi2/Fisher Exact test

Observation code: M-Malformation, V-Variation, A-Anomaly

Table 66: Summary of fetal skeletal observations (study # 8).

### Pathology of does

#### *Macroscopic findings*

No treatment-related macroscopic observation or changes at the injection sites in the treated Cesarean and littering sub-groups were reported.

In groups 1 and 2, ovarian cystic areas were reported in several females. Alopecia and pale liver was also reported.

#### *Maternal organ weights*

No treatment-related effects on mean absolute or relative to body weight ovary weights were reported.

Group	Sex		Necropsy BW (g)	Ovaries ABS (g)	Ovaries Relat. Bod. %
<u>Day: 50 relative to Start Date</u>					
1	f	Mean	4372.8	1.02397	0.02335
		S.D.	317.8	0.27434	0.00579
		N	21	21	21
		----	-----	-----	-----
2	f	Mean	4467.7	0.92161	0.02060
		S.D.	357.3	0.21006	0.00413
		N	25	25	25
<u>Day: 81 relative to Start Date</u>					
1	f	Mean	3750.2	0.52382	0.01391
		S.D.	281.7	0.14430	0.00351
		N	25	25	25
		----	-----	-----	-----
2	f	Mean	3830.6	0.54042	0.01408
		S.D.	295.9	0.19131	0.00480
		N	30	30	30

Table 67: Summary of body/organ weights (study # 8).

### Post-partum litter data

#### *Pre-birth loss*

No treatment-related effects on the mean numbers of implantation sites and delivered kits were reported. No treatment-related effects on pre-birth loss were reported.

Group	N	Group 1 (0 mcg)	Group 2 (45 mcg)
Pregnant	N	27	30
Dams with delivered pups	N	26	30
Pre-birth loss	Total	11	19
No. per animal	MEAN	0.4	0.6
	S.D.	0.6	0.9
% per animal	MEAN%	3.8	6.1
	S.D.	5.8	8.6

Table 68: Mean pre-birth loss (study # 8).

### *Parturition and gestation length*

No treatment-related effects on parturition and gestation length were reported. On day 0 of lactation, one control female (no. 6790) was found dead delivering. There were 26 and 30 females that completed delivery in groups 1 and 2, respectively. The mean duration of gestation was comparable (approximately 31 days) in groups 1 and 2.

Group	N	Group 1 (0mcg)	Group 2 (45mcg)
Females on study	N	30	30
Females mated	N	28f	30
Mating index	%	93.3	100.0
Females pregnant	N	27f	30
Female fertility index	%	96.4	100.0
Females with liveborn	N	26f	30
Gestation index	%	96.3	100.0
Females completing delivery	N	26f	30
	%	86.7	100.0
With stillborn pups	N	5f	4
	%	19.2	13.3
With all stillborn	N	0f	0
	%	0.0	0.0
Litters with live born, but no pups alive			
day 4	N	0 f	0
	%	0.0	0.0
day 28	N	1 f	0
	%	3.8	0.0



Group	N	Group 1 (0mcg)	Group 2 (45mcg)
Duration of gestation	MEAN	31.5 d	31.6
	S.D.	0.5	0.7
	N	26	30
Litters with liveborn pups	N	26	30
Pups delivered (total)	N	234	252
	MEAN	9.0 d	8.4
	S.D.	2.6	2.2
Liveborn	N	226 f	248
Live birth index	%	96.6	98.4
Stillborn	N	8 f	4
	%	3.4	1.6
Pups dying, missing, and/or cannibalized			
day 0	N	0 f	0
	%	0.0	0.0
days 1-4	N	7 f	6
	%	3.0	2.4
days 1-7	N	8 f	14
	%	3.5	5.6
days 8-28	N	24 f	22
	%	10.6	8.9
days 1-28	N	32 f	36
	%	14.2	14.5
Pups Surviving 4 days	N	219 f	242
Viability Index	%	96.9	97.6
Pups Surviving 28 days	N	194 f	212
Lactation Index	%	88.6	87.6
Implantation sites per litter	MEAN	245	271
	N	9.4 d	9.0
	S.D.	2.8	2.5
Live pups/litter Day 4	MEAN	8.4 d	8.1
	N	2.2	2.0
	S.D.	26	30
Day 7	MEAN	8.4 d	7.8
	N	2.2	2.0
	S.D.	26	30
Day 11	MEAN	8.3 d	7.7
	N	2.1	2.0
	S.D.	26	30
Day 14	MEAN	7.9 d	7.3
	N	2.1	2.0
	S.D.	26	30
Day 21	MEAN	7.7 d	7.1
	N	2.4	1.9
	S.D.	26	30
Day 28	MEAN	7.5 d	7.1

