

## **Toxicology Review of Dengvaxia**

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**Through:** Martin Green

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4.2.3.2 Repeated dose toxicity studies  
4.2.3.5. Reproductive and Developmental Toxicity

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**Sponsor:** Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA, USA

**Proposed indication:** Prevention of dengue disease caused by dengue virus serotypes 1,2,3 and 4 in individuals 9-16 years of age with lab-confirmed previous dengue infection and living in endemic areas.

**Division name:** OVRR/DVRPA

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## EXECUTIVE SUMMARY:

For the nonclinical safety evaluation the CYD dengue vaccine was evaluated in a general repeat dose toxicity study in monkeys (review included in this document), distribution, persistence and shedding studies (reviewed by the CMC reviewer); studies evaluating the viscerotropism, neurotropism and neurovirulence (reviewed by the CMC reviewer) as well as developmental and reproductive toxicity (DART) studies which included two immunogenicity/viremia studies, two investigational and two pivotal reproductive developmental toxicity studies in mice and rabbits as well as a lactation study in mice (reviews included in this document).

CYD dengue vaccine was evaluated in a repeat-dose toxicity study in the monkey in which systemic toxicity and local tolerance were assessed after three full human dose SC administrations of Phase II vaccine lot. The vaccine was well tolerated, and no vaccine related systemic or local toxicity was identified. Safety endpoints supportive of systemic and local toxicity evaluation, were also included as part of the biodistribution and shedding study following one SC injection of the Phase III vaccine lot, in the neurovirulence study following one IC injection of Phase I vaccine lot and in all immunogenicity studies in the monkey (reviewed by the CMC reviewer).

In the immunogenicity/viremia studies and the investigative, preliminary dose-range data, the rabbit and the mouse were confirmed as models for DART studies with a robust antibody response in the rabbit and detectable viremia in the mouse after intravenous administration. The rabbit was therefore selected for the evaluation of the effects of the antibody response, but not the viremia. The mouse was selected for the evaluation of the effects of the viremia, but not the antibody response. In the rabbit, no indication of maternal systemic toxicity, no test article-effects on mating performance and fertility, and no indication of teratogenic potential of the test vaccine as well as no effect on pre and post-natal development of the pups were reported when a full human dose was administered twice before mating and three times during gestation. The mouse was selected to investigate the exposure to the virus after one IV injections at a dose of 5 (one full human dose), 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on Day of Gestation (DG) 6, 9 or 12. The doses of 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> induced reductions in maternal body weight gains and food consumption and increases in post-implantation loss. The most pronounced effects occurred in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 and were associated with reduced fetal body weights in litters of females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 or 12. Changes at the fetal examination were limited to delays in skeletal ossification at 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> of CYD Dengue vaccine where reductions in the fetal body weights and maternal toxicity occurred, but no fetal abnormalities. At 5 log<sub>10</sub> CCID<sub>50</sub> CYD Dengue vaccine, there were no changes of toxicological significance.

One intravenous injection of CYD Dengue vaccine at 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> in lactating mice on lactation day 14 was generally tolerated with treatment-related effects limited to a transient body weight loss on the day after injection in females given 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> and no treatment-related changes in litter parameters at any dose.

## INTRODUCTION:

Dengue which is transmitted primarily by the *Aedes aegypti* mosquito, is an acute, systemic viral infection caused by 4 closely related but antigenically distinct virus serotypes {1, 2, 3, and 4}

(1). Dengue is spreading globally during the past 30 years because of changes in human ecology  
 (2). Dengue inflicts a significant public health, economic and social burden on the populations of endemic areas. Half of the world's population is now considered at risk of infection by the dengue viruses. Every year, an estimated 390 million dengue infections occur worldwide, of which around 100 million are associated with clinical manifestation of dengue. The available prevention of dengue by vector control, up to the end of 2015, has proven to be of limited success (3, 4). As recognized by the World Health Organization (WHO), there is an urgent need to develop a safe and effective vaccine against the four serotypes of dengue virus to protect people in endemic countries (5, 6).

Dengue fever is an acute febrile illness usually non-specific, accompanied by headache, myalgia, and a rash. After an incubation period of 2-7 days, there is a sudden onset of fever, chills, frontal and retro orbital headache, myalgia, back pain and abdominal distress. A rash commonly appears after 3-4 days with defervescence and petechiae may become evident on the limbs. In the presence of peptic ulcer disease, some minor bleeding from the nose, gums, and gastrointestinal tract and severe spontaneous bleeding may occur.

Most patients recover following a self-limiting non-severe clinical course. However, a small proportion progress to severe disease (DHF/DSS) via a pathophysiological host response to infection leading to vascular permeability, plasma leakage, microvascular bleeding and reduced functioning of the coagulation cascade. This is defined by one or more of the following: (i) plasma leakage that may lead to shock and/or fluid accumulation (DSS), and/or (ii) severe bleeding, and/or (iii) severe organ impairment (liver, CNS, heart). In <1% of infections, these severe manifestations may involve bleeding of multiple organs and fluid accumulation within body cavities. The decreased severity (including mortality) of dengue disease might be related to the improvement of dengue case detection, identification of warning signs, and early initiation of appropriate treatment (7).

Dengue virus could be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days after the onset of illness. Virus isolation, nucleic acid, or antigen detection could be used to diagnose the infection during the early stages of the disease. Serology is the method of choice for diagnosis at the end of the acute phase of infection (7).

CYD dengue vaccine (contains serotypes 1, 2, 3, and 4, live attenuated) is a prophylactic, tetravalent, live attenuated viral vaccine. Each monovalent CYD dengue virus was obtained separately via recombinant deoxyribonucleic acid (DNA) technology. The CYD dengue viruses were constructed by replacing the gene encoding the pre-membrane (prM) and envelope (E) proteins of the structural proteins in the attenuated yellow fever (YF) 17D virus genome by the corresponding genes of the 4 wild type dengue serotypes 1, 2, 3 and 4. Of each live, attenuated, dengue serotype 1, 2, 3 and 4 viruses, the final formulation contains ~5 log<sub>10</sub> cell-culture

infectious dose 50% (CCID<sub>50</sub>)<sup>1</sup>. The CYD dengue vaccine, initially developed as (b) (4) batches, is a sterile and freeze-dried product to be reconstituted before injection with a sterile solution of 0.4% sodium chloride. After reconstitution, one dose (0.5 mL) is to be administered by the subcutaneous (SC) route.

No adjuvant or preservatives is included in the CYD dengue vaccine. In the manufacturing process of the CYD dengue virus seed lot system, vero cell banking system and CYD drug substance (DS) and drug product (DP), no material of biological origin (animal or human origin) was used. No material of porcine origin was included in the CYD dengue vaccine.

## OVERVIEW OF THE CLINICAL DEVELOPMENT PROGRAM:

In accordance with guidance from the European Medicines Agency (EMA) (8) and the WHO (5, 9, 10), the CDP of the CYD dengue vaccine was initiated in 2002. To characterize the dengue vaccine in terms of efficacy, safety and immunogenicity profiles, when assessed in different regions, in different age groups and in populations with various degrees of endemicity, from highly endemic to non-endemic was the objectives of the CDP. The efficacy of the CYD dengue vaccine was assessed in endemic areas in one proof of concept (PoC) Phase IIb monocenter study (CYD23 conducted in Thailand) and 2 pivotal Phase III studies performed in 10 countries of southeast Asia Pacific (AP) and Latin America (LatAm) (CYD14 in AP and CYD15 in LatAm). The majority of studies were randomized, controlled, and at least blind-observer studies. All serology testing was performed in a blinded manner. Over the duration of the study, during the long-term follow-up of the efficacy studies, investigators and subjects remain blinded to group allocation. Vaccine virus replication was assessed in relation to the safety profile of the vaccine in selected clinical studies. The clinical development was conducted in a stepwise approach with respect to the age of subjects included in the studies, as well as the regions (non-endemic, then endemic) in which they were conducted.

As of December 2017, the CDP included 31 clinical studies, completed {22} or on-going {9}: 5 Phase I, 17 Phase II, and 9 Phase III. A total of 26 clinical studies for which results are available are submitted in this application. A total of more than 41,000 subjects have been enrolled in the clinical studies. These studies included more than 28,500 subjects from 9 months through 60 years of age exposed to at least one injection of the final tetravalent CYD dengue vaccine formulation, regardless of the administration schedule. Among these subjects, 20,974 subjects were aged 9 years through 45 years and received at least one injection of the final formulation of the CYD dengue vaccine, regardless of the schedule. Sixteen out of 26 studies are considered as the main studies. No results are available for CYD56 (a study conducted in non-endemic areas), and for CYD65, CYD66, CYD67, and CYD71 ongoing trials.

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<sup>1</sup> The infectious titers specifications ranged from 4 to 6 log<sub>10</sub> CCID<sub>50</sub> for Phase II lots and from 4.5 to 6 log<sub>10</sub> CCID<sub>50</sub> for Phase III lots.

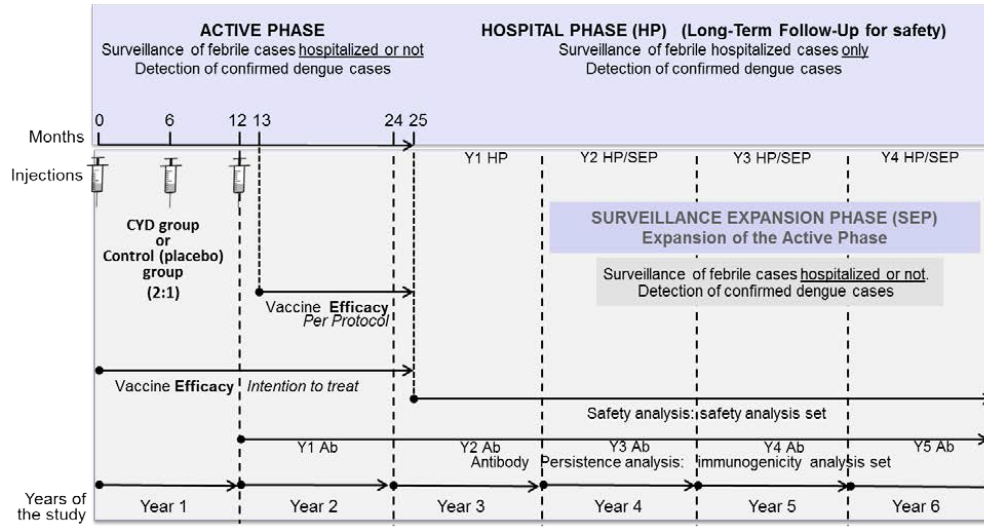


Figure 1: Outline of CYD14 and CYD15 trial design and important timelines

## STABILITY SUMMARY:

(b) (4) storage stability data have been generated for the drug substance manufactured at  
(b) (4)

## **TOXIXITY STUDIES SUBMITTED TO SUPPORT THIS BLA:**

### **General repeat-dose toxicity studies:**

- A Safety Study of Tetravalent ChimeriVax™-DEN Vaccine Following Multiple Subcutaneous Administrations to (b) (4) Monkeys (Study # RQH00006 )

### **Reproductive and developmental toxicity studies and supportive studies:**

#### **Reproductive and developmental Toxicity: immunogenicity studies**

- CYD Dengue Vaccine - Immunogenicity and Viremia Study Following Repeated Intravenous or Subcutaneous Injections in the Rabbit (Study number: 906)
- CYD Dengue Vaccine - Immunogenicity and Viremia Study Following One Intravenous or Repeated Subcutaneous Injections in the Mouse (Study number: 907)

#### **Reproductive and developmental toxicity: investigative Studies**

- CYD Dengue Vaccine – Preliminary Developmental Toxicity Study in the (b) (4) Rabbit Following Three Intravenous Injections (Study number: 1002)
- CYD Dengue Vaccine – Preliminary Development Toxicity Study in (b) (4) Mice Following One Intravenous Injection (Study number: 1003)

#### **Reproductive and developmental toxicity - fertility and early embryonic development to implantation (pivotal)**

- CYD Dengue Vaccine: Developmental and Reproductive Toxicity Study in (b) (4) Rabbits Following Repeated Intravenous Administrations (Study number: 1013)
- CYD Dengue Vaccine: Developmental and Reproductive Toxicity Study in (b) (4) Mice Following One Intravenous Administration (Study number: 1014)

#### **Lactation toxicity study in mice**

- CYD Dengue Vaccine: Lactation Study in (b) (4) Mice Following One Intravenous Administration (Study number: 1109)

**GENERAL TOXICOLOGY STUDY REVIEWS:****A Safety Study of Tetravalent ChimeriVax™-DEN Vaccines Following Multiple Subcutaneous Administrations to (b) (4) Monkeys (Study # RQH00006 )****Reviewer:** *Nabil Al-Humadi*

Performing laboratory: (b) (4)

Initiation date: February 6, 2006

Final report date: November 2, 2006

**Batch/lot number of test article:**

Test Article	Lot No.	Concentration of Live ChimeriVax™-Dengue Serotypes
Tetravalent ChimeriVax™-Dengue 1, 2, 3 and 4 Vaccines	TV5555	1 x 10 <sup>5</sup> TCID50/serotype/mL (for serotypes 1, 2, 3, and 4)

(b) (4)  
(b) (4)

(b) (4)

(b) (4)  
(b) (4)(b) (4)  
(b) (4)  
(b) (4)(b) (4)  
(b) (4)(b) (4)  
(b) (4)Diluent Mixture (b) (4)  
of Water for Injection,  
(b) (4)

Not reported

Animal species and strain: (b) (4) monkeys (b) (4)

Breeder/supplier: Imported from China and obtained via (b) (4)  
(b) (4), on December 20, 2005.

Number of animal per sex per group: 12 per sex per group

Age: 3.5 to 4.5 years of age for the males and 2.9 to 4.9 years of age for the females

Body weight range: 2.9 to 3.8 kg for the males and 2.5 to 3.6 kg for the females at day-2

Route and site of administration: Subcutaneous

Volume of administration: 0.5 mL/dose

Frequency of administration and study duration: Monkeys in groups 1 and 4 received 3 injections {1 injection/day on days 1, 27, and 55} of the tetravalent vaccine mixture and the control article, respectively. Monkeys in group 2 received 2 injections of tetravalent vaccine mixture, 1 injection/day on days 1 and 27. Monkeys in group 3 received 1 injection of a (b) (4) of (b) (4) vaccines on day 1, and 1 injection of a (b) (4) of (b) (4) vaccines on day 27.

Dose/animal: See study design

Stability: According to the stability analysis, the infectious titer of 4 filled products remained stable after a storage of 13 weeks at a temperature (b) (4).

Means of administration: Subcutaneous

Report status: Final

## Methods:

### Study design

Animals were randomized and assigned to 4 different groups. Each group consisted of 3 animals/sex. Animals were dosed by subcutaneous route. The details of the study design are listed in the following table:

*Table 1: Experimental design:*

Group No.	Set	Number of Monkeys (M/F) <sup>1</sup>	Dose	Concentration <sup>2</sup> (TCID <sub>50</sub> /mL)	Number of Injections
1	B	3/3	Tetavalent ChimeriVax™-Dengue Vaccine	Den1 1 x 10 <sup>5</sup> Den2 1 x 10 <sup>5</sup> Den3 1 x 10 <sup>5</sup> Den4 1 x 10 <sup>5</sup>	3
2	A	3/3	(b) (4)	(b) (4)	2
3	A	3/3	(b) (4) (b) (4)	(b) (4)	2
4	B	3/3	Diluent (Control)	0.0	3

<sup>1</sup> Males/Females; <sup>2</sup> TCID<sub>50</sub> = tissue culture infectious dose; concentrations of each mixture are given for vaccine serotypes specific to dengue 1, 2, 3 and 4; dose volume was 0.5 mL per dose; <sup>3</sup> (b) (4)

Randomization procedure: Yes.

Statistical analysis plan: Yes.

The following parameters were evaluated: Cage side observations (twice daily), injection site evaluations (once daily), body weights (prior to the first dose (day -2) and weekly thereafter), food consumption (once daily from day -5 to day 37, day 49, and day 65 or day 76), ophthalmoscopy (pre-study and within 4 days prior to scheduled euthanasia), clinical chemistry, hematology, and coagulation (days -1, 3, 9, 26, and 30, and on the day prior to necropsy {i.e., on days 36 and 48 for groups 2 and 3, respectively; and on days 64 and 75 for groups 1 and 4, respectively}), serology (day -1 and just prior to euthanasia). Urinalysis and gross anatomy at termination and organ weights and histopathology were evaluated/determined on selected tissues.

*Table 2: Parameters evaluated (study # 1)*

Parameters	Frequency of Testing
Cage-side observations	Twice daily
Injection site evaluations	Once daily
Body weight	Prior to the first dose (day -2) and weekly thereafter
Food consumption	Once daily from day -5 to day 37, day 49, and day 65 or day 76
Clinical chemistry	Days -1, 3, 9, 26 and 30, and on the day prior to necropsy (i.e., on days 36 and 48 for groups 2 and 3, respectively; and on days 64 and 75 for groups 1 and 4, respectively)
Hematology	Days -1, 3, 9, 26 and 30, and on the day prior to necropsy (i.e., on days 36 and 48 for groups 2 and 3, respectively; and on days 64 and 75 for groups 1 and 4, respectively)
Coagulation	Days -1, 3, 9, 26 and 30, and on the day prior to necropsy (i.e., on days 36 and 48 for groups 2 and 3, respectively; and on days 64 and 75 for groups 1 and 4, respectively)
Urinalysis	At necropsy
Immunogenicity	Day -1 and just prior to euthanasia
Ophthalmic examination	Pre-study and within 4 days prior to scheduled euthanasia
Necropsy	Days 36 and 48 for groups 2 and 3, respectively; and on days 64 and 75 for groups 1 and 4, respectively
Tissues for histopathology	Days 36 and 48 for groups 2 and 3, respectively; and on days 64 and 75 for groups 1 and 4, respectively

**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 ‘!’.

*Table 3: Tissues collected at necropsy (study #1):*

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone marrow smear (7 <sup>th</sup> rib)	!	
Bone marrow (sternum)	!	
Bone (femoral head)	!	
Bone (7 <sup>th</sup> rib)	!	
Brain	!*	
Cecum	!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!*	
Esophagus	!	
Eyes	!	
Fallopian tubes		X
Gall bladder	!	
Gut Associated Lymphoid Tissue (GALT) (Peyer's patches)		X
Gross lesions	!	
Harderian gland		X
Heart	!*	
Ileum	!	
Injection site <sup>b</sup>	!	
Jejunum	!	
Kidneys	!*	
Large intestine	!	
Liver	!*	
Lungs	!*	
Lymph nodes (Inguinal)	!	
Lymph nodes (mesenteric)	!	
Mammary gland	!	
Optic nerve	!	
Ovaries	!*	
Pancreas	!	
Parathyroid glands <sup>a</sup>	!	
Pituitary gland	!*	
Prostate	!	
Rectum	!	
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicle	!	

Organ/Tissue	Collected	Not collected
Skeletal muscle (psoas and diaphragm)	!	
Skin	!	
Small intestine	!	
Spinal cord (thoracic)	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid <sup>a</sup>	!*	
Tongue	!	
Trachea	!	
Ureters		X
Uterus	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland		X

<sup>a</sup> The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section. <sup>b</sup> Deltoid region of right arm

## Results:

### Morbidity and mortality:

No test article-related morbidity or mortality was reported.

