

# Toxicology Review of Trivalent, Inactivated Subunit-Influenza Vaccine (OPTAFLU)

**BLA:** 125408

**Sponsor:** Novartis Vaccines and Diagnostics, Inc.

**Product:** Trivalent, Inactivated Subunit-Influenza Vaccine

**Cross references:** BB-IND 11580

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**Proposed use:** Optaflu is a trivalent, surface antigen, inactivated, influenza vaccine for interpandemic (seasonal) use. Novartis is seeking an indication for active immunization for persons 18 years of age and older against influenza disease caused by influenza virus subtypes A and B contained in this vaccine.

## **Précis:**

Study number 1: In this repeated dose toxicology study, -----(b)(4)----- rabbits (6/dose/group) were treated with placebo, influenza cell culture subunit vaccine, or influenza subunit vaccine (Agrippal®). Animals were dosed with 0.5 mL of control or test article on study days (SD's) 1 and 8. Test article were administered intramuscularly into the hindlimb of each animal by alternating legs. Terminal sacrifice necropsies were conducted on SD's 10 and 11, and recovery sacrifice necropsies were conducted on SD's 22 and 23. The dose selected was the planned clinical dose, but the two doses administered to rabbits in this study exceeded the intended number (one) in the currently proposed clinical regimen.

Study number 2: In this repeated dose reproductive toxicology study, -----(b)(4)----- rabbits (24/group) were treated with saline or FCC vaccine containing purified influenza virus surface antigens (haemagglutinin and neuraminidase) of three interpandemic influenza strains A/New Caledonia/20/99 IVR-116 (H1N1), A/New York 55/2004x-157, and B/Jiangsu/10/2003 prepared in MDCK cell culture – containing 15 micrograms haemagglutinin per strain. Animals were dosed with 0.5 mL of control or test article on study days (SD's) 1, 15, and 29 at pre-mating and on SD's 7 and 20 of presumed gestation. Test article were administered intramuscularly into the hindlimb of each animal by alternating legs. Terminal sacrifice necropsies were conducted on gestation day (DG) 29 and on lactation day (DL) 29. The dose selected was the planned clinical dose.

**Introduction:** The original inactivated influenza vaccines have been produced in the allantoic cavity of embryonated hen eggs. However, the efficiency of this method is often low and requires one or more eggs for each dose of the vaccine produced. The new production methods using mammalian cell lines (WHO, 1995) can eliminate the reliance on the supply of embryonated eggs and generate more flexibility, adequate availability of substrate for virus growth and the possibility of high virus yields. In addition, cell culture-derived influenza vaccines do not require extensive advance planning and can, in principle, be viable for responding to the threat of an emerging pandemic. Influenza vaccine produced in a specifically developed cell line cloned from Madin Darby Canine Kidney (MDCK) tissue has been manufactured by Novartis. A subunit influenza vaccine, which is derived from a mammalian cell culture and contains three influenza virus strain: two A strains (H3N2 and H1N1) and a B strain, is the investigational product described in this application.

**Proposed clinical study:** In all clinical studies, a full dose of CCI vaccine was administered in 0.5 mL volume and contained the purified viral envelop glycoproteins, neuraminidase (NA) and hemagglutinin (HA) including 15 µg HA for each of the three strains (i.e., A/H1N1, A/H3N2, and B). All study subjects were aged ≥ 18 years and received one full dose of vaccine.

The CCI vaccine clinical development program included six randomized, blinded, controlled clinical studies, one of which was conducted in the US (phase 2; V58P5), one in New Zealand (phase 2; V58P2), and four in countries within the European Union (EU) (one phase 1 and 2 [V58P1] and three phase 3 [V58P4, V58P4E1, and V58P9]). In each study, a subunit egg-derived inactivated trivalent influenza vaccine was used as the control. Agrippal™ (Novartis) was selected as the control in the first five studies (V58P1, V58P2, V58P4, V58P4E1, and V58P9), which were originally designed to support the European development program. A sixth study that was conducted in the US (V58P5) used a US licensed control, Fluvirin™ (influenza virus vaccine; purified surface antigen vaccine, trivalent, types A and B). This study was conducted in subjects 18-45 years of age. The data for the studies presented in this submission have led to the approval of the CCI vaccine (June 01, 2007) in the European Community under the brand name of Optaflu™.

One adult dose of each of the influenza vaccines used in the studies of the CCI vaccine clinical development program contained 15 µg HA of each of the three strains (i.e., A/H1N1, A/H3N2, and B) in 0.5 mL volume formulated in phosphate buffered saline (PBS) at pH (b)(4) The CCI vaccine does not contain thimerosal and antibiotics are not used in the manufacturing process.

**Studies reviewed within this submission:**

- 1- Two dose intramuscular toxicity study of influenza vaccine formulations in -----(b)(4)----- rabbits.
- 2- Intramuscular reproductive and developmental toxicity study of FCC vaccine in rabbits, including a postnatal evaluation.

**Studies not reviewed within this submission:** None

**Toxicology Study Review:**

**Study number 1:** Two dose intramuscular toxicity study of influenza vaccine formulations in -----(b)(4)----- rabbits. Study No. 191-44

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

**Study initiation date:** April 15, 2002  
**Study completion date:** August 28, 2002

**Test article batch/lot:**

<u>Test article</u>	<u>Lot/Batch number</u>	<u>Expiration date</u>
Placebo (PBS):	172	-----
Influenza cell culture subunit vaccine:	522 900/FLU-Triv-01	Sept. 20, 2002
Influenza subunit vaccine (Agrippal®):	3505	June, 2002

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

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

**Number of animal per group and sex:** 18 males and 18 females

**Age:** approximately 13 weeks

**Body weight range:** 2.47-3.62 kg

**Route and site of administration:** Intramuscular injection into hindlimb (left, 1<sup>st</sup> injection; right, 2<sup>nd</sup> injection).

**Volume of injection:** 0.5 mL

**Frequency of administration and study duration:** Animals (6/sex/group) received two doses on SD's 1 and 8. Terminal sacrifice necropsies were conducted on SD's 10 and 11, and recovery sacrifice necropsies were conducted on SD's 22 and 23.

**Dose:** Optaflu vaccine has been formulated to contain 45 µg hemagglutinin (HA) per 0.5 mL dose in the recommended ratio of 15 µg HA of influenza type A (H1N1), influenza type A (H3N2), and influenza type B.

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and ---(b)(4)--- control as used in this study. The stability study shows that the FCC vaccine without needle (lot # 522 008 011) is stable for (b)(4) months at 2-8°C.

Receipt and expiry dates were provided as:

<u>Test article</u>	<u>Receipt date</u>	<u>Expiry date</u>
Placebo (PBS):	April 12, 2002	-----
Influenza cell culture subunit vaccine:	April 12 & 15, 2002	Sept. 20, 2002
Influenza subunit vaccine (Agrippal®):	April 12, 2002	June, 2002

**Means of administration:** Intramuscular injection

**Report status:** Final

## **METHODS:**

### **Experimental design:**

Animals were randomized and assigned to three different groups. Each group consisted of 6 animals and 3 animals were necropsied at each termination time point (SD's 10, 11 and 22, 23). Animals were dosed on SD's 1 and 8 and study duration was 23 days. The details of the study design are listed in the following table:

Group	Treatment	Dose Level* (µg/antigen)	Dose Volume	Number of Animals (#/sex/group) Necropsy Days 10 and 11 Animals /sex	Number of Animals (#/sex/group) Necropsy Days 22 and 23 Animals /sex
1	Placebo (PBS)	0	0.5 mL	3	3
2	Influenza cell culture subunit vaccine	15	0.5 mL	3	3
3	Influenza subunit vaccine (Agrippal®)	15	0.5 mL	3	3

**Table 1: Experimental design. \* Each formulation contains 3 antigens at equal concentration (15 µg/antigen) for a total dose of 45 µg.**

**Randomization procedure:** Yes

**Statistical analysis plan:** t-test was used to compare the following parameters: mean body weights, mean food consumption, and mean absolute organ weights. Wilcoxon's test is used for the mean clinical pathology (hematology, coagulation, and clinical chemistry) parameters.

**Parameters evaluated:**

The following parameters were evaluated: clinical signs (once daily), skin reactions at the intramuscular site of injection (prior to treatment, approximately 24 and 48 hours after dosing, and prior to each necropsy), body weights (prior to treatment, on SD's 8 and 15, and prior to each necropsy), food consumption (once weekly), body temperature (prior to dosing, approximately 24 and 48 hours after dosing, and prior to each necropsy), ophthalmologic evaluation (prior to treatment and prior to each necropsy), haematology, coagulation, and clinical chemistry (during pretest, 48 hours after each application, and on SD 22), gross anatomy at termination, organ weights and histopathology on a selection of tissues. Blood samples for antibody-determination were taken and analyzed (non-GLP) under the responsibility of the sponsor (SD 1, before 2<sup>nd</sup> treatment, on SD 15, and before each necropsy).

Parameters	Frequency of Testing
Cageside observation <sup>1</sup>	Once daily
Clinical observations <sup>2</sup>	Once daily

<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.