Group	N	Group 1 (0mcg)	Group 2 (45mcg)
	N	2.6	1.9
	S.D.	26	30
Sex ratio-male pups: Total pups			
Day 4	N	100 f	126
	%	45.7	52.0
Day 28	N	86 f	110
	%	44.3	51.9

Statistical key: d=Anova/Dunnett test, f=Chi2/Fisher Exact test

Table 69: Summary of delivery and litter data (study # 8).

### Kit observations

No treatment-related clinical observations amongst the kits were reported.

### *Kit viability and litter sizes*

In groups 1 and 2, there was no treatment-related effect on kit viability since the numbers of stillborn and pup deaths during lactation were comparable in both groups. In both groups, the pup live born index and subsequent survival indices at PND 4 and PND 28 were comparable. In both groups, the mean percentage of males per litter and litter size were comparable.

### *Kit weights*

In groups 1 and 2, mean kit weight was comparable at birth and throughout lactation.

### Physical and functional development

No treatment-related effects on pre-weaning physical or functional development of the kits were reported.

Group		1	2
Dose level		0 mcg	45 mcg
Incisor eruption - % of pups positive: day 4 <i>post-partum</i>		100	100
Fur growth - % of pups positive: day 4 <i>post-partum</i>		100	100
Eye opening - % of pups positive:			
day 4	<i>post-partum</i>	0	0
day 7	<i>post-partum</i>	0	0

Group		1	2
Dose level		0 mcg	45 mcg
day 8	<i>post-partum</i>	0	0
day 9	<i>post-partum</i>	0	8
day 10	<i>post-partum</i>	18	29
day 11	<i>post-partum</i>	64	73
day 12	<i>post-partum</i>	91	88
day 13	<i>post-partum</i>	98	96
day 14	<i>post-partum</i>	100	100
Surface righting reflex - day 11 <i>post-partum</i> - % of pups		100	100
Positive: Auditory reflex - day 14 <i>post-partum</i> - % of pups		100	100
Positive: Pupillary reflex - day 22 <i>post-partum</i> - % of pups positive:		100	100

Table 70: Summary of reflex and physical development (study # 8).

Kit necropsy observations

No treatment-related effects on the incidence and type of kit necropsy observations were reported.

Parameters	N	Group 1 (0mcg)	Group 2 (45mcg)
Litters Evaluated	N	25	30
Pups Evaluated	N	228	252
Live	N	220	248
Stillborn	N	8	4
Abdominal cavity			
Litter Incidence	N	7	9
Pup Incidence	N	8	11
Abdominal cavity : Autolysis			
Pup Incidence	N	5	10
	%	2.2	4.0
Litter Incidence	N	4f	8
	%	16.0	26.7
Kidney(s) : Abnormal position			
Pup Incidence	N	0	1
	%	0.0	0.4
Litter Incidence	N	0f	1
	%	0.0	3.3
Hindlimb(s) : Cannibalized			
Pup Incidence	N	2	0
	%	0.9	0.0

Parameters	N	Group 1 (0mcg)	Group 2 (45mcg)
Litter Incidence	N	2 f	0
	%	8.0	0.0
Liver: Dark depressed area			
Pup Incidence	N	1	0
	%	0.4	0.0
Litter Incidence	N	1 f	0
	%	4.0	0.0

Statistical key: f = Chi2/Fisher exact test

Table 71: Summary of pup necropsy observations (study # 8).

### Immunogenicity:

To quantify the antibody response of a single strain (A/H1N1) to vaccination, a standard hemagglutination Inhibition (HI) assay, using red blood cells and influenza virus of one of the strains included in the vaccine, was used. The antibody levels in the fetuses and kits were compared with those from the corresponding does in order to confirm the exposure of fetuses and kits to vaccine antibodies.