Clinical Chemistry, hematology, and coagulation:

Table 4: Clinical chemistry results

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 Greater than 1.5 so Indicated Otherwise $\leq 1.5$ ))	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose

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 Greater than 1.5 so Indicated Otherwise $\leq 1.5$ ))	NOT OF NOTE
<p>LIVER FUNCTION:</p> <p>A) HEPATOCELLULAR</p> <p>B) HEPATOBILIARY</p>	<p>Aspartate aminotransferase (AST or SGOT)</p> <p>SD36 M <math>\downarrow \leq 0.6</math> G1</p> <p>SD-1 F <math>\uparrow \geq 2.5</math> G2</p> <p>SD3 F <math>\uparrow \geq 2.1</math> G2</p> <p>SD26 F <math>\uparrow \geq 1.7</math> G1</p> <p>SD75 F <math>\uparrow \geq 1.9</math> G1</p> <p>Alanine aminotransferase (ALT or SGPT)</p> <p>SD3 M <math>\downarrow \leq 0.5</math> G1</p> <p>SD3 M <math>\downarrow \leq 0.5</math> G2</p> <p>SD3 M <math>\downarrow \leq 0.6</math> G3</p> <p>SD26 M <math>\downarrow \leq 0.5</math> G1</p> <p>SD26 M <math>\downarrow \leq 0.5</math> G2</p> <p>SD26 M <math>\downarrow \leq 0.5</math> G3</p> <p>SD30 M <math>\downarrow \leq 0.6</math> G3</p> <p>SD36 M <math>\downarrow \leq 0.6</math> G1</p> <p>SD36 M <math>\downarrow \leq 0.5</math> G2</p> <p>SD36 M <math>\downarrow \leq 0.6</math> G3</p> <p>SD48 M <math>\downarrow \leq 0.6</math> G3</p> <p>SD-1 F <math>\downarrow \leq 0.5</math> G1</p> <p>SD-1 F <math>\uparrow \geq 2.0</math> G2</p> <p>SD-1 F <math>\downarrow \leq 0.6</math> G3</p> <p>SD3 F <math>\uparrow \geq 2.6</math> G2</p> <p>SD9 F <math>\uparrow \geq 2.8</math> G2</p> <p>SD26 F <math>\downarrow \leq 0.5</math> G2</p> <p>SD30 F <math>\downarrow \leq 0.5</math> G2</p> <p>SD48 F <math>\uparrow \geq 1.9</math> G1</p> <p>SD48 F <math>\uparrow \geq 1.7</math> G3</p> <p>SD64 F <math>\downarrow \leq 0.6</math> G1</p>	

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 Greater than 1.5 so Indicated Otherwise $\leq 1.5$ ))	NOT OF NOTE
	Alkaline phosphatase (ALP) SD48 M $\uparrow \geq 1.8$ G3 SD-1 F $\uparrow \geq 3.6$ G1 SD-1 F $\uparrow \geq 2.0$ G2 SD-1 F $\uparrow \geq 2.0$ G3 SD3 F $\uparrow \geq 3.7$ G1 SD3 F $\uparrow \geq 1.9$ G2 SD3 F $\uparrow \geq 2.0$ G3 SD9 F $\uparrow \geq 3.6$ G1 SD9 F $\uparrow \geq 2.0$ G2 SD9 F $\uparrow \geq 2.2$ G3 SD26 F $\uparrow \geq 3.2$ G1 SD26 F $\uparrow \geq 2.1$ G2 SD26 F $\uparrow \geq 2.5$ G3 SD30 F $\uparrow \geq 3.1$ G1 SD30 F $\uparrow \geq 1.9$ G2 SD30 F $\uparrow \geq 2.2$ G3 SD36 F $\uparrow \geq 3.4$ G1 SD36 F $\uparrow \geq 2.0$ G2 SD36 F $\uparrow \geq 2.3$ G3 SD48 F $\uparrow \geq 3.3$ G1 SD48 F $\uparrow \geq 2.1$ G3 SD64 F $\uparrow \geq 3.3$ G1 SD75 F $\uparrow \geq 3.5$ G1	Total bilirubin
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation) C-reactive protein*
KIDNEY FUNCTION	Blood Urea Nitrogen SD75 M $\uparrow \geq 1.7$ G1	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 Greater than 1.5 so Indicated Otherwise $\leq 1.5$ ))	NOT OF NOTE
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	<p>Fasting Triglycerides</p> <p>SD36 M <math>\downarrow \leq 0.5</math> G2</p> <p>SD36 M <math>\downarrow \leq 0.3</math> G3</p> <p>SD48 M <math>\uparrow \geq 1.7</math> G2</p> <p>SD64 M <math>\uparrow \geq 1.7</math> G1</p> <p>SD75 M <math>\uparrow \geq 1.9</math> G1</p> <p>SD36 F <math>\downarrow \leq 0.6</math> G3</p> <p>SD75 F <math>\downarrow \leq 0.6</math> G1</p> <p>GGT</p> <p>SD3 M <math>\downarrow \leq 0.6</math> G1</p> <p>SD3 M <math>\downarrow \leq 0.5</math> G2</p> <p>SD3 M <math>\downarrow \leq 0.5</math> G3</p> <p>SD9 M <math>\downarrow \leq 0.6</math> G1</p> <p>SD9 M <math>\downarrow \leq 0.6</math> G2</p> <p>SD9 M <math>\downarrow \leq 0.6</math> G3</p> <p>SD48 M <math>\downarrow \leq 0.6</math> G3</p> <p>SD75 M <math>\downarrow \leq 0.5</math> G1</p> <p>SD3 F <math>\downarrow \leq 0.6</math> G2</p> <p>SD3 F <math>\downarrow \leq 0.6</math> G3</p> <p>SD26 F <math>\downarrow \leq 0.6</math> G2</p> <p>SD30 F <math>\downarrow \leq 0.6</math> G2</p> <p>SD36 F <math>\downarrow \leq 0.6</math> G2</p> <p>SD48 F <math>\downarrow \leq 0.4</math> G2</p> <p>SD48 F <math>\downarrow \leq 0.6</math> G3</p> <p>SD75 F <math>\uparrow \geq 1.8</math> G1</p> <p>Lactate dehydrogenase</p> <p>SD-1 F <math>\uparrow \geq 2.8</math> G2</p> <p>SD3 F <math>\uparrow \geq 1.8</math> G2</p> <p>SD9 F <math>\uparrow \geq 2.0</math> G1</p> <p>SD26 F <math>\uparrow \geq 2.1</math> G1</p> <p>SD30 F <math>\uparrow \geq 2.1</math> G1</p> <p>SD36 F <math>\uparrow \geq 1.9</math> G1</p> <p>SD48 F <math>\uparrow \geq 2.0</math> G1</p>	<p>Albumin (A)</p> <p>Total protein</p> <p>Carbon dioxide</p> <p>Globulin</p> <p>A/G ratio</p> <p>Creatine kinase</p> <p>Total cholesterol</p>

\* *Not measured.*

Clinical chemistry results show a decrease in AST levels in group 1 males at study day 36. AST levels were increased in group 2 females at study days -1 and 3. AST levels were increased in group 1 females at study days 26 and 75. ALT levels were decreased in groups 1, 2, and 3 males at study days 3, 26, and 36. ALT levels were decreased in group 3 males at study days 30 and 48. ALT levels were decreased in groups 1 and 3 females at study day -1. ALT levels were increased in group 2 females at study days -1, 3, and 9. ALT levels were decreased in group 2 females at study days 26 and 30. At study day 48, ALT levels were increased in groups 1 and 3 females. ALT levels were decreased in group 1 females at study day 64. ALP levels were increased in

group 3 males at study day 48. At study day -1, ALP levels were higher in groups 1, 2, and 3 females when compared to group 4 (control). At study days 3, 9, 26, 30, and 36, ALP levels were increased in groups 1, 2, and 3 females. At study day 48, ALP levels were increased in groups 1 and 3 females. At study days 64 and 75, ALP levels were increased in group 1 females. At study day 75, BUN levels were increased in group 1 males.

Triglyceride levels were decreased in groups 2 and 3 males at study day 36. Triglyceride levels were increased in groups 2 and 1 males at study days 48 and 64, respectively. Triglyceride levels were increased in group 1 males at study day 75. Triglyceride levels were decreased in groups 3 and 1 females at study days 36 and 75, respectively. GGT levels were decreased in groups 1, 2, and 3 males at study days 3 and 9. GGT levels were decreased in groups 3 and 1 males at study days 48 and 75, respectively. GGT levels were decreased in groups 2 and 3 females at study day 3. In group 2 females, GGT levels were decreased at study days 26, 30, 36, and 48. GGT levels were decreased in group 3 females at study day 48. GGT levels were increased in group 1 females at study day 75.

LDH levels were increased in group 2 females at study days -1 and 3. In group 1 females, LDH levels were increased at study days 9, 26, 30, 36, and 48.

*Table 5: Hematology results*

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 or Less than 1.52, ie, $\geq 1.6$ or $\leq 1.6$	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	Lymphocyte count SD-1 M $\downarrow \leq 0.4$ G1 SD-1 M $\downarrow \leq 0.4$ G2 SD-1 M $\downarrow \leq 0.6$ G3 SD3 M $\downarrow \leq 0.5$ G1 SD3 M $\downarrow \leq 0.4$ G2 SD9 M $\downarrow \leq 0.5$ G1 SD9 M $\downarrow \leq 0.4$ G2 SD9 M $\downarrow \leq 0.6$ G3 SD26 M $\downarrow \leq 0.4$ G1 SD26 M $\downarrow \leq 0.4$ G2 SD26 M $\downarrow \leq 0.6$ G3 SD30 M $\downarrow \leq 0.4$ G1	Macrophage Leukocytes Large unstained cells (LUC)

<sup>2</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 or Less than 1.52, ie, $\geq 1.6$ or $\leq$ 1.6	Not of NOTE
	SD30 M $\downarrow \leq 0.4$ G2 SD36 M $\downarrow \leq 0.4$ G1 SD36 M $\downarrow \leq 0.4$ G2 SD36 M $\downarrow \leq 0.6$ G3 SD48 M $\downarrow \leq 0.4$ G1 SD48 M $\downarrow \leq 0.5$ G2 SD64 M $\downarrow \leq 0.4$ G1 SD36 F $\downarrow \leq 0.6$ G2 SD36 F $\downarrow \leq 0.6$ G3 SD48 F $\downarrow \leq 0.5$ G2 SD48 F $\downarrow \leq 0.4$ G3  White Blood Cells (WBC) SD-1 M $\downarrow \leq 0.5$ G1 SD-1 M $\downarrow \leq 0.5$ G2 SD3 M $\downarrow \leq 0.5$ G1 SD3 M $\downarrow \leq 0.6$ G2 SD9 M $\downarrow \leq 0.5$ G1 SD9 M $\downarrow \leq 0.4$ G2 SD26 M $\downarrow \leq 0.5$ G1 SD26 M $\downarrow \leq 0.5$ G2 SD26 M $\downarrow \leq 0.6$ G3 SD30 M $\downarrow \leq 0.6$ G1 SD30 M $\downarrow \leq 0.5$ G2 SD36 M $\downarrow \leq 0.5$ G1 SD36 M $\downarrow \leq 0.4$ G2 SD36 M $\downarrow \leq 0.6$ G3 SD48 M $\downarrow \leq 0.5$ G1 SD48 M $\downarrow \leq 0.6$ G2 SD64 M $\downarrow \leq 0.6$ G1 SD36 F $\downarrow \leq 0.6$ G2  Monocyte count: SD-1 M $\downarrow \leq 0.6$ G1 SD-1 M $\downarrow \leq 0.4$ G2 SD-1 M $\downarrow \leq 0.6$ G3 SD26 M $\downarrow \leq 0.4$ G2 SD26 M $\downarrow \leq 0.6$ G3 SD30 M $\downarrow \leq 0.6$ G2 SD36 M $\downarrow \leq 0.4$ G2 SD36 M $\downarrow \leq 0.6$ G3 SD48 M $\downarrow \leq 0.3$ G2 SD3 F $\uparrow \geq 2.9$ G1 SD3 F $\uparrow \geq 2.5$ G2 SD9 F $\uparrow \geq 1.9$ G1 SD26 F $\uparrow \geq 2.1$ G1 SD30 F $\uparrow \geq 1.9$ G1	

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 or Less than 1.52, ie, $\geq 1.6$ or $\leq$ 1.6	Not of NOTE
	SD36 F $\uparrow \geq 1.8$ G1 SD36 F $\downarrow \leq 0.6$ G2 SD36 F $\downarrow \leq 0.6$ G3 SD48 F $\downarrow \leq 0.5$ G2 SD48 F $\downarrow \leq 0.6$ G3  Neutrophil count SD3 M $\downarrow \leq 0.6$ G1 SD9 M $\downarrow \leq 0.6$ G1 SD9 M $\downarrow \leq 0.6$ G2 SD26 M $\downarrow \leq 0.6$ G1 SD26 M $\downarrow \leq 0.6$ G2 SD36 M $\downarrow \leq 0.6$ G1 SD36 M $\downarrow \leq 0.6$ G2 SD36 M $\downarrow \leq 0.6$ G3 SD48 M $\uparrow \geq 2.7$ G3 SD64 M $\uparrow \geq 2.3$ G1 SD-1 F $\uparrow \geq 1.8$ G1 SD-1 F $\uparrow \geq 2.1$ G3 SD36 F $\uparrow \geq 2.2$ G3 SD48 F $\uparrow \geq 2.3$ G1 SD48 F $\uparrow \geq 2.0$ G2 SD48 F $\uparrow \geq 3.2$ G3  Eosinophils count SD-1 M $\downarrow \leq 0.2$ G1 SD-1 M $\downarrow \leq 0.2$ G2 SD-1 M $\downarrow \leq 0.6$ G3 SD3 M $\downarrow \leq 0.2$ G1 SD3 M $\downarrow \leq 0.2$ G2 SD9 M $\downarrow \leq 0.6$ G1 SD9 M $\downarrow \leq 0.4$ G2 SD26 M $\downarrow \leq 0.3$ G2 SD30 M $\downarrow \leq 0.4$ G1 SD30 M $\downarrow \leq 0.5$ G2 SD36 M $\downarrow \leq 0.4$ G2 SD48 M $\downarrow \leq 0.5$ G1 SD48 M $\downarrow \leq 0.1$ G2 SD48 M $\uparrow \geq 1.9$ G3 SD64 M $\downarrow \leq 0.3$ G1 SD-1 F $\downarrow \leq 0.5$ G1 SD-1 F $\downarrow \leq 0.3$ G3 SD3 F $\downarrow \leq 0.5$ G1 SD3 F $\downarrow \leq 0.6$ G2 SD3 F $\downarrow \leq 0.3$ G3 SD9 F $\downarrow \leq 0.5$ G1	

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 or Less than 1.52, ie, $\geq 1.6$ or $\leq$ 1.6	Not of NOTE
	<p>SD9 F <math>\downarrow \leq 0.5</math> G2  SD9 F <math>\downarrow \leq 0.3</math> G3  SD26 F <math>\downarrow \leq 0.5</math> G2  SD26 F <math>\downarrow \leq 0.6</math> G3  SD36 F <math>\downarrow \leq 0.5</math> G2  SD36 F <math>\downarrow \leq 0.3</math> G3  SD48 F <math>\downarrow \leq 0.3</math> G2  SD48 F <math>\downarrow \leq 0.3</math> G3</p> <p>Basophils  SD-1 M <math>\downarrow \leq 0.3</math> G1  SD-1 M <math>\downarrow \leq 0.3</math> G2  SD-1 M <math>\downarrow \leq 0.6</math> G3  SD3 M <math>\downarrow \leq 0.4</math> G1  SD3 M <math>\downarrow \leq 0.5</math> G2  SD9 M <math>\downarrow \leq 0.5</math> G1  SD9 M <math>\downarrow \leq 0.3</math> G2  SD9 M <math>\downarrow \leq 0.6</math> G3  SD26 M <math>\downarrow \leq 0.3</math> G1  SD26 M <math>\downarrow \leq 0.3</math> G2  SD30 M <math>\downarrow \leq 0.4</math> G1  SD30 M <math>\downarrow \leq 0.3</math> G2  SD36 M <math>\downarrow \leq 0.2</math> G1  SD36 M <math>\downarrow \leq 0.2</math> G2  SD36 M <math>\downarrow \leq 0.6</math> G3  SD48 M <math>\downarrow \leq 0.5</math> G1  SD48 M <math>\downarrow \leq 0.5</math> G2  SD64 M <math>\downarrow \leq 0.4</math> G1  SD-1 F <math>\uparrow \geq 1.7</math> G1  SD3 F <math>\uparrow \geq 2.1</math> G1  SD9 F <math>\uparrow \geq 2.5</math> G1  SD26 F <math>\downarrow \leq 0.5</math> G2  SD30 F <math>\uparrow \geq 2.2</math> G1  SD36 F <math>\downarrow \leq 0.5</math> G2  SD36 F <math>\downarrow \leq 0.6</math> G3  SD48 F <math>\uparrow \geq 2.4</math> G1</p> <p>Unclassified cells (Unc)  SD-1 M <math>\downarrow \leq 0.3</math> G1  SD-1 M <math>\downarrow \leq 0.4</math> G2  SD-1 M <math>\downarrow \leq 0.5</math> G3  SD3 M <math>\downarrow \leq 0.4</math> G1  SD9 M <math>\downarrow \leq 0.3</math> G1  SD9 M <math>\downarrow \leq 0.4</math> G2  SD9 M <math>\downarrow \leq 0.6</math> G3  SD26 M <math>\downarrow \leq 0.4</math> G1  SD26 M <math>\downarrow \leq 0.4</math> G2</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 or Less than 1.52, ie, $\geq 1.6$ or $\leq 1.6$	Not of NOTE
	SD26 M $\downarrow \leq 0.6$ G3 SD30 M $\downarrow \leq 0.4$ G1 SD30 M $\downarrow \leq 0.5$ G2 SD36 M $\downarrow \leq 0.3$ G1 SD36 M $\downarrow \leq 0.4$ G2 SD48 M $\downarrow \leq 0.6$ G1 SD48 M $\downarrow \leq 0.6$ G2 SD64 M $\downarrow \leq 0.5$ G1 SD26 F $\downarrow \leq 0.6$ G2 SD36 F $\downarrow \leq 0.4$ G2 SD36 F $\downarrow \leq 0.5$ G3	
Clotting potential	Platelet count SD48 M $\downarrow \leq 0.6$ G2 SD75 M $\downarrow \leq 0.6$ G1	Prothrombin time Activated partial-thromboplastin time clotting time Fibrinogen Mean platelet volume
Others		Bone marrow cytology

Hematology results show low levels of lymphocytes in groups 1, 2 and 3 males at study days -1, 9, 26, and 36. Lymphocytes levels were decreased in groups 1 and 2 males at study days 3, 30, and 48. Lymphocytes levels were decreased in group 1 males at study day 64. Lymphocytes levels were decreased in groups 2 and 3 females at study days 36 and 48. WBC levels were decreased in groups 1 and 2 males at study days -1, 3, 9, 30, and 48. WBC levels were decreased in groups 1, 2, and 3 males at study days 26 and 36. WBC levels were decreased in group 1 males at study 64. WBC levels were decreased in group 2 females at study 36.

Monocyte levels in groups 1, 2, and 3 males were lower than the control group (group 4) at study day -1. Monocyte levels were decreased in group 2 males at study days 26, 30, 36, and 48. Monocyte levels were decreased in group 3 males at study days 26 and 36. Monocyte levels were increased in group 1 females at study days 3, 9, 26, 30, and 36. Monocyte levels were increased in group 2 females at study day 3. Monocyte levels were decreased in groups 2 and 3 females at study days 36 and 48.

Neutrophil levels were decreased in group 1 males at study days 3, 9, 26, 36, and 64. Neutrophil levels were decreased in group 2 males at study days 9, 26, and 36. Neutrophil levels were decreased in group 3 males at study days 36 and 48. Neutrophil levels were higher in group 1 females than the control group at study day -1. Neutrophil levels were higher in group 3 females, when compared to control group, at study days -1, 36, and 48. At study day 48, neutrophil levels were increased in groups 1 and 2 females

At study day -1, eosinophil levels were lower than the control group in groups 1, 2, and 3 males. Eosinophil levels were decreased in group 1 males at study days 3, 9, 30, 48, and 64. Eosinophil levels were decreased in group 2 males at study days 3, 9, 26, 30, 36, and 48. Eosinophil levels were increased in group 3 males at study day 48. At study day -1, eosinophil levels were lower than the control group in groups 1 and 3 females. Eosinophil levels were decreased in groups 1, 2, and 3 females at study days 3 and 9. Eosinophil levels were decreased in groups 2 and 3 females at study days 26, 36, and 48.

At study day -1, basophil levels were lower than the control group in groups 1, 2, and 3 males. Basophil levels were decreased in group 1 males at study days 3, 9, 26, 30, 36, 48, and 64. Basophil levels were decreased in group 2 males at study days 3, 9, 26, 30, 36, and 48. At study day -1, basophil levels were lower than the control group in group 1 females. Basophil levels were increased in group 1 females at study days 3, 9, 30, and 48. Basophil levels were decreased in group 2 females at study days 26 and 36. Basophil levels were decreased in group 3 females at study day 36.

At study day -1, unclassified cells (Unc) levels were lower than the control group in groups 1, 2, and 3 males. Unc levels were decreased in group 1 males at study days 3, 9, 26, 30, 36, 48, and 64. Unc levels were decreased in group 2 males at study days 9, 26, 30, 36, and 48. Unc levels were decreased in group 3 males at study days 9 and 26. Unc levels were decreased in group 2 females at study days 26 and 36. Unc levels were decreased in group 3 females at study day 36.

Platelet levels were decreased in groups 2 and 1 males at study days 48 and 75, respectively.

#### **Systemic toxicity:**

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, dermal scores, body weight (gain), food consumption, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

**Organ Weight:***Table 6: Male's organ weights:*

SEX		Males			
GROUPS		1 Day 65/76	2 Day 37/49	3 Day 37/49	4 (CONTROL) Day 65/76
NUMBER OF ANIMALS		2/1	2/1	2/1	2/1
BODY WEIGHT (terminal)		3450/4100	3600/3100	3700/3400	3400/3700
BRAIN		67.7/65.6	64.8/68.7	64.0/57.7	65.4/69.6
ADRENALS		0.447/0.617	0.640/0.338	0.530/0.371	0.383/0.360
EPIDIDYMIDES		1.81/1.98	2.07/0.61	1.83/2.82	1.34/1.01
HEART		12.8/14.6	11.0/11.2	12.5/10.5	10.2/12.3
KIDNEYS		12.3/16.2	14.2/10.9	14.2/10.8	12.8/16.4
LIVER		68.6/75.7	67.6/49.6	72.4/53.8	66.6/70.6
LUNGS		20.1/25.3	17.8/17.3	18.8/19.1	22.3/23.3
ILLIAC LYMPH NODES	Right	NC	NC	NC	NC
	Left	NC	NC	NC	NC
PROSTATE		NC	NC	NC	NC
SPLEEN		2.93/4.51	3.99/2.64	4.66/5.81	4.60/6.09
TESTES		8.65/6.34	13.5/0.92	7.84/12.4	2.02/2.42
PITUITARY		0.055/0.067	0.053/0.031	0.068/0.043	0.039/0.045
THYROID and PARATHYROID		0.365/0.958	0.53/0.384	0.32/0.291	0.580/0.320
THYMUS		3.24/3.04	1.30/0.626	2.88/4.24	5.48/6.69
OVARIES					
UTERUS					

*Absolute weights are expressed as mean (grams). NC = Not collected. Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase at study days 37 and 49 for groups 2 and 3 and study days 65 and 76 for groups 1 and 4.*

In group 1 males, adrenal weight was increased 17% and 71% at study days 65 and 76, respectively. In group 1 males, epididymides weight was increased 35% and 96% at study days 65 and 76, respectively. In group 1 males, heart weight was increased 25% and 19% at study days 65 and 76, respectively. In group 1 males, spleen weight was decreased 36% and 26% at study days 65 and 76, respectively. In group 1, testis weight was increased 328% and 162% at study days 65 and 76, respectively. In group 1 males, pituitary weight was increased 41% and 49% at study days 65 and 76, respectively. In group 1 males, thyroid weight was decreased 37% and increased 199% at study days 65 and 76, respectively. In group 1 males, thymus weight was decreased 41% and 55% at study days 65 and 76, respectively.

No test article-related changes in body weights were reported. Other organ weight fluctuations were within the degree of variation commonly reported in laboratory-housed members of this species that undergo similar study procedures.