Parameters	Frequency of Testing
Body weight	Prior to treatment, on SD's 8 and 15, and prior to each necropsy
Food consumption	Once weekly
Body temperature	Prior to dosing, approximately 24 and 48 hours after dosing, and prior to each necropsy
Ophthalmologic exam	Prior to treatment and prior to each necropsy
Clinical chemistry*	During pretest, 48 hours after each application, and on SD 22
Hematology*	During pretest, 48 hours after each application, and on SD 22
Coagulation*	During pretest, 48 hours after each application, and on SD 22
Immunological response	SD 1, before 2 <sup>nd</sup> treatment, on SD 15, and before each necropsy
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Prior to treatment, approximately 24 and 48 hours after dosing, and prior to each necropsy

**Table 2: Parameters evaluated. \* Sites of blood collection were not reported**

**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 and pons)	!*	
Cervix		X
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		X
Gall bladder	!	
Gross lesions (if any)	!	
Harderian gland (if		X

Organ/Tissue applicable)	Collected	Not collected
Heart	!*	
Ileum	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		X
Larynx		X
Liver	!*	
Lung (main-stem; bronchi)	!*	
Lymph nodes (cervical)	!	
Lymph nodes (iliac)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)		X
Pituitary gland	!	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!	
Tongue		X
Ureters		X
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		X

**Table 3: Histology results – Tissues listed above were collected from all animals and examined microscopically. Any abnormalities, seen during histology processing, not noted during macroscopic examinations, were recorded.**

**RESULTS:**

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

**Clinical chemistry results:**

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
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	<p><b>Lactate dehydrogenase (LDH):</b> SD 1 M <math>\downarrow \leq 0.6</math> G2, SD 3 M <math>\downarrow \leq 0.6</math> G2, SD 10 M <math>\downarrow \leq 0.6</math> G2, SD 10 M <math>\downarrow \leq 0.7</math> G3, SD 1 F <math>\uparrow \geq 1.6</math> G3, SD 10 F <math>\uparrow \geq 2.6</math> G2, SD 10 F <math>\uparrow \geq 1.9</math> G3</p> <p><b>Aspartate aminotransferase (AST):</b> SD 22 M <math>\downarrow \leq 0.6</math> G2, SD 10 F <math>\downarrow \leq 0.7</math> G2, SD 10 F <math>\downarrow \leq 0.7</math> G3, SD 22 F <math>\downarrow \leq 0.4</math> G2, SD 22 F <math>\downarrow \leq 0.4</math> G3</p> <p><b>Alanine aminotransferase (ALT):</b> SD 22 F <math>\downarrow \leq 0.6</math> G2, SD 22 F <math>\downarrow \leq 0.6</math> G3</p>	Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids
B) HEPATOBILIARY	<p><b>Total bilirubin:</b> SD 1 M <math>\uparrow \geq 2.0</math> G3, SD 3 M <math>\uparrow \geq 1.6</math> G3, SD 10 M <math>\uparrow \geq 2.1</math> G2, SD 10 M <math>\uparrow \geq 2.1</math> G3, SD 1 F <math>\uparrow \geq 2.0</math> G3, SD 3 F <math>\uparrow \geq 1.8</math> G3, SD 10 F <math>\uparrow \geq 2.1</math> G2, SD 10 F <math>\uparrow \geq 2.1</math> G3</p>	Gamma-glutamyl transferase (GGT) Total bile acids Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen

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
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	<p><b>Creatine kinase:</b>                      SD 1 M <math>\downarrow \leq 0.6</math> G2,                      SD 10 M <math>\downarrow \leq 0.7</math> G2,                      SD 22 M <math>\downarrow \leq 0.7</math> G2,                      SD 10 F <math>\uparrow \geq 2.4</math> G2</p> <p>Total Cholesterol                      SD 1 M <math>\downarrow \leq 0.7</math> G2</p> <p>Fasting Triglycerides                      SD 22 F <math>\downarrow \leq 0.7</math> G2</p>	Albumin (A) Globulin (G, calculated) or A/G ratio Cholinesterase Total protein Fasting triglycerides

**Table 4:** Clinical Chemistry Results

**Hematology results:**

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
RED BLOOD CELLS	<b>Reticulocytes:</b> SD3 M $\uparrow \geq 2.3$ G2, SD3 M $\uparrow \geq 1.8$ G3, SD3 F $\uparrow \geq 1.9$ G2, SD3 F $\uparrow \geq 1.8$ G3, SD10 M $\uparrow \geq 1.6$ G2, SD10 M $\uparrow \geq 1.6$ G3, SD22 M $\uparrow \geq 2.5$ G2, SD22 M $\uparrow \geq 2.0$ G3, SD22 F $\uparrow \geq 3.2$ G2, SD22 F $\uparrow \geq 1.8$ G3	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	<b>Neutrophil count:</b> SD3 M $\uparrow \geq 2.4$ G3, SD22M $\downarrow \leq 0.7$ G2 <b>Basophils:</b> SD1M $\downarrow \leq 0.7$ G2, SD1M $\downarrow \leq 0.5$ G3, SD3M $\uparrow \geq 1.8$ G2, SD22M $\uparrow \geq 1.8$ G2, SD22M $\downarrow \leq 0.7$ G3, SD1F $\uparrow \geq 1.7$ G3, SD3F $\downarrow \leq 0.6$ G2, SD10F $\uparrow \geq 1.7$ G2, SD10F $\uparrow \geq 1.7$ G3 <b>Lymphocyte count:</b> SD1M $\downarrow \leq 0.2$ G2, SD1M $\downarrow \leq 0.5$ G3, SD3M $\downarrow \leq 0.7$ G2, SD3M $\downarrow \leq 0.4$ G3, SD10M $\downarrow \leq 0.5$ G2, SD10M $\downarrow \leq 0.3$ G3, SD22M $\downarrow \leq 0.3$ G2, SD22M $\downarrow \leq 0.3$ G3, SD1F $\downarrow \leq 0.7$ G3, SD3F $\downarrow \leq 0.6$ G2, SD10F $\uparrow \geq 1.7$ G2, SD10F $\downarrow \leq 0.6$ G3, SD22F $\uparrow \geq 1.5$ G2, SD22F $\downarrow \leq 0.5$ G3	Macrophage Total leukocytes (WBC) Large Unstained Cells (LUC) Eosinophils count Monocyte count
CLOTTING POTENTIAL	Platelet count SD22M $\downarrow \leq 0.7$ G3	Activated partial-thromboplastin time clotting time Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

**Table 5: Hematology Results**

**Systemic toxicity:**

No treatment-related, mortality, nor any toxicologically relevant changes in body weight, clinical signs, relative food consumption, ophthalmoscopic parameters, or body temperature were found.

Females' lung weights were decreased 22 % and 15 % in G's 2 and 3, respectively. Males' heart weight was decreased 21 % in G 2. Spleen to brain weight was increased 21 % in G 3 females. Thymus weight was decreased 25 % in G's 2 and 3 males and 19 % and 27 % in G's 2 and 3 females, respectively. At recovery, reduction in adrenals (30 % and 23 % in G's 2 and 3 females, respectively), and spleen (30% in G 3 females) were reported. An increase in thymus (49 % in G 3 males and 22 % in G's 2 and 3 females) and ovary (59 % in G 2) weights were reported. None of these changes were statistically different.

Lactate dehydrogenase was significantly reduced in group 2 males on study days (SD's) 1, 3, and 10. In females, lactate dehydrogenase was significantly increased in G's 2 (SD 10) and 3 (SD's 1 and 10). Aspartate aminotransferase (AST) was significantly decreased on SD 22 in G 2 males and G's 2 and 3 females. In G's 2 and 3 females, alanine aminotransferase (ALT) was significantly decreased on SD 22. Total bilirubin levels were increased significantly in G's 3 (SD's 1, 3, and 10) and 2 (SD 10) males and females. Creatine kinase was significantly increased in G 2 males (SD 1) and females (SD10).

Reticulocytes levels were increased significantly in G's 2 and 3 of males and females at SD's 3 and 22. This increase was also seen at SD 10 in G's 2 and 3 males. Neutrophils were increased significantly in group 3 males on SD 3. Basophils were increased significantly in G 2 males (SD's 3 and 22), G 3 females (SD's 1 and 10), and G 2 females (SD 10). There were significant decrease in basophil levels in G 3 males (SD 1) and G 2 females (SD 3). Lymphocyte count was significantly decreased in G's 2 (SD's 1, 10 and 22) and 3 (SD's 1, 3, 10, and 22) males and G's 2 (SD 3) and 3 (SD's 10 and 22) females. Lymphocytes were significantly increased in G 2 females on SD 10 only.

One kidney and one testis with epididymis (right side) were undeveloped in one male of G2. One testis with epididymis (left side) was undeveloped and several discolorations in both testes were reported in G3 males. In G's 1 and 2 females, reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg were reported in one animal. Emphysema in all lobes of the lungs was reported in one female of G 2. At recovery, the followings were reported in G 1 females: reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg (1/3), reddened areas in the skeletal muscle (beside the injection site) (1/3), watery cyst in both kidneys (1/3), and hemorrhagic thymus (1/3). Watery cyst in kidneys and discolored and enlarged iliac lymph node were reported in 1 female of G 3.

Slight inflammatory foci in liver were reported in 1 animal of G's 1 and 3 males and G's 1, 2, and 3 females. Moderate focal fatty change in liver of 1 animal was reported in G 1 males. Moderate interstitial infiltration in lungs was seen in 2 animals of G's 1 and 2 males and G's 1, 2, and 3 females. Slight interstitial infiltration in lungs was reported in 1 animal of G's 2 males and G's 1 and 2 females and in 2 animals of G 3 males. Slight granuloma and slight alveolar emphysema in lungs of 1 animal was reported in G 3 males and G 1 females, respectively. Moderate (G's 1 and 2 males) and slight (G 2 females) hemorrhage in iliac lymph nodes were reported in 1 animal. Slight (G 2 males) and minimal (G 3 males) hemorrhage in mesenteric lymph nodes were reported in 1 animal. Marked tubular atrophy in testes of 1 animal was reported in G 3 males. In females, slight to moderate tubular degeneration (G's 1 [3/3], 2 [2/3], and 3 [2/3]) and moderate cortical cyst (G 1 [1/3]) were reported in kidneys.