No antibody titers were detected in sera of the control group and the does (in the treated group prior to vaccination) were reported. Antibodies were detected in sera from treated Cesarean does group after vaccination at G29 (Geometric Titer Mean [GTM] = 844.5) and from littering does group at G20 (GTM = 1650.4) and at PND 29 (GTM = 376.2). Antibodies were detected in sera from fetus of Cesarean does group 2 at G29 (GTM = 1039.7) and from kits of littering does group 2 at PND 29 (GTM = 113.7).

Group	Does					Fetus	Kits
	Cesarean		Littering			Cesarean	Littering
	M 21	G 29	M 21	G 20	PND 29	G 29	PND 29
1		12.3 (n = 10)		15.3 (n = 15)	16.6 (n = 15)		
2	15.3 (n = 10)	844.5 (n = 10)	20.9 (n = 15)	1650.4 (n = 15)	376.2 (n = 15)	1039.7 (n = 10)	113.7 (n = 108)

M 21 = Day 21 before mating. G 20 = Day 20 of gestation. G 29 = Day 29 of gestation. PND = Post natal day 29.

Table 72: Geometric titer mean for anti-A/California/7/2009 (study # 8).

In conclusion, hemagglutination inhibition (HI) assay was used to assess the presence of influenza-neutralizing antibodies in rabbit sera (obtained from does, fetus and kits). No antibody titers were detected in sera from does in the control group, or in sera from does in the treated group prior to vaccination. Antibodies were detected in sera from treated does after vaccination (at G 20 or PND 29), and in sera from their fetuses and kits.

**Summary:**

The objective of this study was to evaluate the effect of Flud<sup>®</sup> vaccine, administered intramuscularly, on pregnant rabbits. Animals (30/ group for littering and 25/ group for Cesarean) were assigned to 2 different groups and treated by 500 µl/rabbit of control or test article (Flud<sup>®</sup>).

Parameters evaluated included clinical signs (daily), mortality (twice daily), injection site observations (before each treatment, 24, and 48 hours post-dose), body weight for littering sub-group (on day 0 [mating day -21], on day 14 [mating day -7], on days 0, 6, 9, 13, 16, 20, 24, 27 and 29 of gestation and when necessary on day 34 of presumed gestation, and on days 4, 7, 11, 14, 17, 21 and 28 of lactation), body weight for Cesarean sub-group (on day 0 [mating day -21], on day 14 [mating day -7], and on days 0, 6, 9, 13, 16, 20, 24, 27 and 29 of gestation), food consumption (daily from the day of arrival to day 29 of gestation for the Cesarean sub-groups and to day 29 of lactation for the littering sub-groups). Pregnancy and parturition, immune responses, and litter data (number of kits born, external abnormalities, number of kits alive, weight of kits a live, physical development of the offspring, and behavioral and functional testing [surface righting reflex, auditory reflex, and pupil reflex]) were reported. Cesarean examinations included; pregnancy status, number of corpora lutea, gravid uterus weight, number and distribution of intrauterine implantations, individual fetal weights, and fetal sex.

**Results**

No test article-related effects on mortality or clinical observations were reported. No test article-related effects on the injection sites were reported. No test article-related effects on body weight gain or food consumption were reported. No test article-related effects on mating performance and fertility parameters were reported. No test article-related effects on gravid uterus weight were reported. No treatment-related effects on the pre-implantation data (mean numbers of corpora lutea and implantations and the corresponding percentage pre-implantation loss) were reported.

No treatment-related effects on the percentage embryo-fetal survival were reported.

**Fetal findings:**

No test article-related effect on mean fetal weight and fetal sex ratio were reported. No test article-related external, visceral, or skeletal malformations were reported.

**Does findings:**

No test article-related effect on the macroscopic observation or the changes at the injection sites in the treated Cesarean and littering sub-groups were reported. No treatment-related effects on mean absolute or relative to body weight ovary weights were reported.

Post-partum litter data

No treatment-related effects on the mean numbers of implantation sites and delivered kits were reported. No treatment-related effects on pre-birth loss were reported. No treatment-related effects on parturition and gestation length were reported.

Kits observations

No treatment-related effects on clinical observations, kits viability, litter sizes, kits weight, kits pre-weaning physical or functional development, or incidence and type of kit necropsy observations were reported.