*Table 7: Female's organ weights:*

SEX		Females			
GROUPS		1 Day 65/76	2 Day 37/49	3 Day 37/49	4 (CONTROL) Day 65/76
NUMBER OF ANIMALS		2/1	2/1	2/1	2/1
BODY WEIGHT (terminal)		3300/3000	3150/2800	2550/2700	3000/3700
BRAIN		68.3/62.2	60.1/60.7	58.2/52.8	57.2/60.9
ADRENALS		0.509/0.393	0.567/0.544	0.428/0.673	0.517/0.533
EPIDIDYMIDES					
HEART		13.7/9.28	10.3/9.65	9.36/9.89	10.0/11.7
KIDNEYS		14.3/15.1	12.1/12.8	11.5/10.9	12.9/16.3
LIVER		73.6/52.9	67.7/57.8	52.7/55.9	63.7/76.7
LUNGS		20.6/17.8	18.0/16.7	14.1/14.5	19.4/20.8
ILLIAC LYMPH NODES	Right	NC	NC	NC	NC
	Left	NC	NC	NC	NC
PROSTATE AND SEMINAL VESICLE					
SPLEEN		3.60/3.54	3.57/3.21	2.54/1.80	4.69/4.14
TESTES					
PITUITARY		0.047/0.045	0.069/0.050	0.052/0.055	0.058/0.059
THYROID and PARATHYROID		0.442/0.331	0.386/0.283	0.379/0.399	0.496/0.364
THYMUS		5.60/4.02	1.97/1.32	3.20/1.43	3.45/4.26
OVARIES		0.264/0.111	0.353/0.368	0.176/0.254	0.359/0.288
UTERUS		NC	NC	NC	NC

*Absolute weights are expressed as mean (grams). NC = Not collected. Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase at study days 37 and 49 for groups 2 and 3 and study days 65 and 76 for groups 1 and 4.*

In group 1 females, body weight was decreased 19% at study day 76. In group 1 females, brain weight was increased 19% at study day 65. In group 1 females, adrenal weight was decreased 26% at study day 76. In group 1 females, heart weight was increased 37% and decreased 21% at study days 65 and 76, respectively. In group 1 females, liver weight was increased 16% and decreased 31% at study days 65 and 76, respectively. In group 1 females, lungs weight was decreased 14% at study day 76. In group 1 females, spleen weight was decreased 23% and 14% at study days 65 and 76, respectively. In group 1 females, pituitary weight was decreased 19% and 24% at study days 65 and 76, respectively. In group 1 females, thymus weight was increased 62% at study day 65. In group 1, ovary weight was decreased 26% and 61% at study days 65 and 76, respectively.

Other organ weight fluctuations were within the degree of variation commonly reported in laboratory-housed members of this species that undergo similar study procedures.

Gross pathology:

Macroscopic findings are listed below:

*Table 8: Male's macroscopic findings*

Groups	Findings
1M Day 65	Animal # 1001: Lung; left apical lobe; Cyst (TGL): 2 in number, 2 and 6 mm Animal # 1002: Pancreas; Nodule; red (TGL): 5 in number, 1 - 2 mm
Day 76	Animal # 1003: Cecum; mucosa; thickness increased; mild (TGL) Ileocecal valve; mucosa; discoloration, red; mild (TGL): Collected Ileum; mucosa; thickness Increased; mild (TGL) Lung; left diaphragmatic lobe; adhesion, fibrous; mild (TGL): To thoracic wall Stomach; mucosa; discoloration, red; multifocal; minimal (TGL) Stomach; mucosa; thickness increased; diffuse; mild (TGL)
2M Day 37	Animal # 2001: Brain; occipital lobe; depression; temporal lobe; left (TGL): 5 cm long by approximately 1.5 cm wide area, with tan, white, and gray mottling Epididymis; nodule; pedunculated; red; left; single (TGL): 1 mm Lung; all right lobes; adhesion, fibrous; moderate (TGL): 2 each other Animal # 2002: Colon; mucosa; nodule; black; multiple (TGL): fewer than 20 total. Same nodules in cecum - not collected. Lung; all lobes; cyst; multiple; moderate (TGL): left diaphragmatic worst affected Thymus; size decreased (TGL)
Day 49	Animal # 2003: Lung; left diaphragmatic lobe; cyst; clear; single (TGL): 3 mm x 5 mm. Stomach; fundus; focus; red; multiple (TGL): less than 10 in number, 1-3 mm.
3M Day 37	Animal #: 3001: Thymus; Size decreased (TGL) Animal #: 3002: Lymph node, axillary; size increased; bilateral (TGL): collected Lymph node, iliac; size increased; bilateral (TGL): not collected Lymph node, inguinal; size increased; bilateral (TGL) Lymph node, mandibular; size increased; bilateral (TGL): collected
Day 49	Animal #: 3003: Brain; occipital lobe; discoloration, red; bilateral; diffuse; minimal (TGL): very light red. Colon; mucosa; focus; red; multifocal (TGL): 2 mm, less than 50, mid-colon only. Collected with section. Duodenum; biliary papilla; focus; red; single (TGL): 3 mm. Spleen; accentuated follicular pattern; diffuse (TGL) Spleen; discoloration, gray; minimal (TGL)
4M Day 65	Animal #: 4001: Colon; nodule; black (TGL): single, probable oesophagostomum, collected extra section Liver; friable; diffuse; minimal (TGL) Animal #: 4002: Duodenum; mucosa; abnormal appearance; diffuse (TGL): prominent villi, white in color Duodenum; papilla; discoloration, red; minimal (TGL) Jejunum; mucosa; abnormal appearance; diffuse (TGL): prominent villi, white in color Liver; capsule; abnormal appearance; left lateral lobe (TGL): white radiating streaks consistent with parasite migration scars, collected with section. Liver; left lateral lobe; nodule; white (TGL): 3 mm, single, collected with section. Lung; all right lobes; adhesion, fibrous; minimal (TGL): To thoracic wall and diaphragm and mediastinum Mesentery; abnormal appearance; diffuse (TGL): Prominent lacteals
Day 76	Animal # 4003: Lung; all left lobes; adhesion, fibrous; mild (TGL): To thoracic wall and diaphragm and mediastinum Spleen; accentuated follicular pattern; diffuse (TGL) Thyroid/parathyroid; absent; right (TGL)

*Table 9: Female's macroscopic findings*

Groups	Findings
1F Day 65	Animal #: 1501: Lung; left apical lobe; cyst; right apical lobe (TGL): less than 5, 2 to 4 mm Animal #: 1502: Omentum; nodule; red (TGL): Collected, 3 mm Pancreas; nodule; red (TGL): 2 mm
Day 76	Animal #: 1503: Cecum; submucosa; nodule; black (TGL): less than 20 in cecum, probable oesophagostomum Colon; mucosa; discoloration, red; multifocal; minimal (TGL): collected with section Colon; submucosa; nodule; black (TGL): less than five in number, probable oesophagostomum Lymph node, mandibular; size increased; bilateral (TGL): collected Lymph node, mesenteric; discoloration, dark; diffuse; mild (TGL)

Groups	Findings
2F Day 37	Animal # 2502: Duodenum; serosa; nodule; white; single (TGL): 1mm, collected with section Ileum; serosa; nodule; white; multiple (TGL): fewer than 10, collected with section Liver; right medial lobe; focus; capsule; white; single (TGL): 1mm, collected extra section Lung; all lobes; cyst; multiple; mild (TGL): left lobes worse, up to 3mm in size, up to 10 in number per lobe.
Day 49	Animal # 2503: Ileum; serosa; nodule; white; multiple (TGL): 2-3 mm, less than 10 Jejunum; serosa; nodule; white; multiple (TGL): 2-3 mm, less than 10 Omentum; nodule; white; multiple (TGL): 2-3 mm, less than 20, collected Omentum; nodule; red; single (TGL): 3 mm, collected Ovary; weighed separately: Right = 0.264g Left = 0.080g, immersed briefly in formalin prior to being weighed separately, original weight taken prior to formalin immersion Ovary; size increased; right (TGL): likely corpus hemorrhagicum Stomach; fundus; focus; red; single (TGL): 1.5 cm
3F Day 37	Animal # 3502: Omentum; nodule; red; single (TGL): 1mm, collected
Day 49	Animal # 3503: Lung; left apical lobe; cyst; clear; single (TGL): 3 mm Lung; left diaphragmatic lobe; adhesion, fibrous; minimal (TGL): To thoracic wall
4F Day 65	Animal # 4501: Colon; parasite; single: probable gastrodiscoides, not collected Lung; left apical lobe; cyst; multiple (TGL): less than 5, 2-3 mm Lung; right apical lobe; discoloration, red; focal; minimal (TGL): dorsal part of lobe, depressed area ~15mm long after inflation Mesentery; parasite (TGL): less than 10 in number, spiral shape, encysted, 10 mm, collected Animal # 4502: Liver; friable; diffuse; mild (TGL) Lung; all lobes; cyst (TGL): less than 30, 2 - 4 mm
Day 76	Animal # 4503: Colon; mucosa; discoloration, red; multifocal; minimal (TGL): Collected with section Liver; accentuated lobular pattern; diffuse; mild (TGL) Liver; friable; diffuse; mild (TGL) Stomach; mucosa; depression; focal; minimal (TGL): with red rim

No test article-related macroscopic findings in monkeys euthanized on days 37, 49, 65 or 76 were reported. However, spontaneously-occurring findings commonly reported in monkeys at the sponsor's facility or other incidental findings not considered related to the administration of Tetravalent ChimeriVax™-DEN vaccines were reported.

Microscopic findings are listed below:

*Table 10: Male's microscopic findings*

Groups	Findings
1M	GALT lymphoid hyperplasia in cecum (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); GALT lymphoid hyperplasia in colon (1/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); focal crypt abscess in colon (1/2); focal lamina propria macrophage infiltrate in colon (1/2); GALT lymphoid hyperplasia in duodenum (2/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); immature epididymis (1/2); bilateral immature spermatozoa in epididymis (1/2); multifocal myofiber degeneration in heart (1/2); mucosa lymphoplasmacytic infiltrate in ileum (2/2); GALT lymphoid hyperplasia in jejunum (2/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); multifocal mononuclear cell infiltrate in kidney (2/2); multifocal mononuclear cell infiltrate in liver (2/2); bronchiolectasis in lung (1/2); multifocal BALT hyperplasia in lung (2/2); multifocal golden pigment in lung (1/2); sinus histiocytosis in inguinal lymph nodes (1/2); lymphoid hyperplasia in mesenteric lymph nodes (2/2); medulla edema in mesenteric lymph nodes

Groups	Findings
	(1/2); paracortical expansion in mesenteric lymph nodes (1/2); spleen ectopic tissue in pancreas (1/2); focal cyst in parathyroid (1/2); focal mononuclear cell infiltrate in parathyroid (1/2); multifocal cytoplasm vacuolation in parathyroid (1/2); focal and multifocal mononuclear cell infiltrate in prostate (1/2); immature prostate (1/2); GALT lymphoid hyperplasia in rectum (1/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); focal mucosa crypt abscess in rectum (1/2); mononuclear cell infiltrate in mandibular salivary gland (2/2); immature seminal vesicle (1/2); focal follicular hypertrophy in spleen (1/2); lymphoid hyperplasia in spleen (1/2); mucosa lymphoplasmacytic infiltrate in stomach (2/2); lymphoid hyperplasia in stomach (1/2); focal lymphoid hyperplasia in stomach (1/2); focal submucosa parasite in stomach (1/2); no spermatogenesis immature testis (1/2); ectopic tissue and multiple follicle cyst in thyroid (1/2); multifocal mononuclear cell infiltrate in tongue (1/2); mononuclear cell infiltrate in trachea (1/2); multifocal mononuclear cell infiltrate in urinary bladder (1/2)
2M	Multifocal unilateral corticomedullary junction hemosiderin pigment in adrenal (1/2); unilateral congestion in adrenal (1/2); myeloid hypercellular in bone marrow (2/2); focal left occipital cortex cyst in brain (1/2); focal left occipital cortex malacia in brain (1/2); multifocal occipital cortex pigmented macrophage infiltrate in brain (1/2); GALT lymphoid hyperplasia in cecum (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); muscularis parasitic granuloma in cecum (1/2); multifocal mucosa eosinophil infiltrate in cecum (1/2); GALT lymphoid hyperplasia in colon (1/2); mucosa lymphoplasmacytic infiltrate in colon (1/2); focal muscularis parasitic granuloma in colon (1/2); GALT lymphoid hyperplasia in duodenum (2/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); immature epididymis (1/2); focal left capsule polyp in epididymis (1/2); multifocal mononuclear cell infiltrate in esophagus (1/2); unilateral posterior synechia in eyes (1/2); focal lymphocyte infiltrate in gallbladder (1/2); multifocal lymphocyte infiltrate in gallbladder (1/2); focal mononuclear cell infiltrate in heart (1/2); multifocal mononuclear cell infiltrate in heart (1/2); Peyer's patches lymphoid hyperplasia in ileum (1/2); mucosa lymphoplasmacytic infiltrate in ileum (2/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); mucosa eosinophil infiltrate in jejunum (1/2); multifocal mononuclear cell infiltrate in kidney (2/2); focal hepatocyte degeneration in liver (1/2); focal mononuclear cell infiltrate in liver (1/2); bronchiolectasis in lungs (1/2); multifocal pleura fibroplasia/fibrosis in lungs (1/2); focal increased alveolar macrophages in lungs (1/2); lymphoid hyperplasia in inguinal lymph nodes (2/2); paracortical expansion in inguinal lymph nodes (2/2); lymphoid hyperplasia in mesenteric lymph nodes (2/2); medulla edema in mesenteric lymph nodes (2/2); sinus histiocytosis in mesenteric lymph nodes (1/2); paracortical expansion in mesenteric lymph nodes (2/2); gland basophilia in mammary glands (1/2); multifocal adipose tissue infiltrate in pancreas (1/2); multifocal mononuclear cell infiltrate in prostate (1/2); immature prostate (1/2); GALT lymphoid hyperplasia in rectum (2/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); multifocal

Groups	Findings
	mononuclear cell infiltrate in mandibular salivary gland (1/2); immature seminal vesicle (1/2); lymphoid hyperplasia in spleen (2/2); mucosa lymphoplasmacytic infiltrate in stomach (1/2); lymphoid hyperplasia in stomach (2/2); immature testis (1/2); lymphoid depletion in thymus (2/2); focal mononuclear cell infiltrate in thyroid (1/2); multifocal mononuclear cell infiltrate in tongue (1/2); focal mononuclear cell infiltrate in urinary bladder (1/2); multifocal mononuclear cell infiltrate in urinary bladder (1/2); multifocal subcutis lymphocyte infiltrate in right deltoid subcutaneous injection site (1/2)
3M	Focal choroid plexus mononuclear infiltrate in brain (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); multifocal mucosa eosinophil infiltrate in cecum (2/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); GALT lymphoid hyperplasia in duodenum (2/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); immature epididymis (1/2); focal mononuclear cell infiltrate in esophagus (1/2); multifocal lymphocyte infiltrate in gallbladder (1/2); multifocal mononuclear cell infiltrate in heart (1/2); Peyer's patches lymphoid hyperplasia in ileum (1/2); mucosa lymphoplasmacytic infiltrate in ileum (2/2); Peyer's patches lymphoid hyperplasia in jejunum (1/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); multifocal papilla mineralization in kidney (1/2); multifocal mononuclear cell infiltrate in liver (1/2); multifocal BALT hyperplasia in lungs (1/2); multifocal black pigment in lungs (1/2); congestion (1/2), lymphoid hyperplasia (1/2), sinus histiocytosis (1/2), and paracortical expansion (1/2) in axillary lymph nodes; congestion in inguinal lymph nodes (1/2); lymphoid hyperplasia in inguinal lymph nodes (2/2); sinus histiocytosis in inguinal lymph nodes (1/2); paracortical expansion in inguinal lymph nodes (2/2); lymphoid hyperplasia (1/2) and paracortical expansion (1/2) in mandibular lymph nodes; lymphoid hyperplasia in mesenteric lymph nodes (2/2); paracortical expansion in mesenteric lymph nodes (2/2); dilated/cystic duct in mammary glands (1/2); immature prostate (1/2); GALT lymphoid hyperplasia in rectum (1/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); mucosa eosinophil infiltrate in rectum (1/2); multifocal mononuclear cell infiltrate in mandibular salivary gland (2/2); immature seminal vesicle (1/2); lymphoid hyperplasia in spleen (1/2); mucosa lymphoplasmacytic infiltrate in stomach (1/2); lymphoid hyperplasia in stomach (1/2); immature testis (1/2); lymphoid depletion in thymus (2/2); focal mononuclear cell infiltrate in thyroid (1/2); focal mononuclear cell infiltrate in tongue (1/2); multifocal mononuclear cell infiltrate in urinary bladder (2/2)
4M (Control)	Multifocal unilateral vascular mineralization in adrenal (1/2); focal choroid plexus mononuclear cell infiltrate in brain (1/2); multifocal meninges mononuclear cell infiltrate in brain (1/2); multifocal brown pigment in brain (1/2); GALT lymphoid hyperplasia in cecum (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); multifocal mucosa eosinophil infiltrate in cecum (2/2); GALT lymphoid hyperplasia in colon (1/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); focal submucosa parasitic granuloma in colon (1/2); GALT lymphoid hyperplasia in duodenum (2/2);

Groups	Findings
	<p>mucosa lymphoplasmacytic infiltrate in duodenum (2/2); focal biliary papilla hemorrhage in duodenum (1/2); diffuse villi cytoplasm vacuolation in duodenum (1/2); immature epididymis (2/2); unilateral interstitium basophilic granules in epididymis (1/2); multifocal mononuclear cell infiltrate in esophagus (1/2); focal mononuclear cell infiltrate in heart (1/2); Peyer's patches lymphoid hyperplasia in ileum (2/2); mucosa lymphoplasmacytic infiltrate in ileum (1/2); GALT lymphoid hyperplasia in jejunum (1/2); Peyer's patches lymphoid hyperplasia in jejunum (1/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); diffuse villi cytoplasm vacuolation in jejunum (1/2); focal papilla mineralization in kidney (1/2); multifocal mononuclear cell infiltrate in kidney (1/2); multifocal bile duct hyperplasia in liver (1/2); multifocal mixed cell infiltrate in liver (1/2); multifocal mononuclear cell infiltrate in liver (1/2); focal lymphoid follicle in liver (1/2); multifocal pleura fibroplasia/fibrosis in lung (1/2); multifocal BALT hyperplasia in lung (1/2); multifocal black pigment in lung (1/2); focal mononuclear cell infiltrate in lung (1/2); congestion in inguinal lymph nodes (1/2); lymphoid hyperplasia in inguinal lymph nodes (1/2); paracortical expansion in inguinal lymph nodes (2/2); lymphoid hyperplasia in mesenteric lymph nodes (1/2); medulla edema in mesenteric lymph nodes (1/2); paracortical expansion in mesenteric lymph nodes (1/2); vascular congestion and lymphatic dilation in mesentery (1/2); multifocal mononuclear cell infiltrate in prostate (1/2); immature prostate (2/2); GALT lymphoid hyperplasia in rectum (2/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); mononuclear cell infiltrate in mandibular salivary gland (1/2); immature seminal vesicle (2/2); ligament mononuclear cell infiltrate in diaphragm (1/2); lymphoid hyperplasia in spleen (1/2); mucosa lymphoplasmacytic infiltrate in stomach (2/2); lymphoid hyperplasia in stomach (1/2); incomplete spermatogenesis and no spermatogenesis immature testis (1/2); ectopic tissue in thyroid (1/2); focal mononuclear cell infiltrate in tongue (1/2); mononuclear cell infiltrate in trachea (1/2)</p>

Terminal sacrifice (day 37 for groups 2 and 3 and day 65 for groups 1 and 4). GALT = Gut-Associated Lymphoid Tissue. BALT = Bronchial-Associated Lymphoid Tissue

*Table 11: Female's microscopic findings.*

Groups	Findings
1F	<p>Myeloid hypercellular in bone marrow (1/2); multifocal lymphoid nodule in bone marrow (1/2); focal choroid plexus mononuclear cell infiltrate in brain (1/2); GALT lymphoid hyperplasia in cecum (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); focal parasitic granuloma in cecum (1/2); GALT lymphoid hyperplasia in colon (1/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); mucosa eosinophil infiltrate in colon (1/2); GALT lymphoid hyperplasia in duodenum (1/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); focal lymphocyte infiltrate in gallbladder (1/2); multifocal mononuclear cell infiltrate in heart (1/2); Peyer's patches lymphoid hyperplasia in ileum (2/2); mucosa lymphoplasmacytic</p>

Groups	Findings
	infiltrate in ileum (2/2); Peyer's patches lymphoid hyperplasia in jejunum (1/2); mucosa lymphoplasmacytic infiltrate in jejunum (1/2); multifocal mononuclear cell infiltrate in kidney (1/2); bilateral congestion in kidney (1/2); diffuse mesangium increased thickness in kidney (1/2); multifocal mononuclear cell infiltrate in liver (1/2); bronchiolectasis in lung (1/2); multifocal BALT hyperplasia in lung (1/2); congestion in inguinal lymph nodes (1/2); lymphoid hyperplasia in inguinal lymph nodes (2/2); paracortical expansion in inguinal lymph nodes (2/2); congestion in mesenteric lymph nodes (1/2); lymphoid hyperplasia in mesenteric lymph nodes (2/2); sinus histiocytosis in mesenteric lymph nodes (2/2); paracortical expansion in mesenteric lymph nodes (2/2); lymphoid hyperplasia and paracortical expansion in pancreatic lymph nodes (1/2); spleen ectopic tissue in omentum (1/2); spleen ectopic tissue in pancreas (1/2); GALT lymphoid hyperplasia in rectum (1/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); multifocal mucosa degeneration in rectum (1/2); multifocal mononuclear cell infiltrate in mandibular salivary gland (1/2); multifocal follicular hypertrophy in spleen (1/2); lymphoid hyperplasia in spleen (2/2); mucosa lymphoplasmacytic infiltrate in stomach (2/2); lymphoid depletion in thymus (1/2); ectopic tissue in thyroid (2/2); multifocal subcutis lymphocyte infiltrate in right deltoid subcutaneous injection site (1/2)
2F	GALT lymphoid hyperplasia in cecum (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); GALT lymphoid hyperplasia in colon (2/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); GALT lymphoid hyperplasia in duodenum (1/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); focal unilateral sclera mononuclear cell infiltrate in eyes (1/2); Peyer's patches lymphoid hyperplasia in ileum (1/2); mucosa lymphoplasmacytic infiltrate in ileum (2/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); multifocal mononuclear cell infiltrate in kidney (1/2); focal subcapsular parasitic granuloma in liver (1/2); bronchiolectasis in lungs (1/2); multifocal peribronchiolar chronic inflammation in lungs (1/2); lymphoid hyperplasia in inguinal lymph nodes (2/2); sinus histiocytosis in inguinal lymph nodes (2/2); paracortical expansion in inguinal lymph nodes (2/2); lymphoid hyperplasia in mesenteric lymph nodes (1/2); medulla edema in mesenteric lymph nodes (1/2); sinus histiocytosis in mesenteric lymph nodes (2/2); paracortical expansion in mesenteric lymph nodes (2/2); multifocal bilateral mineralization in ovary (1/2); GALT lymphoid hyperplasia in rectum (2/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); mononuclear cell infiltrate in mandibular salivary gland (1/2); focal lymphoid follicle in mandibular salivary gland (1/2); mucosa lymphoplasmacytic infiltrate in stomach (2/2); lymphoid hyperplasia in stomach (2/2); lymphoid depletion in thymus (2/2); multifocal cyst in thymus (1/2); multifocal mononuclear cell infiltrate in urinary bladder (1/2); multifocal mononuclear cell infiltrate in urinary bladder (1/2)
3F	Focal unilateral cortical epithelium hypertrophy in adrenal (1/2); multifocal bilateral cortical epithelium hypertrophy in adrenal (1/2); intima increased thickness in aorta (1/2); focal lymphoid nodule in bone marrow (1/2); GALT