### Organ weights at terminal sacrifice:

OBSERVATION		MALES GROUP 1 (CONTROL)	MALES GROUP 2	MALES GROUP 3	FEMALES GROUP 1 (CONTROL)	FEMALES GROUP 2	FEMALES GROUP 3
NUMBER OF ANIMALS		3	3	3	3	3	3
BODY WEIGHT (gram) <sup>a</sup>		2732	2772	2823	3006	3049	2995
<b>BRAIN</b>							
Absolute Weight <sup>a</sup>	gram	9.80	9.53	10.15	10.36	9.86	9.65
Per Body Weight <sup>a</sup>	%	0.36	0.34	0.36	0.34	0.32	0.32
<b>ADRENALS</b>							
Absolute Weight <sup>a</sup>	gram	0.15	0.16	0.18	0.24	0.21	0.24
Per Body Weight <sup>a</sup>	%	0.0055	0.0059	0.0064	0.0078	0.0070	0.0080
Per Brain Weight <sup>a</sup>	%	1.53	1.71	1.78	2.27	2.18	2.48
<b>LUNGS</b>							
Absolute Weight <sup>a</sup>	gram	10.47	10.11	9.62	12.49	9.73	10.59
Per Body Weight <sup>a</sup>	%	0.38	0.36	0.34	0.42	0.32	0.35
Per Brain Weight <sup>a</sup>	%	107.1	106.1	95.03	121.2	98.71	109.7
<b>HEART</b>							
Absolute Weight <sup>a</sup>	gram	8.95	7.04	8.43	7.61	7.13	6.89
Per Body Weight <sup>a</sup>	%	0.33	0.25	0.30	0.25	0.23	0.23
Per Brain Weight <sup>a</sup>	%	91.31	74.15	83.54	73.67	72.28	71.38
<b>KIDNEYS</b>							
Absolute Weight <sup>a</sup>	gram	15.44	14.67	15.51	17.43	18.18	17.64
Per Body Weight <sup>a</sup>	%	0.57	0.53	0.55	0.58	0.60	0.59
Per Brain Weight <sup>a</sup>	%	157.8	153.6	153.0	168.2	184.1	182.9
<b>LIVER</b>							
Absolute Weight <sup>a</sup>	gram	69.78	64.09	69.30	90.32	83.58	88.22
Per Body Weight <sup>a</sup>	%	2.54	2.31	2.45	3.01	2.75	2.93
Per Brain Weight <sup>a</sup>	%	710.7	671.0	684.8	871.6	847.2	913.0
<b>SPLEEN</b>							
Absolute Weight <sup>a</sup>	gram	0.92	0.99	0.86	1.40	1.46	1.58
Per Body Weight <sup>a</sup>	%	0.03	0.04	0.03	0.05	0.05	0.05

OBSERVATION		MALES GROUP 1 (CONTROL)	MALES GROUP 2	MALES GROUP 3	FEMALES GROUP 1 (CONTROL)	FEMALES GROUP 2	FEMALES GROUP 3
NUMBER OF ANIMALS		3	3	3	3	3	3
Per Brain Weight <sup>a</sup>	%	9.40	10.28	8.57	13.55	14.81	16.35
<b>TESTES</b>							
Absolute Weight <sup>a</sup>	gram	4.62	3.71	4.10			
Per Body Weight <sup>a</sup>	%	0.17	0.13	0.14			
Per Brain Weight <sup>a</sup>	%	47.01	38.58	41.05			
<b>THYMUS</b>							
Absolute Weight <sup>a</sup>	gram	3.53	2.64	2.64	3.60	2.92	2.64
Per Body Weight <sup>a</sup>	%	0.13	0.10	0.09	0.12	0.10	0.09
Per Brain Weight <sup>a</sup>	%	36.25	27.78	26.06	34.71	29.53	27.36
<b>OVARIES</b>							
Absolute Weight <sup>a</sup>	gram				0.3023	0.3107	0.3080
Per Body Weight <sup>a</sup>	%				0.0101	0.0103	0.0103
Per Brain Weight <sup>a</sup>	%				2.92	3.15	3.19

**Table 6: Organ weight and their normalization (terminal sacrifice). Absolute weights are expressed as mean(grams).**

**Organ weights at recovery sacrifice:**

OBSERVATION		MALES GROUP 1 (CONTROL)	MALES GROUP 2	MALES GROUP 3	FEMALES GROUP 1 (CONTROL)	FEMALES GROUP 2	FEMALES GROUP 3
NUMBER OF ANIMALS		3	3	3	3	3	3
BODY WEIGHT (gram) <sup>a</sup>		2928	2945	2998	3284	3361	3194
<b>BRAIN</b>							
Absolute Weight <sup>a</sup>	gram	10.29	10.15	10.15	10.01	10.18	9.97
Per Body Weight <sup>a</sup>	%	0.35	0.35	0.34	0.31	0.30	0.31
<b>ADRENALS</b>							
Absolute Weight <sup>a</sup>	gram	0.23	0.26	0.24	0.27	0.19	0.21
Per Body Weight <sup>a</sup>	%	0.0078	0.087	0.0080	0.0083	0.0055	0.0064
Per Brain Weight <sup>a</sup>	%	2.20	2.51	2.34	2.64	1.84	2.06
<b>LUNGS</b>							
Absolute Weight <sup>a</sup>	gram	10.74	11.10	11.65	12.50	11.75	11.82
Per Body Weight <sup>a</sup>	%	0.37	0.38	0.39	0.38	0.35	0.37
Per Brain Weight <sup>a</sup>	%	104.2	109.3	115.2	125.2	115.5	118.8
<b>HEART</b>							
Absolute Weight <sup>a</sup>	gram	8.28	8.09	9.19	9.32	8.85	7.95
Per Body Weight <sup>a</sup>	%	0.28	0.28	0.31	0.28	0.26	0.25
Per Brain Weight <sup>a</sup>	%	80.42	79.67	90.65	93.35	86.88	79.94
<b>KIDNEYS</b>							
Absolute Weight <sup>a</sup>	gram	15.41	18.26	16.22	18.56	18.74	18.58
Per Body Weight <sup>a</sup>	%	0.53	0.62	0.54	0.56	0.56	0.58
Per Brain Weight <sup>a</sup>	%	150.1	180.1	160.1	186.0	184.2	186.3
<b>LIVER</b>							
Absolute Weight <sup>a</sup>	gram	67.01	76.45	78.53	93.85	102.0	83.46

OBSERVATION		MALES GROUP 1 (CONTROL)	MALES GROUP 2	MALES GROUP 3	FEMALES GROUP 1 (CONTROL)	FEMALES GROUP 2	FEMALES GROUP 3
NUMBER OF ANIMALS		3	3	3	3	3	3
Per Body Weight <sup>a</sup>	%	2.29	2.59	2.62	2.81	3.03	2.61
Per Brain Weight <sup>a</sup>	%	652.2	754.9	775.0	943.7	998.9	838.7
<b>SPLEEN</b>							
Absolute Weight <sup>a</sup>	gram	1.01	0.89	1.06	1.55	1.66	1.09
Per Body Weight <sup>a</sup>	%	0.03	0.03	0.04	0.05	0.05	0.03
Per Brain Weight <sup>a</sup>	%	9.82	8.73	10.43	15.55	16.26	11.03
<b>TESTES</b>							
Absolute Weight <sup>a</sup>	gram	5.24	4.60	4.64			
Per Body Weight <sup>a</sup>	%	0.18	0.15	0.16			
Per Brain Weight <sup>a</sup>	%	51.04	45.58	45.51			
<b>THYMUS</b>							
Absolute Weight <sup>a</sup>	gram	2.91	3.20	4.33	3.52	4.28	4.30
Per Body Weight <sup>a</sup>	%	0.10	0.11	0.14	0.11	0.13	0.13
Per Brain Weight <sup>a</sup>	%	28.35	31.58	42.74	35.36	42.12	43.16
<b>OVARIES</b>							
Absolute Weight <sup>a</sup>	gram				0.3513	0.5557	0.3713
Per Body Weight <sup>a</sup>	%				0.0107	0.0164	0.0116
Per Brain Weight <sup>a</sup>	%				3.53	5.43	3.72

**Table 7: Table of organ weight and their normalization (recovery sacrifice). Absolute weights are expressed as mean (grams).**

**Gross pathology:**

No clinical observations at the left or right injection sites were reported in any of the treated groups.

**Macroscopic findings****Terminal sacrifice**

<b>Group</b>	<b>Findings</b>
1M	NF
2M	One kidney and one testis with epididymis (right side) were undeveloped (1/3)*
3M	One testis with epididymis (left side) was undeveloped (1/3); several discolorations in both testes (1/3)
1F	Reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg (1/3); emphysema in all lobes of the lungs (1/3)
2F	Reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg (1/3)
3F	NF

**Table 8: Macroscopic findings at terminal sacrifice. NF = no findings.  
\* (number of animals with the observation/total number of animals in the group).**

**Recovery sacrifice**

<b>Group</b>	<b>Findings</b>
1M	NF
2M	NF
3M	NF
1F	Reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg (1/3)*; reddened areas in the skeletal muscle (beside the injection site) (1/3); watery cyst in both kidneys (1/3); hemorrhagic thymus (heart puncture: hemothorax, hemopericard) (1/3)
2F	NF
3F	Watery cyst in kidneys and discolored and enlarged iliac lymph node (1/3)

**Table 9: Macroscopic findings at recovery sacrifice. NF = no findings.  
\* (number of animals with the observation/total number of animals in the group).**

**Microscopic findings at injection sites****Terminal sacrifice**

<b>Group</b>	<b>Findings</b>
1M	NF
2M	Slight hemorrhage at right injection site (1/3)*
3M	Slight necrosis at left injection site (1/3)
1F	Slight necrosis at left injection site (1/3)
2F	Slight necrosis at left injection site (1/3)
3F	Minimal to slight necrosis at left injection site (2/3)

**Table 10: Microscopic findings at terminal sacrifice. NF = no findings.  
\* (number of animals with the observation/total number of animals in the group).**

**Recovery sacrifice**

<b>Group</b>	<b>Findings</b>
1M	Slight hemorrhage at right injection site (1/3)*
2M	NF
3M	Slight necrosis at left injection site (1/3)
1F	NF
2F	Minimal necrosis at left injection site (1/3)
3F	NF

**Table 11: Microscopic findings at recovery sacrifice. NF = no findings.  
\* (number of animals with the observation/total number of animals in the group).**

**Microscopic finding at different organs:****Terminal sacrifice**

<b>Groups</b>	<b>Findings</b>
1M	<u>Liver</u> Slight inflammatory foci (1/3)*; moderate focal fatty change (1/3) <u>Lung</u> Moderate interstitial infiltration (2/3) <u>Iliac lymph node</u> Moderate hemorrhage (1/3)
2M	<u>Lung</u> Slight interstitial infiltration (1/3); moderate interstitial infiltration (2/3) <u>Mesenteric lymph node</u> Slight hemorrhage (1/3) <u>Iliac lymph node</u> Moderate hemorrhage (1/3)
3M	<u>Liver</u>

<b>Groups</b>	<b>Findings</b>
	Slight inflammatory foci (1/3) <u>Lung</u> Slight interstitial infiltration (2/3); slight granuloma (1/3) <u>Mesenteric lymph node</u> Minimal hemorrhage (1/3) <u>Testes</u> Marked tubular atrophy (1/3)
1F	<u>Kidneys</u> Slight to moderate tubular degeneration (2/3); moderate tubular dilation (1/3); moderate cortical cyst (1/3) <u>Liver</u> Slight inflammatory foci (1/3) <u>Lung</u> Slight interstitial infiltration (1/3); moderate interstitial infiltration (2/3); slight alveolar emphysema (1/3)
2F	<u>Kidneys</u> Moderate tubular degeneration (1/3); slight tubular dilation (1/3) <u>Liver</u> Slight inflammatory foci (1/3) <u>Lung</u> Slight interstitial infiltration (1/3); moderate interstitial infiltration (2/3) <u>Iliac lymph node</u> Slight hemorrhage (1/3)
3F	<u>Kidneys</u> Slight tubular degeneration (2/3) <u>Liver</u> Slight inflammatory foci (1/3) <u>Lung</u> Moderate interstitial infiltration (2/3)