No antibody titers were detected in sera from does in the control group, or in sera from does in the treated group prior to vaccination. Antibodies were detected in sera from treated does after vaccination (at G 20 or PND 29), and in sera from their fetuses and kits.

Conclusion:

No maternal changes indicative of an adverse effect due to intramuscular administration of Fluad® vaccine (0.5 mL/animal) to the (b) (4) rabbits at 21 and 7 days before mating and on days 7 (time of implantation) and 20 (end of major organogenesis) of gestation were reported. No adverse effects of the Fluad® vaccine on embryo-fetal development (including an evaluation of teratogenicity) or early postnatal development of the offspring were reported.

Antibody titers were reported in sera from treated does after vaccination (at G 20 or PND 29), and in sera from their fetuses and kits.

**OVERALL SUMMARY:**

**GENERAL TOXICOLOGY:**

Five studies were submitted to support this BLA. Four of these studies related to the product for licensure and one includes one group related to the adjuvant (MF59) used with the test article. All studies of the test articles (Agrippal or Fluad®) with or without the adjuvant MF59 were repeated dose studies. Animals were treated with 2 or 3 doses of the test article and studies durations were 30 or 43 days.

Adequate nonclinical toxicology data were included in these studies. Based on nonclinical toxicity assessments of the above mentioned studies there were no significant safety issues to preclude the BLA from approval. The delivery of an active dose of the product was verified.

**GENOTOXICOLOGY STUDIES:**

Two micronucleus studies and two Ames testing studies were submitted to support this BLA. Because the two studies of each test article were the same

with the exception of using different lot numbers of the test article, only one study of each testing was reviewed.

There was no genotoxicity found in the micronucleus testing assay. The results of the bacterial reverse mutation assay testing indicate that MF59C.1 did not cause a positive response with any of the tester strains in the presence and absence of (b) (4).

#### REPRODUCTIVE STUDIES:

One reproductive study was submitted to support this BLA. Animals were treated with the test article at 21 days (M-21) and 7 days (M-7) before mating, then on days 7 and 20 of gestation and the study duration was 83 days.

Adequate nonclinical toxicology data were included in this study. Based on nonclinical toxicity assessments of the above mentioned study, there were no significant safety issues to preclude the BLA from approval. The delivery of an active dose of the product was verified.

#### PREGNANCY CATEGORY: B

Justification: No data are available from adequate and well-controlled studies for Flud<sup>®</sup> vaccine in pregnant women. The reproductive study included in this BLA showed no adverse effects on mating, female fertility, pregnancy, parturition, lactation parameters, and embryo-fetal developments. There were no vaccine-related fetal malformations or other evidence of teratogenesis.

#### PROPOSED LABEL WORDING:

##### Pregnancy Category B

A reproductive and developmental toxicity study has been performed in female rabbits using full human dose and has shown no evidence of impaired fertility or harm to the fetus due to Flud<sup>®</sup> vaccine. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, Flud<sup>®</sup> vaccine should only be continued during pregnancy if clearly needed. Treatment with Flud<sup>®</sup> vaccine should not be initiated in pregnant women.

#### CARCINOGENESIS AND MUTAGENESIS

Micronucleus cytogenetic assay in mice using the test article showed no genotoxicity. The Ames testing results indicate that MF59C.1 did not cause a positive response with any of the tester strains in the presence and absence of (b) (4) in the bacterial reverse mutation assay.

**OVERALL CONCLUSION:**

Based on the nonclinical toxicity assessments of the Flud<sup>®</sup> 65 influenza vaccine submitted in this BLA, there are no significant safety issues to preclude the BLA from being approved.

**Concurrence:** Martin D. Green

**References:**

- 1- Stanford Cancer Center. "Cancer Diagnosis - Understanding Cancer". *Understanding Cancer*. Stanford Medicine.
- 2- "Nomenclature of Lipids". IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Retrieved 2007-03-08.
- 3- Drummond *et al.* (2014) *Nutrition for Foodservice and Culinary Professionals* 8th Ed., John Wiley & Sons.
- 4- "Boston scientists say triglycerides play key role in heart health". The Boston Globe. Retrieved 2014-06-18.