Groups	Findings
	<p>lymphoid hyperplasia in cecum (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); multifocal mucosa eosinophil infiltrate in cecum (1/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); GALT lymphoid hyperplasia in duodenum (2/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); unilateral ciliary body mononuclear cell infiltrate in eyes (1/2); multifocal lymphocyte infiltrate in gallbladder (1/2); mucosa lymphoplasmacytic infiltrate in ileum (2/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); multifocal mononuclear cell infiltrate in kidney (1/2); multifocal mononuclear cell infiltrate in liver (1/2); congestion in inguinal lymph nodes (2/2); lymphoid hyperplasia in inguinal lymph nodes (1/2); sinus histiocytosis in inguinal lymph nodes (1/2); paracortical expansion in inguinal lymph nodes (1/2); lymphoid hyperplasia in mesenteric lymph nodes (1/2); paracortical expansion in mesenteric lymph nodes (2/2); spleen ectopic tissue in omentum (1/2); GALT lymphoid hyperplasia in rectum (1/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); multifocal mononuclear cell infiltrate in mandibular salivary gland (1/2); mucosa lymphoplasmacytic infiltrate in stomach (2/2); lymphoid depletion in thymus (1/2); focal mononuclear cell infiltrate in urinary bladder (1/2); focal lymphoid follicle in vagina (1/2); focal subcutis fibroplasia in right deltoid subcutaneous injection site (1/2)</p>
4F (Control)	<p>Multifocal bilateral corticomedullary junction hemosiderin pigment in adrenal (1/2); unilateral congestion in adrenal (1/2); multifocal bilateral medulla nuclear atypia in adrenal (1/2); myeloid hypercellular in bone marrow (1/2); multifocal choroid plexus mononuclear cell infiltrate in brain (1/2); mucosa lymphoplasmacytic infiltrate in cecum (2/2); GALT lymphoid hyperplasia in colon (1/2); mucosa lymphoplasmacytic infiltrate in colon (2/2); GALT lymphoid hyperplasia in duodenum (1/2); mucosa lymphoplasmacytic infiltrate in duodenum (2/2); multifocal lymphocyte infiltrate in gallbladder (1/2); Peyer's patches lymphoid hyperplasia in ileum (1/2); mucosa lymphoplasmacytic infiltrate in ileum (2/2); Peyer's patches lymphoid hyperplasia in jejunum (1/2); mucosa lymphoplasmacytic infiltrate in jejunum (2/2); mucosa eosinophil infiltrate in jejunum (1/2); multifocal mononuclear cell infiltrate in kidney (1/2); bilateral congestion in kidney (1/2); multifocal bile ductule hyperplasia in liver (1/2); multifocal single cell necrosis in liver (1/2); focal hemorrhage in lung (1/2); bronchiolectasis in lung (2/2); focal alveolar wall increased thickness in lung (1/2); lymphoid hyperplasia in inguinal lymph nodes (2/2); paracortical expansion in inguinal lymph nodes (2/2); lymphoid hyperplasia in mesenteric lymph nodes (1/2); medulla edema in mesenteric lymph nodes (2/2); brown pigment in mesenteric lymph nodes (2/2); sinus histiocytosis in mesenteric lymph nodes (2/2); paracortical expansion in mesenteric lymph nodes (2/2); dilated/cystic duct in mammary gland (1/2); pentastome parasite (armillifer) in mesentery (1/2); mucosa lymphoplasmacytic infiltrate in rectum (2/2); lymphoid hyperplasia in spleen (1/2); mucosa lymphoplasmacytic infiltrate in stomach (1/2); lymphoid hyperplasia in stomach (1/2); lymphoid depletion in thymus (1/2); multifocal mononuclear cell infiltrate in urinary bladder (1/2)</p>

Terminal sacrifice (day 37 for groups 2 and 3 and day 65 for groups 1 and 4). GALT = Gut-Associated Lymphoid Tissue. BALT = Bronchial-Associated Lymphoid Tissue

An extensive number of tissues were examined for histology. No test article-related microscopic findings in monkeys euthanized on days 37, 49, 65 or 76 were reported. However, spontaneously-occurring findings commonly reported in monkeys at the sponsor's facility or other incidental findings not considered related to the administration of tetravalent ChimeriVax™-DEN vaccines were reported.

#### **Body temperature:**

Body temperature was not measured.

#### **Serology:**

The antibody titers specific to dengue strains 1, 2, 3 and 4 were quantified using a (b) (4) assay.

Prior to dosing on day 1, three monkeys (one monkey each from groups 2, 3, and 4) had minimal anti-flavivirus antibody titers. Following administration of tetravalent dengue vaccine to these monkeys, the pre-existing antibody titers for the group 2 and 3 monkeys (anti-Japanese Encephalitis virus titers of 1:40 and 1:10, respectively) did not seem to affect the level of immune induction. For group 4, the anti-dengue 2 antibody titer (1:20 pre-dose) persisted through day 64, when the anti-dengue 2 antibody titer was 1:10.

For groups 2 and 3, similar low-titer antibodies to all 4 strains of dengue virus were reported on day 36. One group 2 animal had no detectable antibody to any dengue strain. On day 36, group 3 monkeys had greater antibody titers to all 4 dengue strains when compared to group 2. The titers of anti-DEN 2 antibody in group 3 were generally greater than the antibody levels detected for the other dengue strains. Days 48 and 36 were similar in the anti-dengue virus antibody titers levels.

At day 64, low-titer antibodies to all four strains of dengue virus were reported in group 1. On day 64, monkey 1002 had slightly higher antibody titers (range 1:160 to 1:320) than the other 3 monkeys (antibody titers ranged from 1:20 to 1:80). However, the antibody titers were equivalent (1:80) for both group 1 monkeys on day 75.

*Table 12: Antibody titer for (b) (4) assay (groups 2 and 3).*

<b>Animal ID</b>	<b>DEN-1</b>	<b>DEN-2</b>	<b>DEN-3</b>	<b>DEN-4</b>
<b>SET A Day 36</b>				
<b>Group 2</b>				
2001-MF40165 M	1:160	1:160	1:80	1:80
2002-MF40168 M	1:80	1:80	1:40	1:80
2501-MF40181 F	0	0	0	0
2502-MF40220 F	1:160	1:160	1:160	1:160
<b>Group 3</b>				
3001-MF40197 M	1:320	1:640	1:320	1:160
3002-MF40148 M	1:20	1:80	1:20	1:40
3501-MF40234 F	1:320	1:640	1:320	1:160

Animal ID	DEN-1	DEN-2	DEN-3	DEN-4
3502-MF40210 F	1:20	1:80	1:20	1:20
<b>Replicate 2</b>				
<b>Group 2</b>				
2001-MF40165 M	1:160	1:160	1:80	1:80
2002-MF40168 M	1:40	1:40	1:40	1:80
2501-MF40181 F	0	0	0	0
2502-MF40220 F	1:160	1:160	1:80	1:80
<b>Group 3</b>				
3001-MF40197 M	1:320	1:640	1:320	1:80
3002-MF40148 M	1:20	1:80	1:20	1:20
3501-MF40234 F	1:320	1:640	1:320	1:160
3502-MF40210 F	1:40	1:40	1:20	1:20
<b>SET A Day 48</b>				
<b>Group 2</b>				
2003-MF40132 M	1:80	1:40	1:40	1:40
2503-MF40183 F	1:160	1:160	1:80	1:160
<b>Group 3</b>				
3003-MF40124 M	1:80	1:160	1:80	1:80
3503-MF40237 F	1:160	1:640	1:160	1:80
<b>Group 2</b>				
2003-MF40132 M	1:80	1:40	1:40	1:40
2503-MF40183 F	1:160	1:160	1:160	1:160
<b>Group 3</b>				
3003-MF40124 M	1:80	1:320	1:80	1:80
3503-MF40237 F	1:160	1:640	1:160	1:160

*DEN-1: Dengue 1; DEN-2: Dengue 2; DEN-3: Dengue 3; DEN-4: Dengue 4*

Table 13: Antibody titer from (b) (4) assay (groups 1 and 4).

Animal ID	DEN-1	DEN-2	DEN-3	DEN-4
<b>SET B Day 64</b>				
<b>Group 1</b>				
1001-MF40189 M	1:40	1:40	1:40	1:40
1002-MF40162 M	1:320	1:320	1:160	1:160
1501-MF40236 F	1:80	1:80	1:40	1:80
1502-MF40212 F	1:40	1:40	1:20	1:40
<b>Group 4</b>				
4001-MF40200M	0	0	0	0
4002-MF40128 M	0	0	0	0
4501-MF40224 F	0	1:10	0	0
4502-MF40221 F	0	0	0	0
<b>Replicate 2</b>				
<b>Group 1</b>				
1001-MF40189 M	1:40	1:40	1:40	1:40
1002-MF40162 M	1:320	1:320	1:160	1:160
1501-MF40236 F	1:80	1:80	1:40	1:80

<b>Animal ID</b>	<b>DEN-1</b>	<b>DEN-2</b>	<b>DEN-3</b>	<b>DEN-4</b>
1502-MF40212 F	1:40	1:40	1:20	1:40
<b>Group 4</b>				
4001-MF40200M	0	0	0	0
4002-MF40128 M	0	0	0	0
4501-MF40224 F	0	1:10	0	0
4502-MF40221 F	0	0	0	0
<b>SET B Day 75</b>				
<b>Group 1</b>				
1003-MF40146 M	1:80	1:80	1:80	1:80
1503-MF40187 F	1:80	1:80	1:80	1:80
<b>Group 4</b>				
4003-MF40144 M	0	0	0	0
4503-MF40195 F	0	0	0	0
<b>Replicate 2</b>				
<b>Group 1</b>				
1003-MF40146 M	1:80	1:80	1:80	1:80
1503-MF40187 F	1:80	1:80	1:80	1:80
<b>Group 4</b>				
4003-MF40144 M	0	0	0	0
4503-MF40195 F	0	0	0	0

*DEN-1: Dengue 1; DEN-2: Dengue 2; DEN-3: Dengue 3; DEN-4: Dengue 4*

*Table 14: Test article-related effects*

Test article related effects	Effects considered incidental
↑ ALP ↓ Lymphocyte ↓ WBC ↑ Monocytes in Females ↓ Monocytes in males ↓ Eosinophils ↓ Basophils in males ↑ Adrenal weight in males ↑ Epididymides weight ↓ Spleen weight ↓ Thymus weight in males ↑ Testis weight ↓ Ovary weight Immune responses	↓ ALT ↓ GGT ↓ Pituitary weight in females ↑ Heart weight in males ↑ Pituitary weight in males

### **Assessment:**

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, dermal scores, body weight (gain), food consumption, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

ALP is an enzyme found in bloodstream and it helps break down proteins in the body. It exists in different forms, depending on where it originates. The liver is one of the main sources of ALP, but some is also made in bones, intestines, pancreas, and kidneys. In pregnant women, ALP is made in the placenta. Abnormal levels of ALP indicates a problem with liver, gallbladder, or bones. However, they may also indicates malnutrition, kidney cancer tumors, intestinal problem, a pancreas problem, or a serious infection. The ALP test can be helpful in identifying conditions such as: hepatitis (inflammation of the liver), cirrhosis (scarring of the liver), cholecystitis (inflammation of the gallbladder), and blockage of bile ducts (from a gallstone, inflammation, or cancer). The normal range of ALP varies from person to person and depends on age, blood type, gender, and pregnancy. The normal range for human serum ALP level is 20 to 140 IU/L, but this can vary from laboratory to laboratory. The normal range runs higher in children and decreases with age.

A lymphocyte is any of 3 types of white blood cell (all 3 are agranulocytes) in a vertebrate's immune system. They include natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). Thus, any decrease, in one or all of these cell types, might affect the immune responses.

GGT is present in the cell membranes of many tissues, including the kidneys, bile duct, pancreas, gallbladder, spleen, heart, brain, and seminal vesicles (11). It is involved in the leukotriene metabolism (13) and transfer of amino acids across the cellular membrane (12). It is also involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water, certain L-amino acids, and peptides, leaving the cysteine product to preserve intracellular homeostasis of oxidative stress (14, 15). GGT, along with ALP, is used as a diagnostic marker for liver disease in medicine. Latent elevations in GGT are typically reported in patients with chronic viral hepatitis infections often taking 12 months or more to present (16). Elevated serum GGT activity can be found in diseases of the liver, biliary system, and pancreas. Because GGT levels were decreased in this study, it is considered as incidental effect.

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. The decrease in ALT levels were considered incidental, because they would only be biologically significant if the levels had increased (and not decreased) by a corresponding amount.

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 increases in the monocyte count in females 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.

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

White blood cells (WBC; also called leukocytes), are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system (17). The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The two commonly used categories of white blood cell disorders divide them quantitatively into those causing excessive numbers (proliferative disorders) and those causing insufficient numbers (leukopenias) (18). Several studies show that the apoptosis of blood cells could be related to an increased production of free radicals (19). WBC proliferative disorders can be classed as myeloproliferative and lymphoproliferative. Some are autoimmune, but many are neoplastic.

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 epididymis is a tube that connects a testicle to a vas deferens in the male reproductive system. It is present in all male reptiles, birds, and mammals. It is a single, narrow, tightly-coiled tube connecting the efferent ducts from the rear of each testicle to its vas deferens. An inflammation of the epididymis is called epididymitis. It is much more common than testicular inflammation, termed orchitis.<sup>3</sup>

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum. In males, at study day 57, the increase in thymus weight might be related to the immune responses due to test article-treatment. However, at study day 29, female's thymus weight was decreased significantly. As the thymus is the organ of T-cell development, any congenital defect in thymic genesis or a defect in thymocyte development can lead to a profound T cell deficiency in primary immunodeficiency disease. Defects that affect both the T cell and B cell lymphocyte lineages result in severe combined immunodeficiency syndrome (SCIDs). Acquired T cell deficiencies can also affect thymocyte development in the thymus.<sup>4</sup>

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>5</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

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<sup>3</sup> <https://en.wikipedia.org/wiki/Epididymis>

<sup>4</sup> <https://en.wikipedia.org/wiki/Thymus>.

<sup>5</sup> Spleen, Internet Encyclopedia of Science.

removal<sup>6</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.

Even though no microscopic findings associated with the increase in testis and ovary weight, the changes were significant (at study days 65 and 76, testis weight was increased 328% and 162% and ovary weight was decreased 26% and 61%, respectively) and might be related to the test article treatment. Changes in heart and pituitary weights were not associated with any macroscopic and/or microscopic (mononuclear cell infiltrate in heart was also reported in the control group) findings. Thus, it was considered incidental.

Immune responses due to test article treatment were reported.

Based on the overall findings in this study, it can be concluded that in (b) (4) monkeys, administration of dengue tetravalent vaccine (3 or 2 doses) had no adverse effects in terms of systemic toxicity.

**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, there are no significant safety issues were reported in this study.

### **Internal communication:**

Body temperature and CRP levels were not measured in this study. Body temperature and CRP are important in determining the cause of inflammation, if any, caused by the test article.

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<sup>6</sup> Mebius RE, Kraal G. (2005). Structure and function of the spleen. Nat Rev Immunol. 5(8):606-16.

# REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY STUDIES AND SUPPORTIVE STUDIES (IMMUNOGENICITY AND VIREMIA STUDIES):

## Reproductive and Developmental Toxicity: Immunogenicity Studies

### CYD Dengue Vaccine - Immunogenicity and Viremia Study Following Repeated Intravenous or Subcutaneous Injections in the Rabbit (Study # SP0056 IS0906)

*Reviewer: Nabil Al-Humadi*

Objective of the study: The objective of the study was to evaluate the rabbit as an appropriate model for developmental and reproductive toxicity (DART) studies with CYD dengue vaccine. The study investigated both the antibody (Ab) response and viremia induced by either repeated subcutaneous (s.c.) or intravenous (i.v.) injections of CYD dengue vaccine to rabbit females and/or by pushing the dose of the vaccine.

Performing laboratory: Sanofi Pasteur, 1541 Avenue Marcel Mérieux, 69280 Marcy l'Etoile, France

Initiation date: July 27, 2009

Final report date: February 19, 2014

Batch/lot number of test article:

- 1- Tetravalent Dengue Vaccine, TV CYD, CYD dengue vaccine

Batch numbers: TV5555 (vaccine): CDE09001 5 log10 CCID50/serotype/mL.

(Phase II product) TV9999 (bulks): (b) (4) (CYD1): (b) (4)

(b) (4) (CYD2): (b) (4)

(b) (4) (CYD3): (b) (4)

(b) (4) (CYD4): (b) (4)

- 2- CYD diluent (0.4 % NaCl (b) (4)): Batch number: (b) (4)

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

Breeder/supplier: (b) (4)

Number of animal per group: 9 females

Age: Not reported

Body weight range: 2 to 2.25 kg (at reception)

Route and site of administration: Subcutaneous or intravenous

Dose concentration: See experimental design

Frequency of administration: On study days 0, 21, and 42

Dose volume/animal: 0.5 mL

Means of administration: Subcutaneous or intravenous

Report status: Final

Schedule of the Study:

Table 15: *Schedule of study*

Animal receipt	16 July 2009
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Days of administration (days 0, 21 and 42)	27 July, 17 August, and 07 September 2009
Blood sampling (days 0, 1, 2, 3, 4, 5, 6, 7, 8, 21, 42 and 56)	27 to 31 July, 01 to 04 and 17 August, 07 and 21 September 2009
Euthanasia (day 56)	21 September 2009

## METHODS

Experimental design

Animal identifications and dose levels

*Table 16: Experimental design*

Group / cage number	Animal number	Route of administration	Treatment	Dose level (log <sub>10</sub> CCID <sub>50</sub> /serotype)	Dose volume (volume/animal)
1 / 1	27	s.c.	TV5555	5	One human dose (approximately 0.5 mL)
1 / 2	28	s.c.	TV5555	5	One human dose (approximately 0.5 mL)
1 / 3	29	s.c.	TV5555	5	One human dose (approximately 0.5 mL)
2 / 4	30	i.v.	TV5555	5	One human dose (approximately 0.5 mL)
2 / 5	31	i.v.	TV5555	5	One human dose (approximately 0.5 mL)
2 / 6	32	i.v.	TV5555	5	One human dose (approximately 0.5 mL)
3 / 7	33	i.v.	TV9999	(b) (4)	0.5 mL
3 / 8	34	i.v.	TV9999	(b) (4)	0.5 mL
3 / 9	35	i.v.	TV9999	(b) (4)	0.5 mL

Randomization procedure: Yes

Statistical analysis: No

GLP: This study is not GLP compliant but was conducted according to the standard operating procedures of Sanofi Pasteur.

The following parameters were evaluated:

*Table 17: Parameters evaluated*

Parameters	Frequency of Testing
Clinical observations	Daily
Viremia investigations	On day 0 (7 hours after the first immunization), day 1 (24 hours after the first immunization) and days 2 to 8
Sero-neutralization assay	At pretest and on days 21, 42, and 56
Body weight	At pretest and on days 0, 21, 42, and 56
Euthanasia	Day 56

**RESULTS:****Mortality and Morbidity**

One animal (no. 29) died during blood sampling and was replaced by another animal (with the same animal number in the report).

**Body Weights**

There were no treatment-related changes in body weight (data not reported).

**Viremia**

In rabbits given the high dose by the i.v. route, viremia was reported at a low level on the day after injection (Table 18). Viruses were also reported in one rabbit 7 hours and 6 days after i.v. administration of the high dose and in two out of three rabbits after s.c. injection of the human dose. Because the second assay showed negative results, these findings were considered of doubtful significance.

*Table 18: Viremia results*

Animal Number (dose and route)	Pretest	Day 0 (+7hours)	Day 1	Day 2 and thereafter
27 (TV5555 – s.c.)	_*	-	_**	-
28 (TV5555 – s.c.)	-	-	-	-
29 (TV5555 – s.c.)	-	-	_**	-
30 (TV5555 – i.v.)	-	-	-	-
31 (TV5555 – i.v.)	-	-	-	-
32 (TV5555 – i.v.)	-	-	-	-
33 (TV9999 – i.v.)	-	_**	4.12 +/- 0.09	-
34 (TV9999 – i.v.)	-	-	3.67 +/- 0.33	_***
35 (TV9999 – i.v.)	-	-	2.80 +/- 0.16	-

\* -: Below the limit of detection (<sup>(b) (4)</sup> genome equivalent/mL)

\*\* Positive in one out of two tests conducted on the same sample

\*\*\* Positive in one out of two tests conducted on the same sample on day 6

**Immunogenicity**

Whatever the route of injection and dose level, all rabbits seroconverted to all serotypes (Table 19).

When compared to the s.c. route, the titers were higher and detected earlier when animals were treated by the i.v. route. By day 21, all animals given the high dose by the i.v. route seroconverted to serotypes.

*Table 19: Sero-neutralization results. \* -: titer <10*

Animal Number (dose and route)	Day 21				Day 42				Day 56			
	1	2	3	4	1	2	3	4	1	2	3	4

27 (TV5555 – s.c.)	40	-*	-	-	>80	16	16	-	>80	25	32	10
28 (TV5555 – s.c.)	-	-	-	-	63	13	-	-	>80	32	25	16
29 (TV5555 – s.c.)	-	-	-	-	40	13	16	-	63	13	32	-
30 (TV5555 – i.v.)	>80	-	-	-	>80	>80	63	50	>80	>80	>80	>80
31 (TV5555 – i.v.)	50	-	-	-	>80	>80	>80	20	>80	>80	>80	40
32 (TV5555 – i.v.)	-	-	-	-	>80	40	>80	20	>80	>80	>80	>80
33 (TV9999 – i.v.)	>80	31	25	-	>80	>80	>80	>80	>80	>80	>80	>80
34 (TV9999 – i.v.)	>80	63	20	20	>80	>80	>80	>80	>80	>80	>80	>80
35 (TV9999 – i.v.)	>80	>80	63	>80	>80	>80	>80	>80	>80	>80	>80	>80

## CONCLUSIONS

In rabbits given the high dose by the i.v. route, viremia was detected at a low level on the day after the injection. Also, whatever the route of injection and the dose level, all rabbits seroconverted to all serotypes. The titers were higher and detected earlier when animals were treated by the i.v. route and at the high dose.