**Table 12: Microscopic findings in different organs at terminal sacrifice. \* (number of animals with the observation/total number of animals in the group).**

### **Recovery sacrifice**

<b>Groups</b>	<b>Findings</b>
1M	<u>Liver</u> Slight inflammatory foci (1/3)* <u>Lung</u> Slight interstitial infiltration (2/3) <u>Iliac lymph node</u> Hemorrhage (1/3)
2M	<u>Liver</u> Minimal to slight inflammatory foci (2/3) <u>Lung</u> Slight interstitial infiltration (1/3)

<b>Groups</b>	<b>Findings</b>
3M	<u>Liver</u> Minimal to slight inflammatory foci (2/3); slight focal fatty change (1/3) <u>Lung</u> Slight interstitial infiltration (1/3); moderate interstitial infiltration (2/3)
1F	<u>Kidneys</u> Slight tubular degeneration (2/3); slight tubular dilation (1/3); marked cortical cyst (1/3) <u>Lung</u> Interstitial infiltration (2/3) <u>Thymus</u> Moderate hemorrhage (1/3)
2F	<u>Kidneys</u> Moderate tubular degeneration (1/3); moderate tubular dilation (1/3) <u>Lung</u> Moderate interstitial infiltration (1/3) <u>Iliac lymph node</u> Slight hemorrhage (1/3)
3F	<u>Kidneys</u> Slight tubular degeneration (2/3); moderate tubular degeneration (1/3); moderate tubular dilation (2/3); moderate cortical cyst (1/3) <u>Liver</u> Slight inflammatory foci (2/3) <u>Lung</u> Moderate interstitial infiltration (1/3) <u>Iliac lymph node</u> Moderate hemorrhage (1/3)

**Table 13: Microscopic findings in different organs at recovery sacrifice. \* (number of animals with the observation/total number of animals in the group).**

### **Body temperature**

Group	SD's 1, 2, and 3	SD's 8, 9, 10, and 11	SD's 22 and 23
1 M & F	0	0	0
2 M & F	0	0	0
3 M & F	0	0	0

**Table 14: Table of occurrences for body temperature > 40 °C**

No test article-related effect on body temperature was reported. Temperature of 40 °C (or above) was not reported in any treated group. An increase (39.9 °C) in body temperature in one animal in group 2 at study day 23 was reported.

### **Local toxicity:**

Macroscopically, reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg were reported in 1 female of G's 1 and 2. No macroscopic findings at the injection sites were reported in terminal and

recovery sacrificed males. At recovery sacrifice, reddened areas in the skeletal muscle (not identical to the injection site) of the upper and lower leg and reddened areas in the skeletal muscle (beside the injection site) were reported in 1 female of G 2.

Microscopically, slight hemorrhage at the right injection site and slight necrosis at the left injection site was reported in 1 animal of G's 2 and 3 males, respectively. In females, at the left injection site, slight necrosis was reported in 1 animal of G's 1 and 2, and minimal to slight necrosis was reported in 2 animals of G 3.

At the recovery sacrifice, slight hemorrhage at the right injection site was reported in 1 male of G 1. Slight necrosis at the left injection site was reported in 1 male of G 3. Minimal necrosis at the left injection site was reported in 1 female of G 2.

**Serology:**

Blood samples (0.5 mL serum) for antibody analysis were collected (from -----(b)(4)----- rabbits) on SD 1 (before first administration), before 2<sup>nd</sup> treatment, on SD 15, and before each necropsy. Another 10 mL serum of each animal was collected immediately before necropsy. Samples were stored at -15 to -25°C before transferring to Chiron Behring for further immunological investigations.

Results for strain A/new Caledonia (H1N1) by (b)(4) showed an increase (but not significant) in the titer at SD 8 in G 3 males and females. This is also true for males at SD 10. In females, the increase in titer at SD 11 was much higher in G's 2 and 3 than the control group. In males' recovery group, the titer was increased in G's 2 and 3 on SD's 8, 15, and 22. The titer in G3 (but not G2) was increased in females on SD 8. An increase in the titer was seen in G's 2 and 3 females on SD's 15 and 23.

Results for strain A/Panama (H2N3) by (b)(4) showed an increase in the titer levels in all treated groups including the control. These increases were reported on SD's 1, 8, 10, and 11. The titer level was also increased in the control group of recovery animals during the study. There was an increase in the titer levels of G 2 on SD's 15, 22, and 23 when compared with the control group. This increase was also reported in males and females of G 3 on SD's 8, 15, 22, and 23 when compared to control group. Since the titer was also detected in the control group, a final conclusion regarding the titer increase in G's 2 and 3 will be inconclusive.

Results for strain B/Guangdong by (b)(4) showed an increase in the titer of G 2 females on SD 11. Males and females titer was increased in G3 on SD's 8, 10, and 11. As for recovery males and females of G's 2 and 3, a gradual increase in the titer levels were observed on SD 8. The increase was higher on SD 15 (M & F) and 22 (M). The highest levels were reported in females on SD 23.

**Results for strain A/new Caledonia (H1N1) by (b)(4)-SOP 101076-01**

Male's and Female's geometric titer mean-Terminal

Group #	SD 1 M*	SD 1 F**	SD 8 M	SD 8 F	SD 10 M	SD 11 F
1	1	1	1	11.7	3.4	1
2	1	2.7	2.7	14.7	3.4	23.4
3	1	1	11.7	16.9	9.3	45.8

Male's and Female's geometric titer mean-Recovery

Group #	SD 1 M	SD 1 F	SD 8 M	SD 8 F	SD 15 M	SD 15 F	SD 22 M	SD 23 F
1	1	1	1	3.1	2.7	1	1	1
2	1	1	9.3	3.4	80	145.4	254	290.7
3	1	2.7	63.5	45.8	50.4	80	115.4	403.2

**Results for strain A/Panama (H2N3) by (b)(4)-SOP 101076-01**

Male's and Female's geometric titer mean-Terminal

Group #	SD 1 M	SD 1 F	SD 8 M	SD 8 F	SD 10 M	SD 11 F
1	80	50.4	100.8	100.8	63.5	100.8
2	57.7	50.4	50.4	80	63.5	127
3	45.8	40	80	115.4	100.8	80

Male's and Female's geometric titer mean-Recovery

Group #	SD 1 M	SD 1 F	SD 8 M	SD 8 F	SD 15 M	SD 15 F	SD 22 M	SD 23 F
1	50.4	63.5	63.5	50.4	50.4	63.5	31.8	40
2	80	50.4	63.5	80	80	145.4	127	202
3	58	63.5	160	115.4	100.8	127	160	160

**Results for strain B/Guangdong by (b)(4)-SOP 101076-01**

Male's and Female's geometric titer mean-Terminal

Group #	SD 1 M	SD 1 F	SD 8 M	SD 8 F	SD 10 M	SD 11 F
1	1	1	1	7.4	1	1
2	1	2.7	1	9.3	2.7	183.2
3	1	1	31.7	40	31.7	72.7

Male's and Female's geometric titer mean-Recovery

Group #	SD 1 M	SD 1 F	SD 8 M	SD 8 F	SD 15 M	SD 15 F	SD 22 M	SD 23 F
1	1	1	1	1	1	1	1	1
2	2.7	1	3.1	15.9	231	231	320	403.2

<b>Group #</b>	<b>SD 1 M</b>	<b>SD 1 F</b>	<b>SD 8 M</b>	<b>SD 8 F</b>	<b>SD 15 M</b>	<b>SD15 F</b>	<b>SD 22 M</b>	<b>SD 23 F</b>
<b>3</b>	2.7	2.7	31.7	31.7	201.6	231	210	581.5

**Table 15: Serology results.** \* M = males. \*\* F = females.

**TEST ARTICLE RELATED EFFECTS:** See page 37

**ASSESSMENT:** See page 38

**CONCLUSIONS:** See page 38

**COMMUNICATIONS TO SPONSOR:** See page 40

## Reproduction toxicity study of Trivalent, Inactivated Subunit-Influenza Vaccine (OPTAFLU)

**Study number 2:** Intramuscular Reproductive and Developmental Toxicity Study of FCC Vaccine in Rabbits, Including a Postnatal Evaluation.

**KEY STUDY FINDINGS:** No significant findings were reported.

**Study no.:** UBA00037

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

**Date of study initiation:** 11/09/2006

**GLP compliance:** Yes

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

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

<u>Test article</u>	<u>Batch/Lot #</u>	<u>Expiration date</u>	<u>Purity %</u>
FCC vaccine	008011	01/31/2007	NR*
Sodium Chloride	J6H649	12/2008	
	J6N607	04/2009	

\* NR = Not reported

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

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

**Number of animal per group and sex:** Ninety-six females. Forty-eight females were assigned to Caesarean-sectioning and 48 were assigned to natural delivery.

**Age:** Approximately 7 months

**Body weight range:** 3.2-4.7 kg

**Route and site of administration:** Intramuscular injection in the hind legs (quadriceps muscle) starting with the right leg.

**Volume of injection:** 0.5 mL

**Frequency of administration and study duration:** Animals (24/group) received two doses on SD's 1, 7, 15, 20, and 29. Terminal sacrifice necropsies were conducted on DG 29 and on DL 29.

**Dose:** Optaflu vaccine has been formulated to contain 45 µg hemagglutinin (HA) per 0.5 mL dose in the recommended ratio of 15 µg HA of influenza type A (H1N1), influenza type A (H3N2), and influenza type B.

### STABILITY SUMMARY

The stability study shows that the FCC vaccine without needle (lot # 522 008 011) is stable for (b)(4) months at (b)(4).