## Historical Control Data

Rabbit (b) (4)

Caesarean data

Study	Year	Number of females				Number of corpora lutea		Number of uterine		Pre-implantation		Early resorptions		Late resorptions		Post-implantation	
		Mated	Pregnant	Aborted	With live fetuses	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A12	2012	7	7	0	7	9.3	1.7	8.1	2.9	14.8	19.5	0.0	0.0	0.1	0.4	1.6	4.2
B12	2012	24	23	0	23	10.3	2.0	8.3	1.7	18.2	15.6	0.2	0.5	0.0	0.2	3.1	7.5
D12	2012	25	23	0	23	10.2	2.0	9.1	2.6	12.6	19.5	0.2	0.4	0.5	1.5	6.2	13.4
A11	2011	20	18	0	18	10.6	1.5	9.3	2.4	12.6	19.2	0.3	0.6	0.1	0.5	4.6	9.0
B11	2011	20	19	0	18	11.2	2.9	10.2	2.6	8.3	12.5	0.5	0.6	0.5	0.9	9.7	9.4
C11	2011	6	6	0	6	11.3	2.3	10.2	2.9	11.2	12.5	1.3	2.8	0.3	0.8	16.5	27.6
D11	2011	22	21	0	20	11.0	3.0	10.1	3.0	8.8	10.7	0.3	0.7	0.3	0.7	7.5	11.0
A10	2010	24	21	1	20	10.1	1.9	9.3	2.0	7.6	9.5	0.3	0.6	0.0	0.0	3.0	5.5
C10	2010	24	22	0	22	9.0	2.2	7.7	2.8	16.2	23.3	0.0	0.2	0.2	0.4	2.7	5.1
E10	2010	25	23	0	23	10.3	2.2	9.3	1.9	8.7	9.3	0.1	0.3	0.1	0.5	2.4	4.7
G10	2010	25	25	2	23	9.8	2.4	8.7	2.4	11.1	15.6	0.1	0.3	0.0	0.0	1.5	4.0
Total		222	208 94%	3 1%	203 91%												
Mean						10.0		9.1		11.8		0.2		0.2		4.6	
SD							2.8		2.5		15.8		0.7		0.7		9.5
<b>2007-2009</b>		576	543 94%	6 1%	527 91%	11.0	3.1	9.5	2.9	12.8	13.1	0.4	0.9	0.5	0.9	9.1	14.0

**Historical Control Data**

Rabbit (b) (4)

Caesarean data

Study	Year	Number of													
		Mated	Pregnant	Aborted	With live fetuses	Dead fetuses	Live litter size		Fetal weight (g)		Uterus weight (g)		Placental weight (g)		Sex ratio
						Total	Mean	SD	Mean	SD	Mean	SD	Mean	SD	%males
A12	2012														
B12	2012					0	8.0	2.9	41.7	8.3	453	104			44.3
D12	2012	7	7	0	7	0	8.0	1.7	42.1	4.0	483	72			43.6
A11	2011	24	23	0	23	0	8.4	2.6	40.0	5.6					48.3
B11	2011	25	23	0	23	0	8.9	2.5	38.6	5.7					48.8
C11	2011	20	18	0	18	0	9.2	2.4	41.7	4.8					46.1
D11	2011	20	19	0	18	0	8.5	3.9	40.5	5.7					
A10	2010	6	6	0	6	0	9.4	3.2	39.1	6.8					45.7
C10	2010	22	21	0	20	0	9.0	2.0	41.9	4.5					47.6
E10	2010	24	21	1	20	0	7.5	2.7	43.9	6.7					46.3
G10	2010	24	22	0	22	0	9.0	1.7	44.0	4.5	562	97			47.1
Total		25	23	0	23	0	8.6	2.4	41.9	5.0	516	120			39.4
		25	25	2	23										
Mean		222	208	3	203		8.6		41.5		514				45.7
SD			94%	1%	91%			2.5		5.6		106			
<b>2007-2009</b>		576	543	6	527	2	8.6	2.9	36.5	8.2	484	107	5.73	0.7	47.5
			94%	1%	91%										

## Historical Control Data

Rabbit (b) (4)