## **CYD Dengue Vaccine - Immunogenicity and Viremia Study Following One Intravenous or Repeated Subcutaneous Injections in the Mouse**

*Reviewer: Claudia Wrzesinski*

This study was performed to evaluate the mouse as an appropriate model for developmental and reproductive toxicity (DART) studies with CYD dengue vaccine. The study investigated both the antibody response and viremia induced by either repeated subcutaneous (s.c.) and/or single intravenous (i.v.) injections of CYD dengue vaccine to mouse females.

Twenty-one female (b) (4) mice were given either three s.c. administrations at 3 week-intervals of Tetravalent Dengue Vaccine (at  $5 \log_{10}$  CCID<sub>50</sub>/mL of each dengue serotype 1 to 4 which corresponds to one human dose) or one i.v. administration of Tetravalent Dengue Bulk (at (b) (4)  $\log_{10}$  CCID<sub>50</sub>/mL of each dengue serotype 1 to 4). Blood samples were taken from vaccinated mice on days 0 (7 hours after injection), 1, 2, 3, 4, 5, 6, 21 and 56. They were analyzed for detection of viremia by qRT-PCR and specific neutralizing antibodies to dengue by seroneutralization assay.

### **RESULTS:**

**Mortality and Morbidity:** Three mice given the human dose by s.c. were found dead (30 minutes to two hours after the second injection. The cause of the death could not be determined at necropsy (no histopathology data are provided with this study). However, since there was no death in the group given the test item by i.v. and in the high dose group, the mortality was considered not related to the vaccine.

**Viremia:** Viremia was detected on the day of the injection (+ 7 hours) and during two days after injection in mice given the high dose by the i.v. route. Viruses were also detected on occasions in the other groups and on day 4 in one high dose i.v. mouse, however the second assay was negative and results considered of doubtful significance.

### **Immunogenicity:**

All mice given the high dose by the i.v. route seroconverted to all serotypes by 21 days after the injection. Seroconversion to serotypes 1 and 3 was also observed on day 21 in all mice given one human dose by i.v.. Only one and two mice given the human dose by i.v. seroconverted to serotypes 2 and 4, respectively. In mice given the human dose by s.c., seroconversion was limited to one or two animals (and against serotypes 1 and/or 3 only), after both one and three injections.

*Table 20: Sero-Neutralization*

Animal Number (dose and route)	Day 21				Day 56			
	1	2	3	4	1	2	3	4
1. TV5555 – s.c.	-*	-	-	-	-	-	-	-
	-	-	-	-	13	-	-	-
	-	-	13	-	63	-	20	-
2. TV5555 – i.v.	>80	10	25	20	NA†	NA	NA	NA
	32	-	16	16	NA	NA	NA	NA
	31	-	13	-	NA	NA	NA	NA
3. TV9999 – i.v.	>80	>80	32	25	NA	NA	NA	NA
	>80	>80	20	13	NA	NA	NA	NA
	>80	>80	25	20	NA	NA	NA	NA

\* -: titer &lt;10

† NA: not applicable (no sample)

**Deviation from the protocol:**

Due to death and lower number of remaining animals, no blood sample was taken on day 42 and all surviving mice were given the third injection. Blood samples were then taken 21 days after the third injection on day 56.

**CONCLUSION:**

In conclusion, viremia was detected on day after the injection and during two days in mice given the high dose by the i.v. route. All mice given the high dose by the i.v. route seroconverted 21 days after the injection. In other groups viremia was not detected and seroconversion was limited to occasional animals and/or to serotypes 1 and 3.

## Reproductive and Developmental Toxicity: Investigative Studies

### CYD Dengue Vaccine - Preliminary Developmental Toxicity Study in the (b) (4) Rabbit Following Three Intravenous Injections (study # 20003200)

**Reviewer:** Nabil Al-Humadi

Performing laboratory: (b) (4)

Initiation date: October 26<sup>th</sup>, 2010

Final report date: April 10<sup>th</sup>, 2012

Test article, diluent, and control article information:

*Table 21: Test article, diluent, and control article information*

Test Article Information						
Name: CYD Dengue Vaccine <sup>a</sup>		Supplier:		Sponsor:		
Description	Batch Number	Date Received	Storage	Retest Date	Study Group	Dose Level (CCID <sub>50</sub> )
White freeze-dried powder	S4316	23 SEP 2010	Refrigerated (2 C to 8 C)	APR 2012	II	Approximately 5
(b) (4)	IND10044	16 SEP 2010	(b) (4)	JAN 2012	III	Approximately 6.5
(b) (4)	IND10045	16 SEP 2010	(b) (4)	JAN 2012	IV	Approximately 8

a. Synonymous with 323 CYD Dengue Vaccine.

Diluent Information						
Name	Description	Lot Number	Supplier	Date Received	Storage	Expiration Date
0.4% Sodium Chloride for Injection, (b) (4)	Clear, colorless liquid	(b) (4)	(b) (4)	12 OCT 2010	Room temperature	FEB 2012

a. (b) (4)

Control Article Information						
Name	Description	Lot Number	Supplier	Date Received	Storage	Expiration Date
0.9% Sodium Chloride for Injection, (b) (4)	Clear, colorless liquid	(b) (4)	(b) (4)	09 JUL 2010	Room temperature	DEC 2011

a. (b) (4)

Animal species and strain: (b) (4) Rabbit (b) (4)

Breeder/supplier: (b) (4)

Number of female animal per group: 6

Age: 4 months

Average body weight: 2.2-2.7 kg

Route and site of administration: Intravenous

Volume of injection: 0.5 ml/animal

Frequency of administration and study duration: DS 1 (36 days before mating), DS 21 (15 days before mating), and DG 6 (6 days after mating). The test article was administered via a bolus injection into the marginal vein of the ear

Dose: See table below and experimental design

Stability: The dosing formulations ((b) (4)) had been confirmed to be stable as described in supplement 1 for (b) (4) when stored at (b) (4) and in supplement 2 for (b) (4) when stored at (b) (4)

A certificate of analysis and dose formulation analysis for each batch of the test article is available in appendix 3. The exact concentration of each serotype is presented in the following table:

Table 22: CYD dengue serotype concentrations (reproductive study # 2)

CYD Dengue Serotype Concentrations						
Batch Numbers	Study Group	Dose Level (log <sub>10</sub> CCID <sub>50</sub> )	Virus Concentration (log <sub>10</sub> CCID <sub>50</sub> )			
			Type 1	Type 2	Type 3	Type 4
S4316	II	Approximately 5	5.7	5.7	5.5	5.3
IND10044 (6.5/6.5/6.5/6.5)	III	Approximately 6.5	6.2	6.5	6.9	6.7
IND10045 (8/8/8/8)	IV	Approximately 8	8.1	8.3	8.2	8.0

Means of administration: Intravenous

Report status: Final

#### METHODS:

##### Experimental design

Table 23: Experimental design

Dosage Group	Concentration (log <sub>10</sub> CCID <sub>50</sub> of each virus serotype /dosage) <sup>a</sup>	Batch Number	Dosage Volume (mL/rabbit)	Number of Rabbits	Assigned Rabbit Numbers
I	0 (Control Article)	(b) (4)	0.5	6	8501 - 8506
II	Approximately 5	S4316	0.5	6	8507 - 8512
III	Approximately 6.5	IND10044	0.5	6	8513 - 8518
IV	Approximately 8	IND10045	0.5	6	8519 - 8524

<sup>a</sup>The test article was considered 100% active/pure for the purpose of dosage calculations. CCID-Cell culture infective dose.

##### Rationale for dosage selection

The dose of approximately 5 log<sub>10</sub> CCID<sub>50</sub> of each CYD Dengue virus serotype corresponds to the human dose of the test vaccine. The dose of approximately 8 log<sub>10</sub> CCID<sub>50</sub> of each CYD

Dengue virus serotype was the maximum feasible dose. The dose of approximately  $6.5 \log_{10}$  CCID<sub>50</sub> of each CYD Dengue virus serotype was an intermediate dose.

Randomization procedure: Yes.

Statistical analysis plan: Yes.

Parameters evaluated:

*Table 24: Parameters evaluated*

Parameters	Frequency of Testing
Mortality	Twice daily
Clinical observations	Before dosing and hourly intervals for the first 4 hours after dosing and at the end of the normal working day on each day of dosing. Also, observations were recorded once daily on non-dosing days
Body weight	Twice during the acclimation period, twice weekly during the premating period (including each day of dosing), and on DGs 0, 6, 9, 12, 16, 20, 24, 27, and 29
Food consumption	Daily
Serology*	Prior to the dosing (baseline), DS 35 (two days before mating), on DG 6 (pre-dosage), and on DG 29
Euthanasia	DG 29
Scheduled maternal euthanasia for uteri and ovaries slides, gross lesion slides, numbers of corpora lutea, implantations, early resorptions, late resorptions, dead/live fetuses, placenta and external anomalies	DG 29
<u>Fetuses</u> Fetuses weighed Gross external alterations Internal examination Blood collected from vena cava for immunogenicity	DG 29

\* Blood was collected from the medial auricular artery.

**RESULTS:****Mortality**

No test article-related mortality, clinical observations, gross lesions, body weight before mating and during gestation in the high dose group, and food consumption (at premating and gestation periods) was reported. Prior to dosing on DG 6, body weight gains in groups 2, 3, and 4 were 138%, 150% and 88% of the control group, respectively. During the post-dosage period (DGs 6 through 29), mean body weight gains in groups 2, 3, and 4 were 49%, 72% and 98% of the control group, respectively. Overall, in groups 2 and 3, the body weight gains throughout the gestation period were lower when compared to the control group. These results did not show dose response relationship. Thus, it is considered questionable.

**Mating and fertility**

Pregnancy rate in the mated rabbits were 6, 3, 5, and 6 in groups 1, 2, 3, and 4, respectively. Fertility index (i.e., rabbits pregnant/rabbits in cohabitation) was 100%, 50%, 83.3% and 100% in groups 1, 2, 3, and 4, respectively. In the absence of any dose-relationship, the reduction in the fertility index in group 2 was considered to be incidental and of no toxicological relevance.

*Table 25: Summary of mating and fertility results*

DOSAGE GROUP CONCENTRATION a		I 0 (Control Article)	II 5 log <sub>10</sub>	III 6.5 log <sub>10</sub>	IV 8 log <sub>10</sub>
RABBITS EVALUATED	N	6	6	6	6
RABBITS THAT MATED b	N(%)	6(100.0)	4( 66.7)	5( 83.3)	6(100.0)
MATED BY MALE					
FIRST PAIRING	N(%)	6(100.0)	4(100.0)	0( 0.0)	6(100.0)
SECOND PAIRING	N(%)	0( 0.0)	0( 0.0)	5(100.0)	0( 0.0)
THIRD PAIRING	N(%)	0( 0.0)	0( 0.0)	0( 0.0)	0( 0.0)
FERTILITY INDEX c	N/N (%)	6/ 6 (100.0)	3/ 4 (75.0)	5/ 5 (100.0)	6/ 6 (100.0)
RABBITS PREGNANT/RABBITS IN COHABITATION	N/N (%)	6/ 6 (100.0)	3/ 6 (50.0)	5/ 6 (83.3)	6/ 6 (100.0)

- CCID50 (cell culture infective dose) of each virus serotype/dosage; the concentration is an approximation. Dosage occurred on day 1 of study (36 days before mating), day 21 of study (15 days before mating), and day 6 of gestation (6 days after mating).
- Each female rabbit was paired with an untreated male breeder rabbit of the same source and strain (up to a maximum of three pairings) and was monitored continuously until mating was confirmed, by observation, to have occurred at least twice.
- Number of pregnancies/number of rabbits that mated.

**Caesarean-sectioning and litter observations**

No test article-related changes in the litter averages for corpora lutea, implantations, the percentage of preimplantation loss, litter sizes, live fetuses, early and late resorptions, fetal body weights, the percentage of resorbed conceptuses, the percentage of live male fetuses and the percentage of post-implantation loss were reported. No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

Table 26: Summary of Caesarean-sectioning observations part 1

DOSAGE GROUP CONCENTRATION <sup>a</sup>		I 0 (Control Article)	II 5 log <sub>10</sub>	III 6.5 log <sub>10</sub>	IV 8 log <sub>10</sub>
RABBITS TESTED	N	6	6	6	6
INCLUDED IN ANALYSES	N	6	4 <sup>b</sup>	5 <sup>b</sup>	6
PREGNANT	N(%)	6(100.0)	3( 75.0)	5(100.0)	6(100.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	6	3	5	6
CORPORA LUTEA	MEAN±S.D.	8.8 ± 2.7	7.7 ± 0.6	8.8 ± 1.3	8.8 ± 2.1
IMPLANTATIONS	MEAN±S.D.	7.3 ± 1.9	6.3 ± 1.2	8.0 ± 1.9	6.8 ± 2.4
% PREIMPLANTATION LOSS	MEAN±S.D.	15.0 ± 11.3	17.9 ± 9.3	9.9 ± 10.5	21.8 ± 21.6
LITTER SIZES	MEAN±S.D.	7.3 ± 1.9	6.3 ± 1.2	7.8 ± 1.6	6.8 ± 2.4
LIVE FETUSES	N	44	19	39	41
	MEAN±S.D.	7.3 ± 1.9	6.3 ± 1.2	7.8 ± 1.6	6.8 ± 2.4
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
EARLY RESORPTIONS	N	0	0	1	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
LATE RESORPTIONS	N	0	0	0	0
% POSTIMPLANTATION LOSS	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 4.5	0.0 ± 0.0

% Pre-implantation loss = [(Number of corpora lutea – number of implantations) / number of corpora lutea] x 100

% Post-implantation loss = [(Number of implantation – number of live fetuses) / number of implantations] x 100

- CCID50 (cell culture infective dose) of each virus serotype/dosage; the concentration is an approximation. Dosage occurred on day 1 of study (36 days before mating), day 21 of study (15 days before mating), and day 6 of gestation (6 days after mating).
- Restricted to rabbits with a confirmed mating date.

Table 27: Summary of Caesarean-sectioning observations part 2

DOSAGE GROUP CONCENTRATION <sup>a</sup>		I 0(Control article)	II 5 log <sub>10</sub>	III 6.5 log <sub>10</sub>	IV 8 log <sub>10</sub>
RABBITS TESTED	N	6	6	6	6
Included in analysis	6	4 <sup>b</sup>	5 <sup>b</sup>	6	6
PREGNANT	N(%)	6(100.0)	3( 75.0)	5(100.0)	6(100.0)
Rabbits pregnant and Caesarean-sectioned					
On day 29 of gestation	N	6	3	5	6
Does with any resorption	N(%)	0( 0.0)	0( 0.0)	1( 20.0)	0( 0.0)
Does with all conceptuses resorped	N(%)	0( 0.0)	0( 0.0)	0( 0.0)	0( 0.0)
Does with viable fetuses	N(%)	6(100.0)	3(100.0)	5(100.0)	6(100.0)
Placentae appeared normal	N(%)	6(100.0)	3(100.0)	5(100.0)	6(100.0)

<sup>a</sup> CCID50 (cell culture infective dose) of each virus serotype/dosage; the concentration is an approximation. Dosage occurred on day 1 of study (36 days before mating), day 21 of study (15 days before mating), and day 6 of gestation (6 days after mating).

<sup>b</sup> Restricted to rabbits with a confirmed mating date.

*Table 28: Summary of litter observations (Caesarean-delivered fetuses)*

Dosage group		I	II	III	IV
Concentration <sup>a</sup>		0 (CONTROL ARTICLE)	5 log <sub>10</sub>	6.5 log <sub>10</sub>	8 log <sub>10</sub>
LITTERS WITH ONE OR MORE LIVE FETUSES					
	N	6	3	5	6
IMPLANTATIONS	MEAN±S D	7.3 ± 1.9	6.3 ± 1.2	8.0 ± 1.9	6.8 ± 2.4
LIVE FETUSES	N	44	19	39	41
	MEAN±S D	7.3 ± 1.9	6.3 ± 1.2	7.8 ± 1.6	6.8 ± 2.4
% LIVE MALE FETUSES/LITTER	MEAN±S D	56.7 ± 12.6	69.5 ± 11.6	53.3 ± 18.3	45.0 ± 13.7
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S D	42.63 ± 5.46	41.86 ± 1.79	43.55 ± 4.18	43.08 ± 6.45
MALE FETUSES	MEAN±S D	42.99 ± 6.02	41.29 ± 3.45	46.16 ± 3.67	43.84 ± 7.55
FEMALE FETUSES	MEAN±S D	41.77 ± 4.95	43.26 ± 3.11	41.42 ± 5.29	43.01 ± 5.90
% RESORBED CONCEPTUSES/LITTER	MEAN±S D	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 4.5	0.0 ± 0.0

<sup>a</sup> CCID50 (cell culture infective dose) of each virus serotype/dosage; the concentration is an approximation. Dosage occurred on day 1 of study (36 days before mating), day 21 of study (15 days before mating), and day 6 of gestation (6 days after mating).

#### Fetal Alterations

Fetal evaluations were based on 44, 19, 39 and 41 live DG 29 Caesarean-delivered fetuses in 6, 3, 5, and 6 litters in groups 1, 2, 3, and 4, respectively. Fetuses were examined for gross external alterations.

No test article-related gross external alterations (malformations or variations) were reported. In group 4, fetal gross external alterations were limited to one fetus (8522-11). This fetus had a meningocele on the head and open eye lids. Meningocele does occur spontaneously in the strain of rabbit and, thus, considered not related to test article treatment. No other fetal gross external alterations occurred.

*Table 29: Summary of fetal gross external alterations (Caesarean-delivered live fetuses (day 29 of gestation))*

DOSAGE GROUP CONCENTRATION a		I 0 (CONTROL ARTICLE)	II 5 log <sub>10</sub>	III 6.5 log <sub>10</sub>	IV 8 log <sub>10</sub>
LITTERS EVALUATED	N	6	3	5	6
LITTERS WITH LIVE FETUSES	N	6	3	5	6
FETUSES EVALUATED	N	44	19	39	41
LIVE	N	44	19	39	41
HEAD: MENINGOCELE					
LITTER INCIDENCE	N(%)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)	1 ( 16.7)
FETAL INCIDENCE	N(%)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)	1 ( 2.4)b
EYE: LID(S) OPEN					
LITTER INCIDENCE	N(%)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)	1 ( 16.7)
FETAL INCIDENCE	N(%)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)	1 ( 2.4)b

a. CCID<sub>50</sub> (cell culture infective dose) of each virus serotype/dosage; the concentration is an approximation. Dosage occurred on day 1 of study (36 days before mating), day 21 of study (15 days before mating), and day 6 of gestation (6 days after mating).  
b. Fetus 8522-11 had other gross external alterations.

### Immunogenicity

Following three intravenous injections of CYD dengue vaccine to groups 2, 3, and 4, the immunogenicity data showed seroconversion and anti-CYD antibody transfer.

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

### CONCLUSION

In conclusion, CYD Dengue vaccine concentrations of 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> did not induce vaccine-related embryo-fetal development effects in (b) (4) female rabbits. These dosage levels induced significant seroconversion and antibody transfer to developing offspring. Therefore, 5 log<sub>10</sub> CCID<sub>50</sub> CYD dengue vaccine was recommended for the pivotal developmental and reproductive toxicity study in rabbits.

**CYD Dengue Vaccine – Preliminary Development Toxicity Study in  
(b) (4) Mice Following One Intravenous Injection (Sponsor's  
Reference Number: SP0056 PS1003)**

*Reviewer: Claudia Wrzesinski*

The provided preliminary development toxicity study was designed to provide a preliminary evaluation of the effects of CYD Dengue Vaccine embryo-fetal development in pregnant (b) (4) female mice administered during gestation. This study evaluated the viremia and immunogenicity induced by CYD Dengue Vaccine in pregnant mice and tested the placental transfer of CYD Dengue Vaccine viruses and specific antibodies in order to provide necessary information for selection of dosage levels to be used in subsequent studies in mice.

*Table 30: Study design (table provided by the sponsor)*

Treatment group	Number of females*	Route of administration	Dose volume (mL)	Concentration (log <sub>10</sub> CCID <sub>50</sub> /dose)
Saline control	10	IV	0.5	0
CYD dengue vaccine	10	IV	0.5	Approximately 5 per dengue serotype
Mid dose	10	IV	0.5	Approximately 6.5 per dengue serotype
Highest feasible dose	10	IV	0.5	Approximately 8 per dengue serotype

\* Five mice per group were euthanized on DG 8; maternal blood samples and embryos were taken for qRT-PCR analysis. The remaining mice in each group were euthanized on DG 18 for caesarean-sectioning

Mice were mated and received either the Control Article, or the test article at 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> of each virus serotype, on gestation day 6 by intravenous administration at a constant dosage volume of 0.5 mL per mouse. The following parameters were evaluated for all mice: viability, clinical observations, maternal body weight and body weight changes, feed consumption, necropsy observations and Caesarean-section and litter parameters for mice euthanized on DG 18, fetal body weights, sex and an examination for fetal gross external alterations were performed. qRT-PCR and immunogenicity analyses were conducted on maternal and/or embryo/fetal samples collected on DG 8 and DG 18, respectively.

**RESULTS:**

All mice survived until scheduled euthanasia on DG 8 or DG 18. There were no clinical signs at any dosage level, and all mice appeared normal at necropsy.

A body weight loss was noted in the 8 log<sub>10</sub> CCID<sub>50</sub> dosage group following the single dose of CYD Dengue Vaccine on DG 6, resulting in slightly lower mean maternal body weight gain for DGs 6 through 18 when compared to controls. Corresponding reductions in absolute and relative feed consumption was noted at 8 log<sub>10</sub> CCID<sub>50</sub> during the same interval, as compared to the control article dosage group. There were no treatment-related changes in body weight, body weight gain and feed consumption in groups given 5 and 6.5 log<sub>10</sub> CCID<sub>50</sub> throughout the study. Pregnancy occurred in 8, 10, 9 and 6 out of 10 mice in the control, 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> dosage groups, respectively.