## METHODS

### Study design:

Rabbits were treated 5 times. Animals were treated on study days 1, 15, and 29 at pre-mating and on study days 7 and 20 of presumed gestation. Animals were assigned to 4 different groups and each group contained 24 animals. The details of the study design are listed in the following table:

DOSAGE GROUP	Days 1, 15 and 29 of Study (Premating) (mcg)/Volume (mL) <sup>a</sup>	Days 7 and 20 of Presumed Gestation (Gestation) (mcg)/Volume (mL) <sup>a</sup>	NUMBER OF FEMALES ASSIGNED PER GROUP
1 Rabbits Assigned to Caesarean-sectioning	0/0.5	0/0.5	24
2 Rabbits Assigned to Caesarean-sectioning	45/0.5	45/0.5	24
3 Rabbits Assigned to Natural Delivery	0/0.5	0/0.5	24
4 Rabbits Assigned to Natural Delivery	45/0.5	45/0.5	24

**Table 16: Study design.** <sup>a</sup> The test article was considered 100% active/pure for the purpose of dosage calculations.

<sup>a</sup> On each day of dosage administration, treated rabbits were given intramuscular injections (0.5 mL) of FCC vaccine containing purified influenza virus surface antigens (haemagglutinin and neuraminidase) of three interpandemic influenza strains A/New Caledonia/20/99 IVR-116 (H1N1), A/New York 55/2004x-157, and B/Jiangsu/10/2003 prepared in MDCK cell culture – containing 15 micrograms haemagglutinin per strain, total 45 micrograms antigen per 0.5 mL dose. Control rabbits were given intramuscular injections (0.5 mL) of 0.9% sodium chloride injection, (b)(4) (saline).

### Parameters and endpoints evaluated:

#### F0 generation:

The following parameters were evaluated: Viability observation (twice each day), general appearance (at least once weekly during the acclimation period), clinical observations, abortion, premature deliveries and deaths (before and 60 minutes after dosing and once daily on all other study days), skin irritation (at 24 and 48 hours after dosing), body weight (weekly during the acclimation period, on GD's 0, 7, 10, 13, 16, and 20 during dosing period, on DG's 23, 26, 29, and 34 [when needed], on DL's 1, 5, 8, 11, 15, 18, 22, 25, and 29 [Gs' 3 and 4], and at the time of euthanasia). Food consumption was recorded daily. Rabbits assigned to groups 3 and 4 were evaluated for adverse clinical signs observed during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter sizes (all pups delivered) and pup viability at birth. Maternal behavior was evaluated on DL's 1, 5, 8, 15, 22 and 29. Variations from expected maternal behavior were recorded, if present, on all other days of the postpartum period.

Blood samples for immunogenicity assays were collected from the medial auricular artery (in-life blood collections) or inferior vena cava (terminal blood collection). Blood samples were collected at pre-study and prior to dosage administration on the following days: DS 15 and 29 and DG 7 and 20. In addition, blood samples were collected following euthanasia on DG 29 (groups 1 and 2) or on DL 29 (groups 3 and 4). Following euthanasia, blood samples were collected from the fetuses (groups 1 and 2) and pups (groups 3 and 4).

**F1 generation:**

Day 1 of lactation (postpartum) was defined as the day of birth. Each litter was evaluated for viability at least twice daily. The pups in each litter were counted once daily. Clinical observations were recorded once daily beginning on DL 5. Pup body weights were recorded on DL's 5, 8, 15, 22, and 29 (terminal weight). The following reflex and developmental observations were recorded; hair growth (from DL 5), eye opening (from DL 9), air righting reflex (from DL 10), acoustic (auditory) startle (from DL 14) and pupil constriction was evaluated once (on DL 22). The number of pups meeting the criterion was recorded on each day of testing. Testing continued daily until the day the criterion was attained by all pups in the litter.

Caesarean-section animals (G's 1 and 2): The followings was performed; Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Blood samples were collected and processed. The gravid uterus was excised and weighed. Uteri of apparently nonpregnant does were examined. The number and distribution of corpora lutea were recorded. The uterus of each rabbit was examined for pregnancy status, number and distribution of corpora lutea and implantation sites, and uterine contents (live and dead fetuses and early and late resorptions). An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Nonresponding term fetuses were considered to be dead. However, there were no dead fetuses in this study. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Placentae were examined for size, color and shape.

Fetuses were removed from the uterus and euthanized. Blood samples were collected from each fetus via the vena cava. Samples from each litter were pooled. Each fetus was subsequently weighed and examined for gross external alterations. All fetuses were examined internally to identify sex. Cavitated organs were evaluated in all fetuses by dissection. A single cross-section was made between the parietal and the frontal bones, and the brain was examined *in situ*. All fetuses were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations.

Natural delivery animals (G's 3 and 4):

After completion of the 29-day lactation period, all surviving female rabbits were euthanized and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Blood samples were collected and processed and the number and distribution of implantation sites were recorded.

On postpartum day 29, blood samples were collected from each pup. Blood was collected after euthanasia from the vena cava. Rabbits that did not deliver a litter were euthanized on DG 34 and examined for gross lesions. Uteri of apparently non-pregnant does were examined. Does with no surviving pups were euthanized after the last pup was found dead or missing, presumed cannibalized. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.

F1 Generation Pups:

Pups that died before examination of the litter for pup viability on DL 1 were evaluated for vital status at birth. F1 generation pups were euthanized on DL 29. Blood samples were collected and a gross necropsy was performed. Gross lesions were retained in neutral buffered 10% formalin. The necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. All other tissues were discarded.

**Randomization:** Yes

**Statistical methods:** Yes

## **RESULTS**

### **F<sub>0</sub> generation females:**

#### **Food consumption:**

No test article-related effects on food consumption during pre-mating, gestation, and lactation periods were reported.

#### **Mortality/Clinical signs:**

No test article-related effects on mortality or clinical observations during the pre-mating period were reported.

No test article-related maternal death, abortion, or total litter loss were reported. The animals that died, aborted, or had total litter loss appeared normal throughout the pre-mating period but then had reduced feed consumption and weight loss beginning on DG 13 to 15. Two animals in group 1 had gastric findings (red striations or eroded areas) which it might be associated with stress. Necropsy observations did not indicate any relevant findings that could be attributed to the test article. Peri-natal mortality was 14.3% (6/42) for control rabbits and 15.6% (7/45) for rabbits receiving FCC Vaccine. Total litter losses occurred in one doe in group 2 and one doe in group 4.

In groups 1, 2, 3, and 4, 2, 4, 1 and 2 does, respectively, aborted a litter with no live fetuses or had the liveborn fetuses die before weighing. A total of 2, 5, 4 and 4 litters in the four respective dosage groups consisted of no live conceptuses or of no offspring surviving to weighing. All other does survived to scheduled euthanasia.

Observations	Group 1 n = 24	Group 2 n = 24	Group 3 n = 24	Group 4 n = 24
Abortion/unscheduled euthanasia	1 aborted and 1 unscheduled euthanasia	4 aborted on DG 26, 27, and 28	3 found dead and 1 unscheduled euthanasia	2 aborted and 2 unscheduled euthanasia
Litters	14 dead fetuses; 7 late resorptions	5 dead fetuses and 17 late resorption	7 dead fetuses and 11 late resorption and 3 dead fetuses <i>in utero</i>	26 dead fetuses
Clinical observations	Weight loss, decreased feed consumption, mucoid, scant, discolored and no feces, red perivaginal substance	Erythema at injection site, abnormal feces, red substance in cage pan	Erythema at injection site, abnormal feces, ungroomed coat, yellow periorbital substance, flaking at injection site, and limited use of limbs	Erythema at injection site, abnormal feces, ungroomed coat, lost righting reflex, dehydration and cold to touch, decreased motor activity, red substance in cage pan, and red/orange perivaginal substance/fur
Body weight/Feed	Weight loss and decreased feed consumption	Weight loss and decreased feed consumption	Weight loss and decreased feed consumption	Weight loss and decreased feed consumption
Necropsy	Black and contained dark red gelatinous material in gallbladder, pale liver kidney, and hear, and eroded areas in stomach	Pale liver and heart	Pale liver, kidneys, and heart; red striations in stomach; pancreas, mesentery and abdominal adipose friable; mottled red and dark red spongy lungs; red perinasal substance	Pale liver, green, red and thick walls of bladder (approximately 20 mL of viscous, yellow, red, chalky material in the bladder), abdominal adipose adhered to the bladder, all adipose tissues friable, trichobezoar present in stomach

**Table 17: Summary of observations for does that aborted, died or were euthanized before scheduled euthanasia. n = group size.**

### **Body weight changes:**

No test article-related effects on body weight changes, gravid uterine weight, or corrected body weight during, pre-mating, gestational, and lactation periods were reported. Mean body weight gain of the rabbits in the vaccine treated group assigned to Caesarean section was significantly increased on study days 29 to 36 when compared to the respective control group value. This difference was not considered test article related because: 1) the anticipated adverse effect is reduced body weight gain; 2) the increase was transient; and 3) overall body weight gains during the pre-mating period were comparable among the four dosage groups.

Gestation Period	Group 1 n = 24	Group 2 n = 24	Group 3 n = 24	Group 4 n = 24
Day 1	4.2 ± 0.4	4.1 ± 0.3	4.1 ± 0.3	4.0 ± 0.3
Day 8	4.2 ± 0.4	4.2 ± 0.3	4.1 ± 0.3	4.1 ± 0.3
Day 15	4.3 ± 0.4	4.2 ± 0.3	4.2 ± 0.3	4.1 ± 0.3
Day 22	4.3 ± 0.4	4.3 ± 0.3	4.3 ± 0.3	4.2 ± 0.3
Day 29	4.4 ± 0.4	4.3 ± 0.4	4.3 ± 0.3	4.2 ± 0.3
Day 36	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.2 ± 0.3

**Table 18:** Body weight (kg, mean ± SD). n = group size.

Gestation Period	Group <sup>a</sup> 1 n = 24	Group <sup>a</sup> 2 n = 24	Group <sup>a</sup> 3 n = 24	Group <sup>a</sup> 4 n = 24
pregnant	n = 23	n = 24	n = 19	n = 21
Included in analysis	n = 22 <sup>b</sup>	n = 24	n = 19	n = 21
Day 0	4.4 ± 0.4	4.4 ± 0.4	4.3 ± 0.4	4.2 ± 0.3
Day 7	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.2 ± 0.3
Day 10	4.5 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.3 ± 0.3 <sup>c</sup>
Day 13	4.5 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.3 <sup>c</sup>
Day 16	4.5 ± 0.4	4.4 ± 0.4	4.4 ± 0.5	4.3 ± 0.3 <sup>c</sup>
Day 20	4.4 ± 0.4	4.3 ± 0.4	4.3 ± 0.5	4.3 ± 0.3 <sup>c</sup>
Day 23	4.4 ± 0.4	4.3 ± 0.4	4.3 ± 0.5	4.3 ± 0.4 <sup>c</sup>
Day 26	4.4 ± 0.4 <sup>c</sup>	4.2 ± 0.5	4.3 ± 0.6	4.2 ± 0.4 <sup>c</sup>
Day 29	4.4 ± 0.4 <sup>c</sup>	4.2 ± 0.5 <sup>c</sup>	4.3 ± 0.6 <sup>c,d</sup>	4.3 ± 0.3 <sup>c</sup>
Gravid uterine weight (g)	395.3 ± 124.6 <sup>c</sup>	369.4 ± 140.4 <sup>c</sup>		
Day 29C <sup>e</sup>	3.96 ± 0.4 <sup>c</sup>	3.88 ± 0.5 <sup>c</sup>		

**Table 19: Maternal body weight (kg, mean ± SD). n = group size.**

- a. Dosage occurred on days 1, 15 and 29 of study and days 7 and 20 of gestation.  
b. Excludes values for doe 9519, which did not have a confirmed mating date.  
c. Excludes values for does that were found dead or sacrificed due to adverse clinical observations, abortion.  
d. Excludes value for doe 9571, which delivered on day 28 of gestation.  
e. 29C = Corrected maternal body weight (day 29 of gestation body weight minus the gravid uterine weight).