### FETAL EXAMINATION – EXTERNAL PERIOD

Number of studies included Number of fetuses examined	N	%	N	%
Observation				
Limb: hyperflexion	3	0.06	2	0.11
Limb: hyperextension	1	0.02	0	0.00
Limb: flexed	1	0.02	0	0.00
Limb: malrotated	5	0.11	0	0.00
Paw: malrotated (marked)	0	0.00	2	0.11
Paw: hyperflexion	1	0.02	0	0.00
Hindpaw: malformed	0	0.00	1	0.06
Ectrodactyly	0	0.00	1	0.06
Digit: bent	0	0.00	1	0.06
Claw: absent	1	0.02	1	0.06
Acaudia	3	0.06	0	0.00
Tail: short	1	0.02	0	0.00
Tail: bent	8	0.17	1	0.06

**Historical Control Data**

Rabbit (b) (4)

**Post-partum litter data**

		Number of females						Gestation		Number of		Number of		Live birth index %	Pup viability index %	Pup lactation index %	Pup sex ratio % males	
		Mated	Pregnant	With				Mean	SD	Mean	SD	Mean	SD					
				Liveborn	at L4	at L11	at L35											
C12	2012	28	27	27	27	27	27	31.8	0.6	8.9	2.3	8.1	2.2	95.0	98.1	93.7	49.8	
E12	2012	29	25	24	24	24	24	31.5	0.7	10.4	2.1	9.6	1.6	90.9	93.8	88.8	54.1	
B10	2010	29	26	26	26	26	26	31.6	0.6	8.4	2.2	8.0	2.5	98.1	97.1	90.4	55.9	
D10	2010	29	21	21	21	21	21	32.0	0.7	7.0	3.3	6.7	3.0	99.3	99.3	88.5	53.6	
F10	2010	29	27	26	26	26	26	31.8	0.7	8.0	2.7	7.5	2.7	94.1	98.9	83.5	48.9	
H10	2010	28	26	25	25	25	25	31.7	0.5	8.6	2.7	8.4	2.7	95.0	92.8	85.9	51.6	
G9	2009	30	26	26	24	24	24	31.9	0.6	8.8	2.3	8.0	2.0	98.1	84.7	85.5	48.8	
J9	2009	30	30	25	23	22	21	32.0	0.6	8.7	2.4	8.1	2.2	96.9	92.1	87.4	47.6	47.6
P8	2008	30	28	25	24	24	24	31.7	0.5	10.0	2.5	9.3	2.4	94.4	92.7	89.7	44.1	
Q8	2008	28	26	26	26	26	26	31.7	0.6	9.5	2.0	8.8	1.6	99.1	94.7	89.4	56.8	
B7	2007	30	30	29	29	29	29	31.6	0.7	9.6	1.9	8.1	2.2	95.9	97.0	92.4	56.9	
A6	2006	10	10	10	10	8	8	31.6	0.8			9.5	1.5	100.0	97.9	69.9	50.8	
B6	2006	10	10	10	10	10	10	32.1	0.6			8.4	1.8	97.6	100.0	74.4	54.1	
C6	2006	10	9	9	9	9	9	32.3	0.9			7.2	2.1	100.0	84.6	90.9	58.0	
D6	2006	10	10	9	9	9	9	31.8	0.6			8.1	3.1	92.6	88.0	95.5	47.6	
A5	2005	30	30	29	29	29	NA	31.6	0.7			8.5	2.3	96.7				
B5	2005	28	28	27	26	25	25	31.9	0.5	10.8	2.7	8.7	2.3	99.6	94.0	79.5		
A4	2004	30	26	24	20	20	20	31.6	1.4	9.3	2.8	8.3	2.9	96.5		98.0	50.0	
A3	2003	18	15	14	14	14	14	31.5	2.3	10.6	2.4	10.2	2.0	90.9		94.0	43.7	
A2	2002	16	13	13	10	10	10	32.4	0.7	8.5	2.4	9.7	1.8	98.4		96.0	56.8	
B2	2002	16	15	15	14	12	12	31.7	2.3	9.1	1.8	8.6	2.1	97.7		89.0	50.5	

Total	498	453	438	425	392	390											
%		91%	88%	85%	79%	78%											
Mean							31.8		9.1		8.4		96.4	95.8	89.7	51.5	
SD								0.9		2.6		2.4					

Studies F10, H10 and C12 were recorded on lactation day 28 instead of 35

L = Lactation day

NA = Not Applicable

**Note:** For complete list of historical control data, please visit appendix 36 on page 476 of the reproductive study report.