Fetal body weights were slightly reduced in the  $8 \log_{10} \text{CCID}_{50}$  dosage group, in comparison to the control article group values. There were no other effects on Caesarean-sectioning or litter parameters at concentrations of CYD Dengue Vaccine as high as  $8 \log_{10} \text{CCID}_{50}$ , and there were no test article-related fetal gross external alterations.

The qRT-PCR analysis showed that CYD dengue viral RNA was detected at low level in serum from 1/3 pregnant females and in 4/5 embryo samples of another female given  $8 \log_{10} \text{CCID}_{50}$ . No viral RNA was detected in lower dosage groups.

The immunogenicity data showed that there were very limited seroconversion and anti-CYD antibody transfer following the intravenous bolus injections of CYD dengue vaccine before mating and during gestation at the dose of 5 and  $6.5 \log_{10} \text{CCID}_{50}$ . The immune response and transfer of antibodies was slightly higher in mice given  $8 \log_{10} \text{CCID}_{50}$ .

**CONCLUSION:**

Based on these data, the CYD dengue vaccine dose levels of 5, 6.5 and  $8 \log_{10} \text{CCID}_{50}$  were recommended for the pivotal developmental and reproductive toxicity study in mice.

## Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation (pivotal):

### CYD Dengue Vaccine: Developmental and Reproductive Toxicity Study in (b) (4) Rabbits Following Repeated Intravenous Administrations (study # 20016549)

Reviewer: Nabil Al-Humadi

Performing laboratory: (b) (4)

Initiation date: September 21<sup>st</sup>, 2011

Final report date: June 28th, 2012

Test article, diluent, and control article information:

Table 31: Test article, diluent, and control article information

Test Article Information						
Name: CYD Dengue Vaccine <sup>a</sup>		Supplier:		Sponsor:		
Description	Batch Number	Date Received	Storage	Retest Date	Study Group	Dose Level (CCID <sub>50</sub> )
White freeze dried powder	S4316	23 SEP 2010	Refrigerated (2 C to 8 C)	APR 2012	II	Approximately 5

a. Synonymous with 323 CYD Dengue Vaccine.

Diluent Information						
Name	Description	Lot Number	Supplier	Date Received	Storage	Expiration Date
0.4% Sodium Chloride for Injection, (b) (4)	Clear, colorless liquid	(b) (4)	(b) (4)	12 OCT 2010	Room temperature	FEB 2012

a. (b) (4)

Control Article Information						
Name	Description	Lot Number	Supplier	Date Received	Storage	Expiration Date
0.9% Sodium Chloride for Injection, (b) (4)	Clear, colorless liquid	(b) (4)	(b) (4)	25 Aug and 22 Oct 2011	Room Temperature	30 Sep 2012

a. (b) (4)

Animal species and strain: (b) (4) Rabbit/(b) (4)

Breeder/supplier: (b) (4)

Number of female animal per group: 55

Age: 6.5-7 months

Average body weight: 2.8-3.8 kg

Route and site of administration: Intravenous

Volume of injection: 0.5 ml/animal

Frequency of administration and study duration: Animals were treated intravenously (in the marginal ear vein) on study day (DS) 1 (30 days prior to mating), DS 21 (10 days prior to mating) and again on gestation days (DGs) 6, 12, and 27. Study duration was 35 days *postpartum*

Dose: Approximately 5 log<sub>10</sub> CCID<sub>50</sub> of each CYD Dengue virus serotype

Stability: The virus concentration shows a decrease below 1 log for each serotype over 3 months. The detailed stability analysis and the certificate of analysis for the test article is available in appendix 2.

Means of administration: Intravenous

Report status: Final

## METHODS

Table 32: Experimental design

Group No.	Test Material (Batch/Lot No.)	Concentration (log <sub>10</sub> CCID <sub>50</sub> per Dengue Virus Serotype)	Dose Volume (mL)	Rabbit Numbers	
				Caesarean- Section	Natural Delivery
1	Control Article	0	0.5	6301 - 6325	6326 - 6330, 9475 <sup>a</sup> , 6332 - 6355
2	CYD Dengue (S4316)	Approximately 5	0.5 <sup>b</sup>	6356 - 6380	6381 - 6391, 2075 <sup>c</sup> , 6393 - 6410

<sup>a</sup>. Rabbit 6331 was excluded from study prior to dose administration because of low food consumption and was replaced with rabbit 9475.

<sup>b</sup>. For group 2, the dose volume was based on the fill volume of the vial (contents of 1 vial/rabbit/dose; approximately 500 µL).

<sup>c</sup>. Rabbit 6392 was found to have aborted 10 pups on DS 5. This rabbit was presumed to be pregnant upon arrival at the testing facility rather than a virgin as specified in the protocol. Rabbit 6392 was excluded from study and was replaced with rabbit 2075.

### Administration of test and control articles

#### F0 generation

Female rabbits were administered the test article formulation or the control article once on DS 1 (30 days prior to mating), DS 21 (10 days prior to mating) and again on DGs 6, 12 and 27. Dose administration was staggered to accommodate the mating schedule, such that the number of days between dose administration and mating were the same for each rabbit.

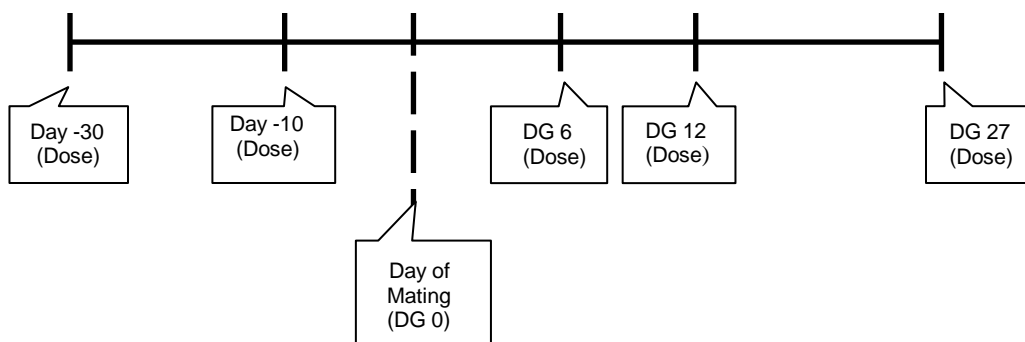


Figure 2: Test article administration procedure

F0 generation female rabbits were administered doses intravenously into the marginal ear vein (without disinfection).

Rationale for dosage selection

The dose of approximately  $5 \log_{10}$  CCID<sub>50</sub> of each CYD Dengue virus serotype corresponds to the human dose of the test vaccine.

Randomization procedure: Yes.

Statistical analysis plan: Yes.

Parameters evaluated

Table 33: Parameters evaluated

Parameters	Frequency of testing		
Mortality	Twice daily		
Clinical observations	Before dosing and hourly intervals for the first 4 hours after dosing and at the end of the normal working day on each day of dosing.		
Maternal observations	Days 4, 7, 10, 14, 18 and 21 <i>postpartum</i>		
Body weight	Pre-mating Period	Gestation Period (subgroup A and B)	<i>Postpartum</i> Period (subgroup B)
	At least twice weekly (including the days of dose administration)	DGs 0, 6, 9, 12, 16, 20, 24, 27, 29 and/or 34	Days 4, 7, 10, 14, 17, 21, 28 and 35 <i>postpartum</i>
Food consumption	Daily		
Serology*	The immunogenicity evaluation was performed for ten F0 generation females and their fetuses (rabbits assigned to ovarian and uterine examinations, subgroup A) and ten F0 generation females and their litters (rabbits assigned to natural delivery observations, subgroup B)		
		Subgroup	Sample Collection Time Points

Parameters	Frequency of testing																																																																													
	Group No.		No. of Females	Prior to Initiation of Dosing	DG 29	DL 35																																																																								
	1	A <sup>a</sup>	10	X	X	-																																																																								
		B <sup>b</sup>	10	X	-	X																																																																								
	2	A <sup>a</sup>	10	X	X	-																																																																								
		B <sup>b</sup>	10	X	-	X																																																																								
X = Sample collected; - = Not applicable. <sup>a</sup> Rabbits assigned to ovarian and uterine examinations (subgroup A). <sup>b</sup> Rabbits assigned to natural delivery (subgroup B).  Fetal samples were collected on DG29 from the vena cava Pup samples were collected on day 35 from the vena cava																																																																														
F1 generation: Viability Clinical observation Body weight  Development	Day 4 postpartum Once daily Pup body weights were recorded on days 4, 7, 10, 14, 18, 21, 28 and 35 postpartum <table><tr><th colspan="3">Parameters</th><th colspan="3">Day Initiated</th></tr><tr><td colspan="3">Hair Growth</td><td colspan="3">Day 5 <i>postpartum</i></td></tr><tr><td colspan="3">Eye Opening</td><td colspan="3">Day 7 <i>postpartum</i></td></tr><tr><td colspan="3">Air Righting</td><td colspan="3">Day 10 <i>postpartum</i></td></tr><tr><td colspan="3">Acoustic (Auditory) Startle</td><td colspan="3">Day 14 <i>postpartum</i></td></tr><tr><td colspan="3">Pupil Constriction</td><td colspan="3">Once on day 22 <i>postpartum</i></td></tr></table>						Parameters			Day Initiated			Hair Growth			Day 5 <i>postpartum</i>			Eye Opening			Day 7 <i>postpartum</i>			Air Righting			Day 10 <i>postpartum</i>			Acoustic (Auditory) Startle			Day 14 <i>postpartum</i>			Pupil Constriction			Once on day 22 <i>postpartum</i>																																						
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F0 generation	Terminal Procedures for All Rabbits Assigned to Ovarian and Uterine Examinations <table><tr><th rowspan="2">Group No.</th><th rowspan="2">No. of Rabbits</th><th rowspan="2">Scheduled Euthanasia Day</th><th colspan="4">Necropsy Procedures</th><th rowspan="2">Histology</th><th rowspan="2">Histopathology</th></tr><tr><th>Ovarian/ Uterine Examination</th><th>Necropsy</th><th>Tissue Collection</th><th>Organ Weights</th></tr><tr><td>1</td><td>25</td><td rowspan="2">DG 29</td><td rowspan="2">Full Exam</td><td rowspan="2">X</td><td rowspan="2">X</td><td rowspan="2">X</td><td>-</td><td>-</td></tr><tr><td>2</td><td>25</td><td>-</td><td>-</td></tr><tr><td colspan="3">Unscheduled Deaths</td><td>Full Exam</td><td>X</td><td>X</td><td>-</td><td>-</td><td>-</td></tr></table> X = Procedure conducted; - = Not applicable. Terminal Procedures for All Rabbits Assigned to Natural Delivery Observations <table><tr><th rowspan="2">Group No.</th><th rowspan="2">No. of Rabbits</th><th rowspan="2">Scheduled Euthanasia Day</th><th colspan="4">Necropsy Procedures</th><th rowspan="2">Histology</th><th rowspan="2">Histopathology</th></tr><tr><th>Ovarian/ Uterine Examination</th><th>Necropsy</th><th>Tissue Collection</th><th>Organ Weights</th></tr><tr><td>1</td><td>30</td><td rowspan="2">Day 35 Postpartum</td><td rowspan="2">Full Exam</td><td rowspan="2">X</td><td rowspan="2">X</td><td rowspan="2">X</td><td>-</td><td>-</td></tr><tr><td>2</td><td>30</td><td>-</td><td>-</td></tr><tr><td colspan="3">Unscheduled Deaths</td><td>Full Exam</td><td>X</td><td>X</td><td>-</td><td>-</td><td>-</td></tr></table> X = Procedure conducted; - = Not applicable.								Group No.	No. of Rabbits	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology	Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights	1	25	DG 29	Full Exam	X	X	X	-	-	2	25	-	-	Unscheduled Deaths			Full Exam	X	X	-	-	-	Group No.	No. of Rabbits	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology	Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights	1	30	Day 35 Postpartum	Full Exam	X	X	X	-	-	2	30	-	-	Unscheduled Deaths			Full Exam	X	X	-	-	-
Group No.	No. of Rabbits	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology																																																																						
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights																																																																								
1	25	DG 29	Full Exam	X	X	X	-	-																																																																						
2	25						-	-																																																																						
Unscheduled Deaths			Full Exam	X	X	-	-	-																																																																						
Group No.	No. of Rabbits	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology																																																																						
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights																																																																								
1	30	Day 35 Postpartum	Full Exam	X	X	X	-	-																																																																						
2	30						-	-																																																																						
Unscheduled Deaths			Full Exam	X	X	-	-	-																																																																						
Scheduled euthanasia	DG2 9 or DL 35																																																																													

\* Blood was collected from the medial auricular artery. DG = Gestation day; DL = Lactation day.

### Tissue collection and preservation

*Table 34: Tissue collection and preservation*

Tissue	Collected	Weighed	Comment
Cervix	X	-	Collected with the uterus. All rabbits.
Gross lesions/masses	X	-	All rabbits.
Heart	X	-	Rabbits found dead or unscheduled euthanized.
Kidneys	X	-	Rabbits found dead or unscheduled euthanized.
Liver	X	-	Rabbits found dead or unscheduled euthanized.
Lungs	X	-	Rabbits found dead or unscheduled euthanized.
Ovaries	X	X	All rabbits. Weighed at scheduled euthanasia only.
Spleen	X	-	Rabbits found dead or unscheduled euthanized.
Stomach	X	-	Rabbits found dead or unscheduled euthanized.
Uterus	X	-	All rabbits.
Vagina	X	-	All rabbits.

X = Procedure conducted; - = Not applicable.

### Tissue collection and preservation for pups

*Table 35: Tissue collection and preservation for pups*

Tissue	Collected	Weighed	Comment
Pups with gross lesions	X	-	Pups found dead on day 1 <i>postpartum</i> .
Gross lesions/masses	X	-	All pups
Heart	X	-	Pups found dead or unscheduled euthanized (Days 2 to 35 <i>postpartum</i> )
Kidney	X	-	Pups found dead or unscheduled euthanized (Days 2 to 35 <i>postpartum</i> )
Liver	X	-	Pups found dead or unscheduled euthanized (Days 2 to 35 <i>postpartum</i> )
Lung	X	-	Pups found dead or unscheduled euthanized (Days 2 to 35 <i>postpartum</i> )
Spleen	X	-	Pups found dead or unscheduled euthanized (Days 2 to 35 <i>postpartum</i> )
Stomach	X	-	Pups found dead or unscheduled euthanized (Days 2 to 35 <i>postpartum</i> )

X = Procedure to be conducted; - = Not applicable.

## RESULTS:

### Mortality

No test article-related mortality, abortion, clinical observations, body weight (during premating, gestation, and lactation period), food consumption (during premating, gestation, and lactation period), mating, fertility, terminal body weight, ovaries weight, and ovaries to body weight ratio was reported.

### Clinical signs

Clinical signs reported were limited to: sparse hair coat (underside, limbs and/or back), abnormal fecal output (scant/reduced, soft or liquid, none), fecal and/or urine staining (genital area or underside of tail), mild dehydration (based on skin turgor), thin body condition, localized alopecia (underside), ungroomed coat, lacrimation and red staining on the fur in the genital area. These observations were commonly reported in this species and strain of rabbit and there was no difference in the incidence of the observations between the control and treated groups.

## Ovarian and Uterine Examinations (tables 34 and 35)

Pregnancy were reported in 23 and 20 does (N=25 per group) in the control and the treated groups, respectively. These rabbits were examined for ovarian and uterine contents on DG 29.

No test article-related effects on ovarian or uterine parameters were reported. No test article-related effects on corpora lutea, implantations, the percentage of preimplantation loss, early and late resorptions, the percentage of post-implantation loss, fetal body weights, the percentage of resorbed conceptuses, and the percentage of live male fetuses were reported. No dead fetuses and no doe had a litter consisting of only resorbed conceptuses were reported. All placentae appeared normal.

No test article-related effects on the number of live fetuses (at DG 29) were reported. Statistically significant increase ( $p \leq 0.05$ ) in the averages of litter size and live fetuses were reported in the test article-treated group. However, this increase might be related to: 1) the distribution of the litters between subgroup A (ovarian and uterine examinations) and subgroup B (natural delivery); 2) slightly few litters that were examined on DG 29 in the treated group (20 litters vs. 23 in controls); and 3) a decrease, rather than an increase, may have been considered of potential toxicological relevance.

Table 36: Summary of Cesarean-sectioning observations-F0 generation

DOSE GROUP TEST MATERIAL (BATCH/LOT NUMBER) CONCENTRATION a,b		1 CONTROL ARTICLE 0 (CONTROL ARTICLE)	2 CYD DENGUE (S4316) 5 log <sub>10</sub>
RABBITS TESTED	N	24c	25
PREGNANT	N(%)	23( 95.8)	20( 80.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	23	20
CORPORA LUTEA	MEAN±S.D.	9.6 ± 1.9	10.2 ± 1.7
IMPLANTATIONS	MEAN±S.D.	9.0 ± 2.3	9.8 ± 1.6
% PREIMPLANTATION LOSS	MEAN±S.D.	7.6 ± 15.6	3.6 ± 6.3
LITTER SIZES	MEAN±S.D.	8.5 ± 2.1	9.8 ± 1.6*
LIVE FETUSES	N	195	19
	MEAN±S.D.	8.5 ± 2.1	5.1 ± 1.6* 9.8 ±
DEAD FETUSES	N	0	0
RESORPTIONS	MEAN±S.D.	0.5 ± 1.0	0.1 ± 0.3
EARLY RESORPTIONS	N	2	1
	MEAN±S.D.	0.1 ± 0.3	0.0 ± 0.2
LATE RESORPTIONS	N	10	1
	MEAN±S.D.	0.4 ± 1.0	0.0 ± 0.2
% POSTIMPLANTATION LOSS	MEAN±S.D.	4.9 ± 8.9	1.1 ± 3.5
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	23	20
DOES WITH ANY RESORPTIONS	N(%)	7(30.4)	2(10.0)

DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0 0)	0(0 0)
DOES WITH VIABLE FETUSES	N(%)	23(100 0)	20(100)
PLACENTAE APPEARED NORMAL	N(%)	23(100 0)	20(100 0)

% Pre-implantation loss = [(Number of corpora lutea – number of implantations) / number of corpora lutea] x 100

% Post-implantation loss = [(Number of implantations – number of live fetuses) / number of implantations] x 100

a CCID<sub>50</sub> (CCID - Cell Culture Infective Dose) of each CYD Dengue virus serotype/dose; the concentration is an approximation

b Dose administration occurred on days 1 and 21 of study and again on days 6, 12 and 27 of gestation

c Excludes rabbit 6302, which was presumed to have been pregnant at arrival to the testing facility and was euthanized due to abortion on day 28 of study (estimated day 30 of gestation)

\* Significantly different from the control group value (p≤0.05)

*Table 37: Summary of litter observations-F1 generation (Caesarean-delivered litters)*

DOSE GROUP TEST MATERIAL (BATCH/LOT NUMBER) CONCENTRATION a,b		1 CONTROL ARTICLE 0 (CONTROL ARTICLE)	2 CYD DENGUE (84316) 5 log <sub>10</sub>
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	20
IMPLANTATIONS	MEAN±S.D.	9.0 ± 2.3	9.8 ± 1.6
LIVE FETUSES	N	195	195
	MEAN±S.D.	8.5 ± 2.1	9.8 ± 1.6*
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	42.8 ± 23.4 [ 22]c	42.3 ± 14.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	40.13 ± 7.18	38.16 ± 4.96
MALE FETUSES	MEAN±S.D.	39.97 ± 6.92 [ 21]c,d	38.15 ± 5.36
FEMALE FETUSES	MEAN±S.D.	39.29 ± 6.57 [ 20]c,e	37.93 ± 4.83
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.9 ± 8.9	1.1 ± 3.5

[ ] = NUMBER OF VALUES AVERAGED

a. CCID<sub>50</sub> (CCID - Cell Culture Infective Dose) of each CYD Dengue virus serotype/dose; the concentration is an approximation.

b. Dose administration occurred on Days 1 and 21 of study and again on Days 6, 12 and 27 of gestation.

c. Excludes values for litter 6306; sex of fetuses 6306-5, 7, 8 and 9 were not recorded.

d. Litter 6318 had no male fetuses.

e. Litters 6304 and 6305 had no female fetuses.

\* Significantly different from the control group value (p≤0.05).

## Summary of fetal examination:

No fetal gross external or fetal soft tissue abnormalities were reported.

## Skeletal Examination

With the exception of two incidences of skeletal abnormalities [malformations and variations] (i.e., a hemivertebra present as a cervical rib and duplicated sternal centra), The litter and fetal skeletal abnormalities were low in incidence and occurred within the historical range of the testing facility (See appendix 24, historical control data). The duplicated sternal centra and the hemivertebra that was present as a cervical rib were reported in groups 1 and 2, respectively.