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Rabbits tested	n = 24	n = 24
Pregnant	n = 19	n = 21
Delivered a litter	n = 17 <sup>b</sup>	n = 16 <sup>b,c</sup>
Included in analyses	n = 15 <sup>d</sup>	n = 15 <sup>d</sup>
Day 1	3.96 ± 0.52	3.94 ± 0.23
Day 5	4.14 ± 0.45 <sup>d</sup>	4.02 ± 0.26
Day 8	4.12 ± 0.45 <sup>d</sup>	4.00 ± 0.31
Day 11	4.09 ± 0.48 <sup>d</sup>	4.00 ± 0.24
Day 15	4.05 ± 0.44 <sup>d</sup>	3.98 ± 0.23
Day 18	4.02 ± 0.44 <sup>d</sup>	3.96 ± 0.22
Day 22	4.04 ± 0.47 <sup>d</sup>	4.00 ± 0.26

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Day 25	4.03 ± 0.46 <sup>d</sup>	4.00 ± 0.28
Day 29	4.00 ± 0.47 <sup>d</sup>	3.93 ± 0.26

**Table 20: Maternal body weight during lactation period (kg, mean ± SD). n = group size.**

- a. Dosage occurred on days 1, 15 and 29 of study and days 7 and 20 of gestation.  
b. Excludes values for does that were found dead during gestation.  
c. Excludes values for doe 9594, which did not deliver a litter and was euthanized on day 34 of gestation.  
d. Excludes values for does that were found dead or euthanized due to no surviving pups.

### Clinical signs and skin reactions:

During the pre-mating period, flaking and erythema grade 1, ungroomed coat, abnormal feces (scant, soft or liquid, none), localized alopecia, lacrimation and red urine were reported. These clinical signs and skin reactions were not considered test article related because: 1) the incidences did not significantly differ from the control group values; 2) the observations occurred in only one or two rabbits; and/or 3) the observation is common in this species and strain of rabbit.

During the gestation period, erythema grade 1, scab at injection site, ungroomed coat, abnormal feces (scant, soft or liquid, none, mucoid, discolored-tan), localized alopecia, decreased motor activity, lost righting reflex, apparent dehydration, cold to touch, yellow periorbital substance, limited use of limbs, orange/red substance on fur and sparse hair coat were reported. These clinical signs and skin reactions were not considered test article-related because: 1) the observations occurred in only one or two rabbits; and/or 2) the observation is common in this species and strain of rabbit. Clinical signs associated in general with an impending abortion or poor clinical condition included red substance in the cage pan (0, 3, 0 and 4 does in the two respective control and vaccine treated groups) and red perivaginal substance (1 doe in the control group assigned to Caesarean-section and 1 doe in the vaccine group assigned to natural delivery).

During the lactation period, sparse hair coat, abnormal feces (scant, soft or liquid, none), ungroomed coat and red perinasal substances were not considered test article-related because the observations did not significantly differ from the control group values.

### Mating and fertility:

No test article-related effects on mating or fertility were reported. Pregnancy in 23, 24, 19, and 21 does were reported in groups 1, 2, 3, and 4, respectively.

Observation	Group <sup>a</sup> 1	Group <sup>a</sup> 2	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Rabbits evaluated (n)	24	24	24	24
Mated rabbits (n)	24(100.0)	24(100.0)	23( 95.8)	23( 95.8)
Mated by male First pairing [n (%)]	20 ( 83.3)	24(100.0)**	21( 91.3)	18( 78.3)
Mated by male Second pairing [n (%)]	3 (12.5)	0 (0.0)	2 (8.7)	4 (17.4)
Mated by male Third pairing [n (%)]	1( 4.2)	0( 0.0)	0( 0.0)	1( 4.3)
Fertility index <sup>b</sup> [n/n (%)]	23/24 ( 95.8)	24/24 (100.0)	19/23 (82.6)	21/23 (91.3)

Observation	Group <sup>a</sup> 1	Group <sup>a</sup> 2	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Rabbits pregnant/rabbits in cohabitation [n/n (%)]	23/24 ( 95.8)	24/24 (100.0)	19/24 (79.2)	21/24 (87.5)

**Table 21: Mating and fertility summary. n = group size.**

a. Dosage occurred on days 1, 15, and 29 of study and days 7 and 20 of presumed gestation.

b. Number of pregnancies/number of rabbits that mated.

\*\* Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 1 and 2.

### Necropsy observation:

Necropsy observations included: friable mesentery, adipose tissue and/or pancreas; pale heart, kidney and/or liver; spongy, mottled red and dark red lungs; numerous eroded areas on mucosal surface of stomach; numerous red striations on mucosal surface of the cardiac region of the stomach; trichobezoar in stomach; tan area on left liver lobe; dark red gelatinous material in gallbladder; clear fluid filled cyst on kidney and a urinary bladder that was adhered to abdominal adipose and had thick, green and red walls, and contained viscous yellow/red chalky material. Because of the low number (one or two does in any dosage group) of these incidents, they were considered not related to test article treatment.

### Caesarean sectioning and litter observations:

Pregnancy rates were 95.8% (23/24) and 100% (24/24) in groups 1 and 2, respectively. Caesarean-sectioning observations were based on 21 and 19 pregnant does with one or more live fetuses in groups 1 and 2, respectively. No test article-related Caesarean-sectioning or litter parameters findings were reported. No significant differences between groups 1 and 2 in the litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were reported. One doe in group 2 had a litter consisting of only resorbed conceptuses. There were no dead fetuses and all placentae appeared normal.

Observation	Group <sup>a</sup> 1	Group <sup>a</sup> 2
Rabbits tested (n)	24	24
Pregnant [n (%)]	23 (95.8)	24 (100)
Unscheduled euthanasia	0	0
Aborted	2 (8.7)	4 (16.7)
Caesarean sectioned on GD 29	21 <sup>b</sup>	20
Corpora lutea	9.2 ± 1.9	8.9 ± 2.5
Implantations	7.5 ± 2.9	7.0 ± 2.8
Litter sizes	7.0 ± 2.6	6.6 ± 2.8
Live fetuses	148 (7.0 ± 2.6)	132 (6.6 ± 2.8)
Dead fetuses	0	0
Resorption	0.4 ± 0.7	0.4 ± 0.7

Observation	Group <sup>a</sup> 1	Group <sup>a</sup> 2
Early resorption	9 (0.4 ± 0.7)	7 (0.4 ± 0.7)
Late resorption	0	0
Does with any resorption [n (%)]	7( 33.3)	5( 25.0)
Does with all conceptuses resorbed [n (%)]	0( 0.0)	1(5.0)
Does with viable fetuses [n (%)]	21 (100.0)	19( 95.0)
Placentae appeared normal <sup>c</sup> [n (%)]	21(100.0)	19(100.0)

**Table 22: Caesarean-sectioning observations (F0 generation)**

a. Dosage occurred on days 1, 15, and 29 of study and days 7 and 20 of gestation.

b. Includes values for doe 9519, which did not have a confirmed mating date.

c. Excludes does with all early resorptions.

### Fetal alterations:

Fetal evaluations were based on 148 and 132 live DG 29 Caesarean-delivered fetuses in 21 and 19 litters in groups 1 and 2, respectively. Fetuses were examined for gross external, soft tissue and skeletal alterations and fetal ossification site averages.

No test article-related gross external, soft tissue, or skeletal fetal alterations (malformations or variations) were reported. There were no significant differences in the litter or fetal incidences of any gross external, soft tissues or skeletal alterations. No differences in the ossification site averages between groups 1 and 2 were reported.

Litters with fetuses with alterations numbered 15 (71.4%) and 13 (68.4%) were reported in groups 1 and 2, respectively. Fetuses with alterations observed were 38 (25.7%) and 32 (24.2%), and the percentages of fetuses with any alteration per litter were 24.0% and 22.2% in groups 1 and 2, respectively.

Observation	Group <sup>a</sup> 1	Group <sup>a</sup> 2
Litters with one or more live fetuses (n)	21 <sup>b</sup>	19
Implantations	7.5 ± 2.9	7.3 ± 2.5
Live fetuses	148 (7.0 ± 2.6)	132 (6.9 ± 2.4)
Live male fetuses	84	66
% Live male fetuses/litter	57.5 ± 19.6	50.1 ± 24.6
Live fetal body weights (grams)/litter	39.76 ± 9.08	39.75 ± 9.58
Males fetuses	39.92 ± 9.67	40.20 ± 8.73 <sup>c</sup>
Female fetuses	38.20 ± 8.85 <sup>d</sup>	37.91 ± 10.13 <sup>d</sup>
% resorbed conceptuses/litter	4.7 ± 8.0	3.5 ± 7.8

**Table 23: Caesarean-delivered fetuses [litter observations (F1 generation fetuses)]**

a. Dosage occurred on days 1, 15, and 29 of study and days 7 and 20 of gestation.

- b. Includes values for litter 9519; the doe did not have a confirmed mating date.
- c. Litter 9543 had no male fetuses.
- d. Litters 9520 and 9527 had no female fetuses.

**Fetal gross external alterations:**

Body and limbs: One fetus in group 1 had a thread-like tail with four caudal vertebrae present at skeletal examination and internasal ossification site. One fetus in group 2 had left forepaw flexed downward. An unossified hyoid body, fused 3rd to 4th sternal centra, small manubrium, and the presence of a small ossification site within the suture of the nasal were reported in this (skeletal examination) fetus.

*Fetal soft tissue alterations:*

Malformations and variations: Absence of innominate artery and the right subclavian arose to the left of the left subclavian and passed dorsal to the trachea and esophagus were reported in one fetus of group 2. Three fetuses (from one doe) of group 1 were reported with absence of the intermediate lobe of the lung. Skeletal examination of 2 fetuses revealed displaced (left) midline nasal suture.

*Fetal skeletal alterations:*

Malformations: One fetus in group 1 was reported with interrelated vertebral/rib malformations (including thoracic hemivertebrae, bifid centrum, fused ribs). One fetus in group 2 was reported with fused ribs too.

Variations: In 21 group 1 fetuses from 11 litters and 13 group 2 fetuses from nine litters, common small irregularities in ossification of the skull (the presence of small ossification sites within the sutures of the nasal or frontal bones and/or displaced midline nasal suture) were reported. One fetus in group 1 had a thread-like tail and only four caudal vertebrae. One fetus in group 1 had fused 3rd and 4th sternal centra and sibling fetus had misaligned 16th caudal vertebra. One fetus in group 2 had additional external alterations and not ossified hyoid body, fused 3<sup>rd</sup> to 4<sup>th</sup> sternal centra, and a small manubrium.

Hyoid: Small hyoid bodies were reported in 3 fetuses from 2 litters in group 2. No ossified hyoid bodies were reported in 8 fetuses from 5 group 1 litters and 4 fetuses from four group 2 litters. Angulated hyoid ala was reported in 1 group 1 fetus. Incomplete ossified 1<sup>st</sup> sternal centrum were reported in 5 group 1 fetuses and 2 group 2 fetuses. Additional skeletal variations were reported in 1 group 2 fetus. Angulated ala of the hyoid occurred in 1 and 3 fetuses from 1 and 2 litters from the control and test article treated groups, respectively. No ossified hyoid body was reported in group 1 fetus.

Vertebrae: Misaligned caudal vertebra was reported in 1 group 1 fetus and 2 group 2 fetuses. A bifid centrum in a caudal vertebra reported in 1 fetus in group 2. Only 4 caudal vertebrae (an observation associated with the external

observation of a thread-like tail) was reported in group 1 fetus. An internasal ossification site was also reported in this fetus.

Sternum: Fused sternal centra occurred in 1 in each of groups 1 and 2 fetuses. Small manubrium and additional alterations were reported in 1 fetus of group 2. The manubrium was also small in 1 fetus in group 1. Delayed sternal ossification (incompletely ossified 1st sternal centrum) was reported in 11 and 8 fetuses from 6 and 6 litters in groups 1 and 2, respectively.

Fetal ossification site averages: No test article-effects on the average numbers of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium, sternal centers and xiphoid), forelimbs (carpals, metacarpals, digits and phalanges) or hindlimbs (tarsals, metatarsals, digits and phalanges) were reported. Significant increase in the average number of sternal centers and xiphoid per fetus per litter was reported in group 2 when compared to control group.

#### **Natural delivery observations:**

In groups 3 and 4, pregnancy occurred in 19/23 and 21/23 of the mated female rabbits, respectively. In each group, 16 does delivered litters. Natural delivery observations were unaffected by test article administration. No test article-related effects on the number of does delivering litters, the duration of gestation, averages for implantation sites per delivered litter and live litter size, the gestation index (number of does with one or more liveborn pups/number of pregnant rabbits), the number of does with stillborn pups and of does with all pups dying during lactation, lactation indices, or pup sex ratios, litter size, and body weights were reported.

Statistically significant reductions in the numbers of pups found dead or euthanized due to adverse clinical observations on day 1 postpartum and number of pups that were stillborn in group 4 were reported. This reduction was not considered test article-related because the number of pups found dead/euthanized in group 4 was less (N=1) than the number observed in group 3 (N=6) and the number of stillborn pups in group 4 was less (N=3) than the number observed in group 3 (N=11). As a result, the viability index in group 4 (99.0%) was greater than that of group 3 (93.6%) and the number of liveborn pups on day 1 postpartum in group 4 was greater than the number of pups in group 3. The lactation index in group 4 (89.20%) was less (not statistically significant) than that of group 3 (97.7%). The number of live pups in group 4 on day 5 postpartum was greater than the number of pups in group 3.

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Animal # (n)	24	24
Pregnant [n (%)]	19 (79.2)	21(87.5)
Included in analysis (n)	18 <sup>b</sup>	21
Delivered a litter [n (%)]	16 (88.9)	16 (76.2) <sup>c</sup>
Duration of gestation <sup>d</sup>	32.2 ± 1.2	32.4 ± 0.9

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Animal # (n)	24	24
(Mean±S.D.)		
Implantation sites per delivered litter (Mean±S.D.)	128 8.0 ± 2.4	128 8.0 ± 1.8
Does with stillborn pups [n (%)]	3 (18.8)	3 (18.8)
Does with no liveborn pups	0 (0.0)	1 (6.2)
Gestation index <sup>e</sup> % n/n	88.9 16/ 18	71.4 15/ 21
Does with all pups dying (days 1-4 postpartum) [n (%)]	1 (6.2)	0 (0.0)
Does with all pups dying (days 5-21 postpartum) [n (%)]	0 (0.0)	0 (0.0)

**Table 24: Natural delivery observations (F0 generation)**

- a. Dosage occurred on days 1, 15, and 29 of study and days 7 and 20 of gestation.  
b. Excludes values for doe 9553, which was found dead before delivery was completed on day 1 of lactation.  
c. Excludes values for doe 9594, which did not deliver a litter and was euthanized on day 34 of gestation.  
d. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.  
e. Number of rabbits with live offspring/number of pregnant rabbits.

#### **Clinical observations (F1 generation pups):**

No test article-related effect on the clinical observations in pups (birth to DL 29) was reported. No milk in the stomach, not nesting or nursing, cold to touch and/or apparent dehydration, and/or reduced maternal care were the findings associated with pup deaths.

Scabs or purple/black discoloration on various areas of the body, cold to touch, not nursing (no milk band present), not nesting, swollen hindlimbs/hindpaw(s) and head tilt (right) were the transient clinical signs reported. Persistent clinical signs were included: missing/short left pinna, one digit present on left forelimb (other digits presumed cannibalized) and short 1st and 2nd digit on right hindpaw.

#### **Reflex and physical development - F1 generation pups:**

No test article-related effect on hair growth, eye opening, air righting, acoustic startle, and pupil constriction were reported in the F1 generation pups. Eye opening in pups that met the criterion was significantly reduced in group 4 on day 9 postpartum. The criterion day (average day that at least 50% of all pups in a litter met the criterion) was significantly increased in group 4 when compared to group 3 values. Because the number of pups that met the criterion on days 10 to 13 postpartum was comparable between the groups 3 and 4 and there were no other apparent delays in physical development in the other evaluated physical development parameter (e.g., hair growth), these differences were not considered related to test article treatment. Because the criterion day was comparable between group 3 and group 4, the statistically significant difference in the number of pups that met criterion for air righting on day 11 postpartum was not considered test article-related.

**Necropsy observations - F1 generation pups:**

No test article-related effect on necropsy observations were reported in the F1 (birth to DL 29) generation pups. Moderate dilation of the ventricle of the brain in a single group 4 pup and accessory spleen in one group 3 pup was reported. No milk present in stomach was the only necropsy observation that occurred in pups that were found dead or stillborn.

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Delivered litters with one or more liveborn pups (n)	16 <sup>b</sup>	15
Pups delivered [n (Mean±S.D.)]	113 (7.1 ± 2.6)	106 (7.1 ± 2.2)
Liveborn [n (%)] (Mean±S.D.)	102 (90.3) (6.4 ± 2.6)	103 (97.2)* (6.9 ± 2.4)
Stillborn [n (%)] (Mean±S.D.)	11 (9.7) 0.7 ± 2.0	3 (2.8)* 0.2 ± 0.6
Pups found dead, unscheduled euthanasia or presumed cannibalized		
Day 1 [n/n (%)]	6/ 94 ( 6.4) <sup>c</sup>	1/103 (1.0)*
Day 6-8 [n/n (%)]	2/ 88 (2.3)	7/102 (6.9)
Day 9-15 [n/n (%)]	0/ 86 (0.0)	2/ 95 (2.1)
Day 16-22 [n/n (%)]	0/ 86 (0.0)	0/ 93 (0.0)
Day 23-29 [n/n (%)]	0/ 86 (0.0)	0/ 93 (0.0)
Viability index <sup>d</sup> [n/n (%)]	88/ 94 <sup>c</sup> (93.6)	102/103* (99.0)
Lactation index <sup>e</sup> [n/n (%)]	86/ 88 (97.7)	91/102* (89.2)

**Table 25: Natural delivery pups observations (F1 generation)**

- a. Dosage occurred on days 1, 15, and 29 of study and days 7 and 20 of gestation.  
b. Excludes values for litter 9553; the doe was found dead prior to completion of delivery on day 1 of lactation.  
c. Excludes values for litter 9557; which doe was found dead on day 1 of lactation.  
d. Number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum.  
e. Number of live pups on day 29 postpartum/number of live pups on day 5 postpartum.  
\* Significantly different from the group 3 value ( $p \leq 0.01$ )

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Litters evaluated (n)	17	16
Total pups stillborn or found dead <sup>b</sup> (n)	21	14
Stillborn (n)	11	2
Found dead	8	11
Unscheduled euthanasia (n)	2	1
No milk in stomach <sup>c</sup> [n (%)]	8 (100)	10 (90.9)
Pups sacrificed and necropsied on day 1 or 29 postpartum		
Litters evaluated (n)	15	15
Pups evaluated (n)	89	91
Appeared normal		
Litter incidence [n (%)]	14 (93.3)	14 (93.3)
Pup incidence [n (%)]	88 (98.9)	90 (98.9)
Brain, ventricle, moderate dilation		
Litter incidence [n (%)]	0 (0.0)	1 (6.7)
Pup incidence [n (%)]	0 (0.0)	1 (1.1)

Observation	Group <sup>a</sup> 3	Group <sup>a</sup> 4
Litters evaluated (n)	17	16
Accessory spleen		
Litter incidence [n (%)]	1 (6.7)	0 (0.0)
Pup incidence [n (%)]	1 (1.1)	0 (0.0)

**Table 26: Necropsy observations (F1 generation pups)**

- a. Dosage occurred on days 1, 15, and 29 of study and days 7 and 20 of gestation.
- b. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full evaluation.
- c. Analysis restricted to pups found dead and necropsied.