*Table 38: Summary of fetal skeletal alterations (day 29 of gestation)-F1 generation (Caesarean-delivered fetuses)*

DOSE GROUP		1	2
TEST MATERIAL (BATCH/LOT NUMBER)		CONTROL ARTICLE	CYD DENGUE (S4316)
CONCENTRATION a,b		0 (CONTROL ARTICLE)	5 log <sub>10</sub>
LITTERS EVALUATED	N	23	20
LITTERS WITH LIVE FETUS(ES)	N	23	20
FETUSES EVALUATED	N	195	195
LIVE	N	195	195
<hr/>			
SKULL: NASAL FRONTAL SUTURE IRREGULAR			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	3 ( 1.5) f
SKULL: NASAL, CONTAINS AN INTRANASAL			
LITTER INCIDENCE	N (%)	0 ( 0.0)	2 ( 10.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	2 ( 1.0) f
HYOID: ALA, ANGULATED			
LITTER INCIDENCE	N (%)	1 ( 4.3)	4 ( 20.0)
FETAL INCIDENCE	N (%)	1 ( 0.5)	5 ( 2.6)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA			
LITTER INCIDENCE	N (%)	3 ( 13.0)	2 ( 10.0)
FETAL INCIDENCE	N (%)	3 ( 1.5) d	2 ( 1.0)
CERVICAL VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION			
LITTER INCIDENCE	N (%)	1 ( 4.3)	0 ( 0.0)
FETAL INCIDENCE	N (%)	1 ( 0.5) d	0 ( 0.0)
CERVICAL VERTEBRAE: HEMIVERTEBRA			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h
CERVICAL VERTEBRAE: ARCHES, FUSED			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h
THORACIC VERTEBRAE: CENTRUM, BIFID			
LITTER INCIDENCE	N (%)	1 ( 4.3)	1 ( 5.0)
FETAL INCIDENCE	N (%)	1 ( 0.5) c	1 ( 0.5) h
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h
THORACIC VERTEBRAE: HEMIVERTEBRA			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h
THORACIC VERTEBRAE: ARCH, SMALL			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h
THORACIC VERTEBRAE: CENTRA, FUSED			
LITTER INCIDENCE	N (%)	1 ( 4.3)	0 ( 0.0)
FETAL INCIDENCE	N (%)	1 ( 0.5) c	0 ( 0.0)
CAUDAL VERTEBRAE: MISALIGNED			
LITTER INCIDENCE	N (%)	2 ( 8.7)	1 ( 5.0)
FETAL INCIDENCE	N (%)	2 ( 1.0)	1 ( 0.5)
RIBS: THICKENED			
LITTER INCIDENCE	N (%)	1 ( 4.3)	1 ( 5.0)
FETAL INCIDENCE	N (%)	1 ( 0.5)	1 ( 0.5)
RIBS: FUSED			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) g
MANUBRIUM: IRREGULARLY SHAPED			
LITTER INCIDENCE	N (%)	1 ( 4.3)	1 ( 5.0)
FETAL INCIDENCE	N (%)	1 ( 0.5) d	1 ( 0.5)
MANUBRIUM: FUSED			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h

DOSE GROUP		1	2
TEST MATERIAL (BATCH/LOT NUMBER)		CONTROL ARTICLE	CYD DENGUE (84316)
CONCENTRATION a,b		0 (CONTROL ARTICLE)	5 log <sub>10</sub>
LITTERS EVALUATED	N	23	20
LITTERS WITH LIVE FETUS (ES)	N	23	20
FETUSES EVALUATED	N	195	195
LIVE	N	195	195
<hr/>			
STERNAL CENTRA: INCOMPLETELY OSSIFIED			
LITTER INCIDENCE	N (%)	2 ( 8.7)	1 ( 5.0)
FETAL INCIDENCE	N (%)	2 ( 1.0) d	2 ( 1.0) e
STERNAL CENTRA: DUPLICATED			
LITTER INCIDENCE	N (%)	1 ( 4.3)	0 ( 0.0)
FETAL INCIDENCE	N (%)	1 ( 0.5) d	0 ( 0.0)
STERNAL CENTRA: FUSED			
LITTER INCIDENCE	N (%)	3 ( 13.0)	2 ( 10.0)
FETAL INCIDENCE	N (%)	3 ( 1.5)	3 ( 1.5) e, g, h
STERNAL CENTRA: ASYMMETRIC			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5) h
XIPHOID: IRREGULARLY SHAPED			
LITTER INCIDENCE	N (%)	1 ( 4.3)	0 ( 0.0)
FETAL INCIDENCE	N (%)	1 ( 0.5) d	0 ( 0.0)
PELVIS: PUBIS, INCOMPLETELY OSSIFIED			
LITTER INCIDENCE	N (%)	0 ( 0.0)	1 ( 5.0)
FETAL INCIDENCE	N (%)	0 ( 0.0)	1 ( 0.5)
PELVIS: PUBIS, NOT OSSIFIED			
LITTER INCIDENCE	N (%)	2 ( 8.7)	0 ( 0.0)
FETAL INCIDENCE	N (%)	2 ( 1.0)	0 ( 0.0)

a CCID<sub>50</sub> (CCID - Cell Culture Infective Dose) of each CYD Dengue virus serotype/dose; the concentration is an approximation

b Dose administration occurred on days 1 and 21 of study and again on days 6, 12 and 27 of gestation

c Fetus 6311-1 had other skeletal alterations

d Fetus 6320-3 had other skeletal alterations

e Fetus 6359-8 had other skeletal alterations

f Fetus 6363-1 had other skeletal alterations

g Fetus 6369-1 had other skeletal alterations

h Fetus 6369-5 had other skeletal alterations

## Malformations

### *Vertebrae*

One fetus in the control group had fused centra in the 7<sup>th</sup> and 8<sup>th</sup> thoracic vertebrae. This fetus also had a bifid centrum in the 8<sup>th</sup> thoracic vertebra. One fetus in group 2 had a hemivertebra (arch only) present as the 4<sup>th</sup> cervical vertebra with a corresponding finding of fused arches in the 4<sup>th</sup> and 5<sup>th</sup> cervical vertebrae. In addition, this fetus had a hemivertebra (arch only) present as the 4<sup>th</sup> thoracic vertebra with a corresponding finding of a small arch in the 4<sup>th</sup> thoracic vertebra. Other skeletal findings included a bifid centrum in the 5<sup>th</sup> thoracic vertebra, a unilaterally ossified centrum in the 1<sup>st</sup> thoracic vertebra, fusion of the manubrium to the 1<sup>st</sup> sternal centrum, an asymmetric 1<sup>st</sup> sternal centrum and fused sternal centra (1<sup>st</sup> through 3<sup>rd</sup>). No other findings occurred in this fetus.

### *Ribs*

One fetus in group 2 had fused ribs (left, 11<sup>th</sup> and 12<sup>th</sup>, base to proximal). In addition, this fetus had fused sternal centra (3<sup>rd</sup> and 4<sup>th</sup>).

## Variations

### *Skull*

One fetus in group 2 had an extra site of ossification in the right nasal bone (i.e., an intranasal). One fetus in group 2 had an irregularly shaped nasal-frontal suture and an extra site of ossification in the right nasal bone (i.e., an intranasal). One fetus in group 2 had an irregularly shaped nasal-frontal suture.

*Hyoid*

One or both hyoid alae were angulated in 1 and 5 fetuses from 1 and 4 litters in the control and treated groups, respectively.

*Vertebrae*

One fetus in each of groups 1 and 2 had a bifid centrum a thoracic vertebra. A misaligned caudal vertebra was present in 2 and 1 fetuses from 2 and 1 litters in groups 1 and 2, respectively. One fetus in group 2 had a unilaterally ossified centrum in the 1<sup>st</sup> thoracic vertebra.

*Ribs*

At least one thickened rib was reported in one fetus in each of the control and the treated groups (fetuses 6315-6 and 6367-4, respectively). A cervical rib at the 7<sup>th</sup> cervical vertebra, a common variation in this strain of rabbit<sup>15</sup>, was present in 3 and 2 fetuses from 3 and 2 litters in groups 1 and 2, respectively. One fetus in the control group had an irregularly shaped manubrium, incompletely ossified sternal centra (1<sup>st</sup> and 2<sup>nd</sup>), duplicated sternal centra (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 5<sup>th</sup>), an irregularly shaped xiphoid process, and a unilaterally ossified centrum in the 4<sup>th</sup> cervical vertebra.

*Sternum*

One fetus in the control group had an irregularly shaped manubrium, incompletely ossified sternal centra (1<sup>st</sup> and 2<sup>nd</sup>), duplicated sternal centra (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 5<sup>th</sup>), and an irregularly shaped xiphoid process, as previously described. One fetus in group 2 had a fused manubrium (to the 1<sup>st</sup> sternal centrum), as previously described. This fetus also had asymmetric 1<sup>st</sup> sternal centrum and fused sternebrae. An incompletely ossified 1<sup>st</sup> sternal centrum was reported in 2 and 2 fetuses from 2 and 1 litters in groups 1 and 2, respectively. Also, one fetus in group 2 had additional skeletal findings, including fused sternal centra. One or more fused sternal centra were reported in 3 and 3 fetuses from 3 and 2 litters in groups 1 and 2, respectively. One fetus in group 2 had an irregularly shaped manubrium.

*Pelvis*

Two fetuses in group 1 did not have ossified pubes. One fetus in group 2 had incompletely ossified pubes.

*Fetal ossification site averages*

In group 2, a statistically significant decrease ( $p \leq 0.05$ ) in the average number of ossified rib pairs and xiphoid process were reported. The average value for ossified rib pairs (12.41) and xiphoid (0.94) was within the historical range of the testing facility. In 30 studies conducted at the testing facility between June 2009 and June 2011 (615 control group litters; 5301 fetuses), the litter averages were 12.45 per fetus (range 12.34- 12.59 per study) for rib pairs and 0.95 per fetus (range 0.89-0.99 per study) for the xiphoid. These decreases in skeletal ossification occurred in the absence of any reductions in fetal body weight or any CYD Dengue vaccine-related fetal abnormalities, and were therefore, not considered to be incidental and of toxicological significance.

There were no statistically significant or biologically important differences among the two dose groups in the average numbers of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), sternum (manubrium and sternal centers), forelimbs (carpals, metacarpals, digits and phalanges) or hindlimbs (tarsals, metatarsals, digits and phalanges).

*Table 39: Summary of fetal ossification sites (day 29 of gestation)-F1 generation (Caesarean-delivered fetuses)*

DOSE GROUP TEST MATERIAL (BATCH/LOT NUMBER) CONCENTRATION a,b		1 CONTROL ARTICLE 0 (CONTROL ARTICLE)	2 CYD DENGUE (84316) 5 log <sub>10</sub>
LITTERS EXAMINED	N	23 195	20 195
OSSIFICATION SITES PER FETUS PER LITTER			
HYOID	MEAN±S D	1 00 ± 0 02	0 99 ± 0 03
VERTEBRAE			
CERVICAL	MEAN±S D	7 00 ± 0 00	7 00 ± 0 00
THORACIC	MEAN±S D	12 63 ± 0 28	12 52 ± 0 28
LUMBAR	MEAN±S D	6 36 ± 0 27	6 48 ± 0 27
SACRAL	MEAN±S D	3 00 ± 0 00	3 00 ± 0 00
CAUDAL	MEAN±S D	16 58 ± 0 45	16 40 ± 0 35
RIBS (PAIRS)	MEAN±S D	12 58 ± 0 28	12 41 ± 0 26*
STERNUM			
MANUBRIUM	MEAN±S D	1 00 ± 0 00	1 00 ± 0 00
STERNAL CENTERS	MEAN±S D	3 90 ± 0 18	3 94 ± 0 12
XIPHOID	MEAN±S D	1 00 ± 0 02	0 94 ± 0 09*
FORELIMB c			
CARPALS	MEAN±S D	0 00 ± 0 00	0 00 ± 0 00
METACARPALS	MEAN±S D	4 90 ± 0 19	4 92 ± 0 16
DIGITS	MEAN±S D	5 00 ± 0 00	5 00 ± 0 00
PHALANGES	MEAN±S D	13 86 ± 0 17	13 92 ± 0 16
HINDLIMB c			
TARSALS	MEAN±S D	1 99 ± 0 04	2 00 ± 0 00
METATARSALS	MEAN±S D	4 00 ± 0 00	4 00 ± 0 00
DIGITS	MEAN±S D	4 00 ± 0 00	4 00 ± 0 00
PHALANGES	MEAN±S D	12 00 ± 0 02	11 98 ± 0 04

a CCID<sub>50</sub> (CCID - Cell Culture Infective Dose) of each CYD Dengue virus serotype/dose; the concentration is an approximation

b Dose administration occurred on days 1 and 21 of study and again on days 6, 12 and 27 of gestation

c Calculated as average per limb

\* Significantly different from the control group value (p≤0.05)

### Natural delivery observations

Of the rabbits assigned to natural delivery observations (N=30/group), pregnancy occurred in 25 and 27 mated female rabbits in groups 1 and 2, respectively. One doe in group 2 was found dead on DG 26. All other pregnant does delivered litters.

No test article-related effects on natural delivery and litter observations were reported. No test article-related effects on the numbers of does delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of does with one or more liveborn pups/number of pregnant rabbits), the numbers of does with stillborn pups, litter sizes, viability and lactation indices, surviving pups per litter, the percentage of male pups per number of pups sexed per litter, live litter size at weighing and pup weight per litter were reported.

Between days 1 and 4 postpartum, two does in the control group had all pups die. One doe group 2 had no liveborn pups and another doe had all pups die between days 5 and 35 postpartum.

*Table 40: Summary of natural delivery observations-F0 generation female rabbits*

DOSE GROUP TEST MATERIAL (BATCH/LOT NUMBER) CONCENTRATION a,b		1 CONTROL ARTICLE 0 (CONTROL ARTICLE)	2 CYD DENGUE (S4316) 5 log <sub>10</sub>
RABBITS ASSIGNED TO NATURAL DELIVERY	N	30	30
PREGNANT	N	25 ( 83.3)	27 ( 90.0)
INCLUDED IN ANALYSES	N	25	26 <sup>c</sup>
DELIVERED LITTERS	N(%)	25(100.0)	26(100.0)
DURATION OF GESTATION d	MEAN±S.D.	32.2 ± 0.6	32.2 ± 0.7
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	191 <sup>e</sup> 8.7 ± 2.1 [ 22] <sup>e</sup>	201 7.7 ± 2.3
DOES WITH STILLBORN PUPS	N(%)	2 ( 8.0)	0 ( 0.0)
DOES WITH NO LIVEBORN PUPS	N	0 ( 0.0)	1 ( 3.8)
GESTATION INDEX f	% N/N	100.0 25/ 25	96.2 25/ 26
DOES WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	2 ( 8.0)	0 ( 0.0)
DOES WITH ALL PUPS DYING DAYS 5-35 POSTPARTUM	N	0 ( 0.0)	1 ( 4.0)

a. CCID<sub>50</sub> (CCID - Cell Culture Infective Dose) of each CYD Dengue virus serotype/dose; the concentration is an approximation.  
b. Dose administration occurred on Days 1 and 21 of study and again on Days 6, 12 and 27 of gestation.  
c. Excludes doe 6386, which was found dead on Day 26 of gestation.  
d. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the day the first pup was delivered.  
e. Excludes values for rabbits 6346, 6347 and 6350; number of implantation sites appeared incorrectly recorded.  
f. Number of rabbits with live offspring/number of pregnant rabbits.

*Table 41: Summary of litter observation (naturally delivered pups)-F1 generation delivered litters*

DOSE GROUP TEST MATERIAL (BATCH/LOT NUMBER) CONCENTRATION a,b		1 CONTROL ARTICLE 0 (CONTROL ARTICLE)	2 CYD DENGUE (S4316) 5 log <sub>10</sub>
DELIVERED LITTERS WITH			
ONE OR MORE LIVEBORN PUPS	N	25	25
PUPS DELIVERED (TOTAL)	N MEAN±S D	220 8.8 ± 2.1	190 7.6 ± 2.3
LIVEBORN	MEAN±S D N(%)	7.8 ± 2.6 195(88.6)	7.0 ± 2.7 174(91.6)
STILLBORN	MEAN±S D N(%)	0.1 ± 0.4 3(1.4)	0.0 ± 0.0 0(0.0)
UNKNOWN VITAL STATUS c	N	22	16
FOUND DEAD OR EUTHANIZED DUE TO ADVERSE CLINICAL OBSERVATIONS ON DAYS 1- 3 POSTPARTUM d	N	8	3
Pups found dead, euthanized due to adverse clinical observations or presumed cannibalized			
DAYS 4- 7	N/N(%)	27/187 (14.4)	17/171 (9.9)
DAYS 8-10	N/N(%)	8/160 (5.0)	9/154 (5.8)
DAYS 11-14	N/N(%)	2/152 (1.3)	1/145 (0.7)
DAYS 15-18	N/N(%)	1/150 (0.7)	2/144 (1.4)
DAYS 19-21	N/N(%)	0/149 (0.0)	0/142 (0.0)
DAYS 22-28	N/N(%)	0/149 (0.0)	0/142 (0.0)
DAYS 29-35	N/N(%)	0/149 (0.0)	0/142 (0.0)

VIABILITY INDEX e	%	85.6	90.0
	N/N	160/187	154/171
LACTATION INDEX f	%	93.1	92.2
	N/N	149/160	142/154

DAYS = Days postpartum

a. CCID50 (CCID - Cell Culture Infective Dose) of each CYD Dengue virus serotype/dose; the concentration is an approximation.

b. Dose administration occurred on days 1 and 21 of study and again on days 6, 12 and 27 of gestation.

c. Further examinations were not performed to determine whether these pups were stillborn or born alive and found dead.

d. These pups were found outside the nesting box on days 1 to 3 postpartum.

e. Number of live pups on day 7 postpartum/number of pups on day 4 postpartum.

f. Number of live pups on day 35 (weaning) postpartum/number of live pups on day 7 postpartum.

## F1 generation pups

No test article-related effects on the clinical signs were reported in the F1 generation pups.

Transient clinical observations included varying degrees of dehydration (based on skin turgor), thin body condition, emaciation, cold to touch (whole body), decreased motor activity, dyspnea, gasping, ataxia, hunched posture, low carriage, impaired and/or lost righting reflex, no use of the limb/paw, splayed forelimbs, ptosis, lack of maternal care (i.e., no milk band present, not nursing or not nesting, ungroomed coat and injuries (i.e., a laceration or scabs at one or more locations along the body, discolored [purple or black] body or tip of tail, malformed/cursed rib cage, swelling [left hindlimb, left hind paw, head], broken left forepaw, severely rotated left forepaw and a hole in the skull cap that extended into the brain) were reported.

*Table 42: Summary of clinical observations from birth to day 35 postpartum-F1 generation (naturally delivered pups)*

MATERNAL DOSE GROUP		1	2
TEST MATERIAL (BATCH/LOT NUMBER)		CONTROL ARTICLE	CYD DENGUE (S4316)
MATERNAL CONCENTRATION		0 (CONTROL ARTICLE)	5 log <sub>10</sub>
LITTERS EXAMINED (N)		25	26
TRANSIENT CLINICAL OBSERVATIONS:		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS	
a			
DEHYDRATION: TOTAL	N/N	96/14	95/11
MILD	N/N	40/11	65/9
MODERATE	N/N	39/11	7/5
SEVERE	N/N	14/5	22/9
SEVERITY NOT NOTED	N/N	3/3	1/1
THIN BODY CONDITION	N/N	36/10	11/6
EMACIATION	N/N	1/1	1/1
WHOLE BODY, COLD TO TOUCH	N/N	7/4	8/6
DECREASED MOTOR ACTIVITY	N/N	4/4	7/6
DYSPNEA	N/N	0/0	3/3
GASPING	N/N	2/2	2/2
ATAXIC	N/N	0/0	1/1
HUNCHED POSTURE	N/N	0/0	1/1
LOW CARRIAGE	N/N	0/0	1/1
IMPAIRED/LOST RIGHTING REFLEX	N/N	1/1	2/2
NO USE OF LEFT FORELIMB, LEFT HINDLIMB AND/OR LEFT	N/N	0/0	2/2
BOTH FORELIMBS, SPLAYED	N/N	0/0	15/1

TRANSIENT CLINICAL OBSERVATIONS: a			TOTAL FREQUENCY X PUPS)/LITTERS WITH (DAYS OBSERVATIONS)
	N/N	0/0	
NO MILK BAND PRESENT			1/1
NOT NURSING	N/N	9/5	3/3
NOT NESTING	N/N	1/1	2/2
UNGROOMED COAT	N/N	4/1	0/0
LEFT HINDLIMB, HEAD AND/OR RIGHT SIDE OF BACK,	N/N	1/1	1/1
SCAB(S)b	N/N	12/3	26/6
TIP OF TAIL, BLACK	N/N	0/0	3/1
BOTH SIDES OF BACK AND/OR BOTH	N/N	0/0	2/1
WHOLE BODY, PURPLE	N/N	0/0	1/1
PTOSIS	N/N	0/0	1/1
RIB CAGE APPEARED	N/N	0/0	1/1
LEFT HINDLIMB AND PAW, SWOLLEN	N/N	0/0	1/1
LEFT FOREPAW APPEARED BROKEN	N/N	0/0	1/1
LEFT FOREPAW SEVERELY ROTATED	N/N	0/0	1/1
HEAD, SWOLLEN	N/N	2/1	0/0
HOLE IN SKULL CAP THAT EXTENDED INTO BRAIN	N/N	1/1	0/0

- 
1. Tabulation restricted to adverse observations; all other pups appeared normal.
  2. Located on the head, neck, left forelimb, back, right side of back, left hindlimb and/or right hindlimb.

### Reflex and physical development

No test article-related effects on the reflex and physical development parameters were reported. The average day postpartum that at least 50% and 100% of the pups had reached the criterion is summarized in table 41.

*Table 43: Developmental landmark parameters*

Parameters	Day Initiated	Criterion Day (at least 50% passed)		Criterion Day (100% passed)	
		Control	Treated	Control	Treated
Hair Growth	Day 5 <i>postpartum</i>	5.0	5.0	5.0	5.0
Eye Opening	Day 7 <i>postpartum</i>	10.0	9.9	13	13
Air Righting	Day 10 <i>postpartum</i>	11.8	11.8	19	19
Acoustic Startle	Day 14 <i>postpartum</i>	14.0	14.0	18	18
Pupil Constriction	Once on Day 22 <i>postpartum</i>	22.0	22.0	22.0	22.0

CCID: cell culture infective dose

### Necropsy Observations

The necropsy findings that were reported in the F1 generation pups were not test article-related. On day 35 postpartum, there were 65 and 55 pups did not survive to scheduled euthanasia in groups 1 and 2, respectively. Of these pups, 6 and 3 did not have milk present in the stomach in groups 1 and 2, respectively. Other necropsy observations in the pups that did not survive to scheduled euthanasia included: the presence of a red gelatinous material on the brain, tan areas on one or more lobes of the liver and a broken left forelimb or left hindlimb. All pups that survived to scheduled euthanasia on day 35 postpartum appeared normal at necropsy examination.

*Table 44: Summary of necropsy observations-F1 generation (naturally delivered pups)*

MATERNAL DOSE GROUP TEST MATERIAL (BATCH/LOT NUMBER) MATERNAL CONCENTRATION		1 CONTROL ARTICLE 0 (CONTROL ARTICLE)	2 CYD DENGUE (S4316) 5 log <sub>10</sub>
LITTERS EVALUATED	N	25	26
TOTAL PUPS STILLBORN, FOUND DEAD OR EUTHANIZED DUE TO ADVERSE CLINICAL OBSERVATIONS <sup>a,b</sup>			
STILLBORN	N	2	0
FOUND DEAD	N	59	51
EUTHANIZED DUE TO ADVERSE CLINICAL OBSERVATIONS	N	4	4
NO MILK IN STOMACH <sup>c</sup>	N(%)	6( 9.5)	3( 5.4)
APPEARED NORMAL	N(%)	57( 87.7)	48( 87.3)
BRAIN AREA: RED GELATINOUS MATERIAL PRESENT	N(%)	1( 1.5)	1( 1.8)
LIVER: MEDIAN AND/OR LEFT LATERAL LOBE(S), TAN AREA(S)	N(%)	1( 1.5)	1( 1.8)
LEFT FORELIMB OR LEFT HINDLIMB: BROKEN	N(%)	0( 0.0)	2( 3.6)
PUPS EUTHANIZED AND NECROPSIED ON DAY 35 POSTPARTUM <sup>b</sup>			
LITTERS EVALUATED	N	23	24
PUPS EVALUATED	N	149	142
APPEARED NORMAL			
LITTER INCIDENCE	N(%)	23(100.0)	24(100.0)
PUP INCIDENCE	N(%)	149(100.0)	142(100.0)

a. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full evaluation.  
b. Refer to the individual pup clinical observations table for external clinical observations confirmed at necropsy.  
c. Analysis restricted to pups found dead, or euthanized due to adverse clinical observations and necropsied.