**Immunology:**

Blood samples for immunogenicity assays were collected from the medial auricular artery (in-life blood collections) or inferior vena cava (terminal blood collection). Blood samples were collected at pre-study and prior to dosage administration on the following days; DS 15 and 29 and DG 7 and 20. In addition, blood samples were collected following euthanasia on DG 29 (groups 1 and 2) or on DL 29 (groups 3 and 4). Following euthanasia, blood samples were collected from the fetuses (groups 1 and 2) and pups (groups 3 and 4). To detect antibodies against interpandemic (influenza A/New Caledonia/20/99-like strain) influenza strain in rabbits’s serum, the hemagglutination inhibition assay (HAI) was used. The geometric and group mean antibody levels are summarized in the following tables:

Observation Day	C-sectioning Range of Individual HAI Titers  Group1	C-sectioning Range of Individual HAI Titers  Group 2	Natural Delivery Range of Individual HAI Titers  Group 3	Natural Delivery Range of Individual HAI Titers  Group 4
Pre-study	-----	<10	-----	-----
Observation day 15	-----	<10 – 40 <sup>a</sup>	-----	-----
Observation day 29	-----	320	-----	-----
Gestation day 7	-----	269	-----	-----
Gestation day 20	-----	519	<10	367
Gestation day 29	<10	226	-----	-----
Pooled fetal samples (Gestation day 29)	-----	293	-----	-----
Lactation day 29	-----	-----	<10	57
Pup samples (Lactation day 29)	-----	-----	-----	<10 – 80 <sup>a</sup>

**Table 27: Immunology results (geometric mean)**

<sup>a</sup> Range of titers

---- Samples collected but not tested

The groups mean antibody levels are summarized in the following table:

Observation Day	C-sectioning Range of Individual HAI Titers Group1	C-sectioning Range of Individual HAI Titers Group2	Natural Delivery Range of Individual HAI Titers Group 3	Natural Delivery Range of Individual HAI Titers Group 4
Pre-study	-----	<10 – <10 <sup>a</sup>	-----	-----
Observation day 15	-----	<10 - 40	-----	-----
Observation day 29	-----	160 - 1280	-----	-----
Gestation day 7	-----	80 - 1280	-----	-----
Gestation day 20	-----	160 - 1280	<10 – <10	160 - 1280
Gestation day 29	<10 – <10	40 – 640	-----	-----
Pooled fetal samples (Gestation day 29)	-----	80-1280 <sup>b</sup>	-----	-----
Lactation day 29	-----	-----	<10 – <10	40 - 160
Pup samples (Lactation day 29)	-----	-----	-----	<10 – 80 <sup>c</sup>

**Table 28: Immunology results (groups mean [OD])**

<sup>a</sup> Range of titers, and where applicable, the mean range of their fetuses or pups.

<sup>b</sup> Range mean of titers from n = 8 pools, each pool made up of sera from fetuses from a single C-sectioned doe

<sup>c</sup> Mean of n = 6 to 9 individual pups

---- Samples collected but not tested

HAI titers were measurable beginning on study day 15 (after one injection of FCC Vaccine) in three of the eight rabbits that were assayed. HAI titers were measurable in all rabbits on study day 29. Immune response to the vaccine was increased and/or remained elevated over the duration of the study [either DG 29 (group 2) or DL 29 (group 4)]. Anti-influenza antibodies were detected in all fetal pooled samples of group 2 does (at levels comparable to those of the respective maternal sample) at the time of Caesarean sectioning. At the time of euthanasia (29 days after birth), antibodies remained elevated in all group 4 pups although titers were lower than those of group 2 fetuses.

Study #1:

**Test article related effects are listed in the table below**

Test article related effects	Effects considered incidental*
Injection site reactions	Increase in creatine kinase
Increase in total bilirubin	Decrease in LDH, AST, and ALT
Increase in reticulocytes	Decrease in lymphocytes
Increase in neutrophils and basophils	Decrease in lungs, heart, thymus, adrenals, spleen, and ovary weights

**Assessment:**

The elevation of creatine kinase levels is considered part of the expected mechanism of toxicity due to transient skeletal muscle damage at the site of immunization due to the means of administration.

Except for the increases in total bilirubin, reticulocytes, neutrophils and basophils, 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 increases in neutrophils and basophils count are not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Other than minimal to slight necrosis and slight hemorrhage at the sites of injection which was attributable to recovery of trauma due to injection, there were no treatment-related effects on histopathology and any histopathology findings were considered as incidental to the study and not related to the test article.

No treatment-related, mortality, nor any toxicologically relevant changes in body weight, clinical signs, relative food consumption, ophthalmoscopic parameters, or body temperature were found.

Based on the overall findings in this study, it can be concluded that in -----(b)(4)--  
----- rabbits repeated intramuscular administration of OPTAFU vaccine had no adverse effects in terms of systemic toxicity and local tolerance at the dose level of 45 µg antigen.

Immunology performed in this study verified that an active dose was administered.

**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 preclude the BLA from going into effect.

Study #2:

**SUMMARY:**

The objective of this study was to evaluate the effect of trivalent, inactivated subunit-influenza vaccine, administered intramuscularly, on pregnant and lactating rats and on the developing offspring during the first pregnancy. Animals (24/group) were assigned to 4 different groups and treated by 0.5 mL intramuscular injections of control or test article. Test article was administered at two doses on SD's 1, 7, 15, 20, and 29. Terminal sacrifice necropsies were conducted on DG 29 and on DL 29.

Serum samples for immunology were collected from dams at pre-study and prior to dosage administration on the following days; DS's 15 and 29 and DG's 7 and 20. Serum samples were also collected following euthanasia on DG 29 (groups 1 and 2) or on DL 29 (groups 3 and 4). Following euthanasia, blood samples were collected from the fetuses (groups 1 and 2) and pups (groups 3 and 4).

Clinical symptoms, mortality, body weight, necropsy findings, skin irritation, food consumption, duration of gestation, litter size, and pup viability at birth were evaluated. Pups were weighed and developmental landmarks (surface-righting reflex, hair growth, acoustic startle, and pupil constriction) were evaluated. Gross necropsy was performed on litter, pups, and fetuses. Blood was collected from the litters, pups, and fetuses for immunology evaluation.

*Caesarian sectioning:*

No test article-related effects on mortality, clinical observations, skin reactions, body weight, body weight changes, or food consumption during the pre-mating, gestation, and lactation periods were reported. Gravid uterine and corrected body weights for the Caesarean-section animals were comparable between the control and treated groups. No test article-related effects on mating and fertility indices were reported. No test article-related effects on necropsy findings were reported. No test article-related effects on pregnancy rates or litter parameters (corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses) were reported. No test article-related effects on gross external, soft tissue, skeletal fetal alterations, or ossification site averages were reported.

No test article-related effects on fetal gross external alterations (body and limbs), fetal soft tissue alterations (malformations and variations), fetal skeletal alterations (Vertebrae/ribs, skull, hyoid, vertebrae, and sternum) were reported.

No test article-related effects on ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium, sternal centers, and xiphoid), forelimbs (carpals, metacarpals, digits, and phalanges) or hindlimbs (tarsals, metatarsals, digits, and phalanges)

were reported. Significant increase in the average number of sternal centers and xiphoid per fetus per litter was reported in group 2.

*Natural delivery:*

No test article-related effects on pregnancy rates, number of litters delivered, or delivery observations (values for the number of does delivering litters, the duration of gestation, averages for implantation sites per delivered litter and live litter size, the gestation index [number of does with one or more liveborn pups/number of pregnant rabbits], the number of does with stillborn pups and of does with all pups dying during lactation, lactation indices, pup sex ratios, and litter size and body weights) were reported. No test article-related effect on viability index was reported. Lactation index in group 4 (89.2%) was less than that in group 3 (97.7%). This reduction was not statistically different.

*F1 generation pups:*

No test article-related effect on the pups' clinical observations, hair growth, eye opening, air righting, acoustic startle, pupil constriction, and necropsy observations were reported.

All rabbits in group 2 showed measurable HAI titers on study day 29. Immune response to the vaccine was increased and/or remained elevated over the duration of the study [either DG 29 (group 2) or DL 29 (group 4)]. In all fetal-pooled samples of group 2 does, anti-influenza antibodies were detected at the time of Caesarean sectioning. Antibodies remained elevated in all group 4 pups (although titers were lower than those of group 2 fetuses) at 29 days after birth.

## **CONCLUSIONS**

The administrations of trivalent influenza vaccine on SD's 1, 7, 15, 20, and 29 at 500µL via the intramuscular route did not give an indication of F<sub>0</sub> and F<sub>1</sub> generations toxicity. Serological analysis data indicated a robust antibody response in dams and conferred passive immunity to their litters.

## **Communications to sponsor:**

You have used SOP 101076-01 to determine the serology of strains A/New Caledonia (H1N1), A/ Panama (H2N3) by (b)(4), and B/ Guangdong by (b)(4), please indicate the type of analysis performed and associated performance parameters such as sensitivity, lower limit of quantitation, and reproducibility.

For study number 1 above, please provide the site/s of blood collection for clinical chemistry, hematology, and coagulation determinations.

In study number 1 above, results for strain A/Panama (H2N3) by (b)(4) showed an increase in the titer levels in all treated groups including the control. However, control group animals were not supposed to have been exposed to any test article. Cross contamination with the test article or with samples taken from

animals treated with the test article might be the cause of these results. Strict laboratory procedures (e.g., gloves must be changed when processing samples for each group) should be followed to prevent this kind of cross contamination. To ensure the scientific integrity of future studies, we recommend that you institute procedures to preclude the potential for cross contamination with test articles.

**Concurrence:** Martin D. Green