### Immunogenicity

Seroconversion and anti-CYD antibody transfer was detected for all samples after intravenous injection of CYD Dengue vaccine.

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

### CONCLUSION

No indication of maternal systemic toxicity was reported in this study. No test article-effects on mating performance and fertility, and no indication of teratogenic potential of the test vaccine as

well as no effect on pre and post-natal development of the pups were reported. A robust specific antibody response against each CYD Dengue virus serotypes were reported in all females. And, an efficient transfer of the vaccine specific maternal antibodies to the fetuses and the pups was reported.

## **CYD Dengue Vaccine: Developmental and Reproductive Toxicity Study in (b) (4) Mice Following One Intravenous Administration**

*Reviewer: Claudia Wrzesinski*

### **SUMMARY:**

After mating, 25 female mice were administered one single intravenous bolus injection of test article formulations at approximately 5, 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> of each CYD dengue virus on day of gestation (DG) 6, 9 or 12 (5 controls and 25 treated mice per timepoint in the main study). Twenty-five control female mice were administered one intravenous bolus injection of the control article (0.9% Sodium Chloride) on DG 6, 9 and 12.

Reductions in body weight gains and food consumption occurred in females given concentrations of 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6, 9 or 12. The most pronounced reductions in body weight gains and food consumption occurred in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9. Postimplantation loss was increased in females given concentrations of 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6 or 9. The most pronounced effects on postimplantation loss occurred in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9.

Fetal body weights were reduced in litters of females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 or 12. There were no treatment-related fetal gross external, soft tissue or skeletal abnormalities at any doses observed. A delay in ossification at 8 log<sub>10</sub> CCID<sub>50</sub> occurred at a concentration where reductions in the fetal body weights were observed. A reduced skeletal ossification also occurred in litters of females given at 5 and/or 6.5 log<sub>10</sub> CCID<sub>50</sub> on DG 9 or 12, but it was not considered to be of toxicological significance because the reductions were minimal, occurred in the absence of reduced fetal body weights, fetal abnormalities, effects on maternal body weight or food consumption, and/or will most likely resolve itself with further growth and development.

YFNS5 RNA was detected by qRT-PCR analysis in 12/13 dams given 8 log<sub>10</sub> CCID<sub>50</sub>, but not in dams given lower doses and no evidence of transfer to fetuses since YFNS5 RNA was not detected in the embryos of mice in any group.

There was a dose-related increase in the incidence of females with detection of specific CYD Dengue antibodies by the seroneutralization assay associated with some antibody transfer to pups.

The maternal and developmental No-Observed-Adverse-Effect Level for CYD dengue vaccine was established at 5 log<sub>10</sub> CCID<sub>50</sub> in this study.

**Study no.:** Sponsor Reference No. SP0056 DV1014; Testing Facility Study No. 20016584

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

**Date of study initiation:** 02 September 2011 (Protocol)

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** CYD Dengue Vaccine (Tetravalent Dengue Chimeric Vaccine): Concentration approximately 5 log<sub>10</sub> CCID<sub>50</sub>/dose: S4316, purity: 100%

Concentration approximately  $6.5 \log_{10}$  CCID<sub>50</sub>/mL: IND10044, purity: 100%

Concentration approximately  $8 \log_{10}$  CCID<sub>50</sub>/mL: IND10045; purity: 100%

**Doses:** Dose selection was based on the response elicited by administration of CYD Dengue Vaccine in previous investigative and preliminary embryo-fetal studies. The dose level of approximately  $5 \log_{10}$  CCID<sub>50</sub> of each CYD Dengue virus serotype corresponds to the human dose of vaccine at which no effects to dams and fetuses, with no antibody or virus detection were seen. The dose of approximately  $6.5 \log_{10}$  CCID<sub>50</sub> was an intermediate dose at which no effects to dams and fetuses, with limited antibody response and transfer, and no virus detection. The dose of approximately  $8 \log_{10}$  CCID<sub>50</sub> was the maximum feasible dose. At this dose level, virus was detected in fetuses with limited antibody response and minimal maternal toxicity and little or no developmental toxicity.

Each mouse in main study Groups 2, 3 and 4 received one intravenous injection at different stages of gestation to cover the period organogenesis in separate cohorts A, B and C (3-day apart), since no virus replication and detection are expected after subsequent injections. Mice in Group 1 received intravenous injections at three occasions to cover the period of organogenesis.

**Species/strain:** (b) (4) mice

**Number/sex/group:** 5 controls and 25 treated female mice per timepoint

**Route, formulation, volume, and infusion rate:** Intravenous

**Study design:** Two hundred and fifty virgin mice (b) (4) were randomly assigned to four dose groups (25 control and 75 mice in each treated group in the main study). After mating, 25 female mice were administered one single intravenous bolus injection of test article formulations at approximately  $5$ ,  $6.5$  or  $8 \log_{10}$  CCID<sub>50</sub> of each CYD dengue virus on day of gestation (DG) 6, 9 or 12 (5 controls and 25 treated mice per timepoint in the main study). Twenty-five control female mice were administered one intravenous bolus injection of the control article (0.9% Sodium Chloride) on DG 6, 9 and 12. An additional fifty satellite mice, used for viral exposure and transfer (qRT-PCR analysis), were randomly assigned to the four dose groups (5 control mice and 15 mice per treated group) and given one intravenous bolus injection on DG 9.

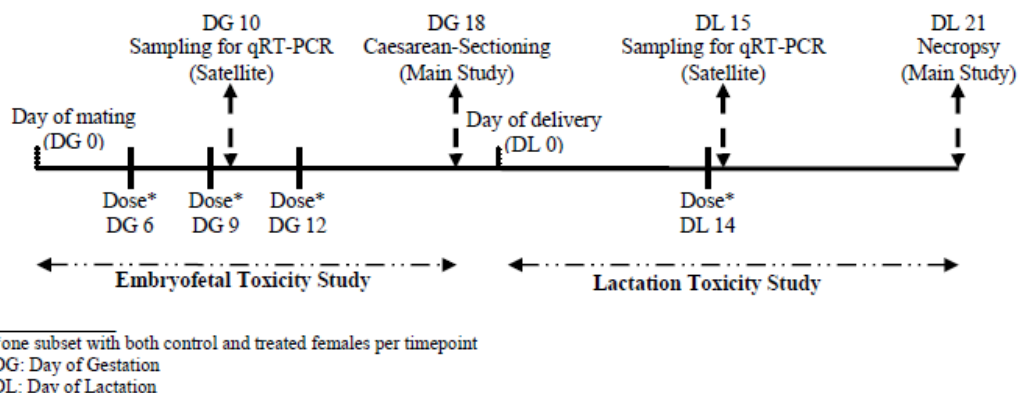


Figure 3: Developmental and reproductive toxicity study design (provided by the sponsor)

*Table 45: Experimental design (table provided by the sponsor)*

Group No.	No. of Mice	Cohort	Test Material	Concentration (log <sub>10</sub> CCID <sub>50</sub> )	Dosing Days		
					DG 6	DG 9	DG 12
1	25	A	Control Article	0	X	X	X
2	25	A	CYD Dengue (S4316)	approximately 5	X	-	-
	25	B			-	X	-
	25	C			-	-	X
3	25	A	CYD Dengue (IND10044)	approximately 6.5	X	-	-
	25	B			-	X	-
	25	C			-	-	X
4	25	A	CYD Dengue (IND10045)	approximately 8	X	-	-
	25	B			-	X	-
	25	C			-	-	X

X = Procedure to be conducted; - = Not applicable.

CCID – Cell culture infective dose; a) In the main study, 25 treated mice per timepoint (DGs 6, 9 and 12) and 25 control mice were administered the test and/or control article formulations on each occasion; 5 controls and 15 treated satellite mice were administered the test and/or control article formulations on DG 9; b) for Group 2, the dose volume was based on the fill volume of the vial (contents of 1 vial/mouse/dose; approximately 500 µL);

*Table 46: Dosing schedule for satellites (table provided by the sponsor)*

Group No.	No. of Mice	Test Material	Concentration (log <sub>10</sub> CCID <sub>50</sub> )	Dosing Day
1	5	Control Article	0	DG 9
2	15	CYD Dengue (S4316)	approximately 5	DG 9
3	15	CYD Dengue (IND10044)	approximately 6.5	DG 9
4	15	CYD Dengue (IND10045)	approximately 8	DG 9

Satellite mice used for viral exposure and transfer (qRT-PCR analysis).

## METHODS:

### Parameters and endpoints evaluated:

**Viability checks:** Twice daily

**Clinical observations:** Mice were observed for general appearance twice during the acclimation period, on DGs 0 and 3, at least once daily during the dose and postdose periods and once daily between DGs 6 and 18.

**Body weight:** Twice during the acclimation period, and on DGs 0, 3, 6, 9, 12, 15 and 18 for main study mice, and DGs 0, 3, 6 and 9 for satellite mice.

**Food consumption:** Daily during the dose and postdose periods (DGs 6 through 18 for main study mice and DGs 6 through 9 for satellite study mice).

**Mating Performance:** Daily during the cohabitation period and confirmed by observation of spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ*.

**Laboratory Evaluations:** qRT-PCR for all satellite dams and litters and immunogenicity for 10 pregnant main study mice (per cohort).

### Terminal Procedures:

Table 47: Terminal procedures for main study and satellite mice

Group No.	No. of Mice	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights		
1	5 <sup>a</sup>	DG 10	Pregnancy Status (implants and number of embryos)	-	-	-	-	-
2	15 <sup>a</sup>						-	-
3	15 <sup>a</sup>						-	-
4	15 <sup>a</sup>						-	-
1	25 <sup>b</sup>	DG 18	Full Exam	X	X	-	-	-
2	75 <sup>b</sup>						-	-
3	75 <sup>b</sup>						-	-
4	75 <sup>b</sup>						-	-

X = Procedure conducted; - = Not applicable.

<sup>a</sup> Satellite mice assigned to the qRT-PCR sample collection.

<sup>b</sup> Following euthanasia, maternal and fetal blood samples for immunogenicity evaluation were collected from the first 10 mice and their litter per cohort in each main study group [See Section 9.12.2 (Immunogenicity -Main Study)].

### Tissue Collection and Preservation:

Representative samples of the tissues were collected from mice that survived to scheduled euthanasia and preserved in 10% neutral buffered formalin, unless otherwise indicated. No tissues were collected or retained from mice assigned to the satellite study.

Table 48: Tissue collection and preservations

Tissue	Collected	Comment
Cervix	X	Collected with uterus. All nonpregnant mice.
Gross lesions/masses	X	All mice.
Ovaries	X	All nonpregnant mice.
Uterus	X	Collected with cervix. All nonpregnant mice.

X = Procedure conducted.

**Fetal Examinations:** Fetuses were examined for sex and for external abnormalities. Late resorptions and dead fetuses were examined for external abnormalities and sex to the extent possible. Following fetal gross external examination, fetal blood samples were collected from 10 litters (where possible) per cohort per dose group.

Approximately one-half of the fetuses in each litter were examined for visceral abnormalities by using a modification of the microdissection technique of Staples. Each fetus was fixed in Bouin's

solution and the heads were subsequently examined by free-hand sectioning; head sections were stored in alcohol. The decapitated carcasses were not retained.

The remaining fetuses (approximately one-half of the fetuses in each litter) were examined for skeletal abnormalities after staining with alizarin red.

### **Statistical analysis:**

Averages and percentages were calculated. Litter values were used where appropriate. Only body weights of live fetuses were used to determine litter mean fetal body weight. Individual data collected for mice assigned to the satellite study were reported but were not summarized or analyzed statistically.

Clinical observations and other proportional data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data (e.g., maternal body weights, body weight changes, food consumption values and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights and fetal anomaly data) were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance when appropriate [i.e., Bartlett's Test was not significant ( $p > 0.001$ )]. If the Analysis of Variance was significant ( $p \leq 0.05$ ), Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test is significant ( $p \leq 0.001$ )], the Kruskal-Wallis Test was used ( $\leq 75\%$  ties). In cases where the Kruskal-Wallis Test was statistically significant ( $p \leq 0.05$ ), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data. Count data were evaluated using the procedures described above for the Kruskal-Wallis Test.

### **RESULTS:**

#### **F0 Generation**

##### Mortality/Clinical signs:

All main study mice survived to scheduled euthanasia on DG 18. All satellite mice survived to scheduled euthanasia on DG 10. There were no adverse clinical signs at any concentration of CYD Dengue Vaccine when given to mice on DG 6, 9 or 12. All mice in the control group and Cohort C (Dosing Day DG 12) appeared normal. Clinical signs observed in Cohorts A (Dosing Day DG 6) and B (Dosing Day DG 9) were limited to individual mice in the 8 log<sub>10</sub> CCID<sub>50</sub> group. These clinical signs included discoloration (purple) at the injection site, lacrimation, a lenticular opacity and ptosis, these findings were seen with a low incidence rate (one female) and short duration (one occasion). A missing tip of the tail also occurred in one control female.

##### **Maternal Body Weights and Body Weight Changes:**

There was a treatment-related decrease in maternal body weight gain in the 6.5 and/or 8 log<sub>10</sub> CCID<sub>50</sub> groups, following the single dose of CYD Dengue Vaccine on DG 6, 9 and/or 12, as compared to the control article dose group. The reduction in body weight gain, which generally correlated with a decrease in food consumption, also correlated with a decrease in litter sizes, in addition to the effect of high dose administration.

**Cohort A:** vaccination on DG 6: A slight transient treatment-related decrease in maternal body weight gains was observed in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6. The mean maternal body weight gains on DGs 6 to 9 and the maternal body weight on DG 9 were significantly reduced ( $p \leq 0.05$  or  $p \leq 0.01$ ), in comparison to the control values. Thereafter, on DGs 9 to 12, body weight gains were significantly increased ( $p \leq 0.05$ ) and then reduced again on DGs 15 to 18, in comparison to the control values. The mean body weight gain on DGs 6 through 18 was 90% of the control value and the mean maternal body weights on DG 18 was comparable to the control value. Body weights and body weight gains were unaffected by the 5 and 6.5 log<sub>10</sub> CCID<sub>50</sub> doses given on DG 6.

*Table 49: Maternal body weights summary cohort A*

		CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
Day of gestation	Control	Approx. 5	Approx. 6.5	Approx. 8
0	25.3 ± 1.4	25.3 ± 1.4	25.3 ± 1.5	25.4 ± 1.5
3	26.6 ± 1.4	26.6 ± 1.4	26.8 ± 1.5	26.7 ± 1.7
6	28.5 ± 1.4	28.7 ± 1.6	28.7 ± 1.7	28.8 ± 1.7
9	31.0 ± 2.0	31.3 ± 1.8	31.1 ± 1.9	29.7 ± 1.9*
12	36.3 ± 2.5	36.6 ± 2.3	36.6 ± 2.3	35.9 ± 2.8
15	43.8 ± 3.6	44.3 ± 3.3	44.5 ± 3.6	43.1 ± 4.5
18	54.7 ± 5.6	55.2 ± 4.9	55.4 ± 5.3	52.4 ± 7.5

\* Significantly different from the Group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (Dosing Day 6).

*Table 50: Maternal body weight gains summary cohort A*

		CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
Day of gestation	Control	Approx. 5	Approx. 6.5	Approx. 8
0-3	+1.3 ± 0.8	+1.3 ± 0.7	+1.5 ± 0.8	+1.3 ± 0.7
3-6	+2.0 ± 0.5	+2.0 ± 0.7	+1.9 ± 0.7	+2.1 ± 0.6
6-9	+2.4 ± 0.8	+2.6 ± 0.8	+2.4 ± 0.7	+0.9 ± 0.9**
9-12	+5.3 ± 1.1	+5.3 ± 1.0	+5.5 ± 1.1	+6.2 ± 1.6*
12-15	+7.5 ± 1.4	+7.8 ± 1.6	+8.0 ± 1.5	+7.2 ± 2.1
15-18	+10.9 ± 2.2	+10.9 ± 2.1	+10.8 ± 2.1	+9.3 ± 3.3
6-18	+26.2 ± 4.8	+26.5 ± 4.3	+26.7 ± 4.5	+23.6 ± 7.0
0-18	+29.4 ± 5.1	+29.9 ± 4.4	+30.0 ± 5.2	+27.0 ± 7.0

\* Significantly different from the Group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 6). \*\* Significantly different from the Group 1 value ( $p \leq 0.01$ ); analyses restricted to Groups 2 through 4 (Dosing Day 6).

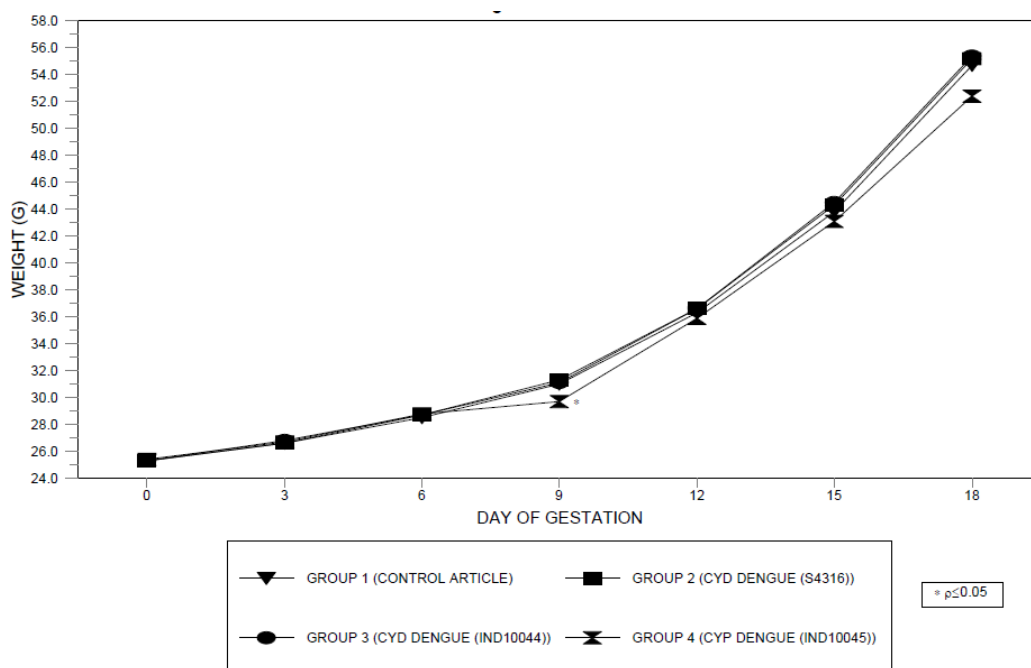


Figure 4: Maternal body weights on cohort A (figure provided by the sponsor)

**Cohort B:** vaccination on DG 9: A treatment-related decrease in maternal body weight gains was observed in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9. The mean maternal body weight gains were significantly reduced ( $p \leq 0.01$ ) on DGs 9 through 18, in comparison to the control values. There was also a transient treatment-related decrease in maternal body weight gains in females given 6.5 log<sub>10</sub> CCID<sub>50</sub> on DG 9. The mean maternal body weight gain on DGs 9 to 12 was significantly reduced ( $p \leq 0.01$ ), in comparison to the control value. However, subsequent body weight gains and maternal body weight on DG 18 were comparable to the control values.

The mean maternal body weights at 8 log<sub>10</sub> CCID<sub>50</sub> on DG 15 and DG 18 were consequently significantly reduced ( $p \leq 0.01$ ), in comparison to the control values (85% and 76% of control values, respectively).

Body weights and body weight gains were unaffected by the 5 log<sub>10</sub> CCID<sub>50</sub> dose given on DG 9.

Table 51: Maternal body weights summary cohort B

Day of gestation	Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
		Approx. 5	Approx. 6.5	Approx. 8
0	25.3 ± 1.4	25.9 ± 1.4	25.9 ± 1.5	26.1 ± 1.4
3	26.6 ± 1.4	26.9 ± 1.8	26.7 ± 1.8	27.3 ± 1.4
6	28.5 ± 1.4	29.0 ± 1.8	28.9 ± 1.9	29.2 ± 1.4
9	31.0 ± 2.0	31.6 ± 2.3	31.8 ± 2.0	31.9 ± 1.8
12	36.3 ± 2.5	36.5 ± 3.2	36.1 ± 2.7	35.2 ± 2.2
15	43.8 ± 3.6	44.5 ± 4.1	43.7 ± 3.4	37.3 ± 4.1##
18	54.7 ± 5.6	55.2 ± 5.3	53.8 ± 5.4	41.8 ± 6.6##

## Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 9).

Table 52: Maternal body gains summary cohort B

Day of gestation	Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
		Approx. 5	Approx. 6.5	Approx. 8
0-3	+1.3 ± 0.8	+0.9 ± 0.6	+0.8 ± 1.0	+1.2 ± 0.7
3-6	+2.0 ± 0.5	+2.2 ± 0.5	+2.2 ± 0.9	+1.9 ± 0.8
6-9	+2.4 ± 0.8	+2.6 ± 0.8	+2.9 ± 1.0	+2.7 ± 0.7
9-12	+5.3 ± 1.1	+4.9 ± 1.3	+4.4 ± 1.1**	+3.4 ± 1.2**
12-15	+7.5 ± 1.4	+8.0 ± 1.6	+7.5 ± 1.7	+2.1 ± 2.7**
15-18	+10.9 ± 2.2	+10.6 ± 1.7	+10.1 ± 2.5	+4.5 ± 2.8**
6-18	+26.2 ± 4.8	+26.1 ± 4.0	+24.9 ± 4.9	+12.7 ± 5.8**
0-18	+29.4 ± 5.1	+29.2 ± 4.3	+27.9 ± 5.1	+15.7 ± 6.2**

\* Significantly different from the group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 9). \*\* Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 9).

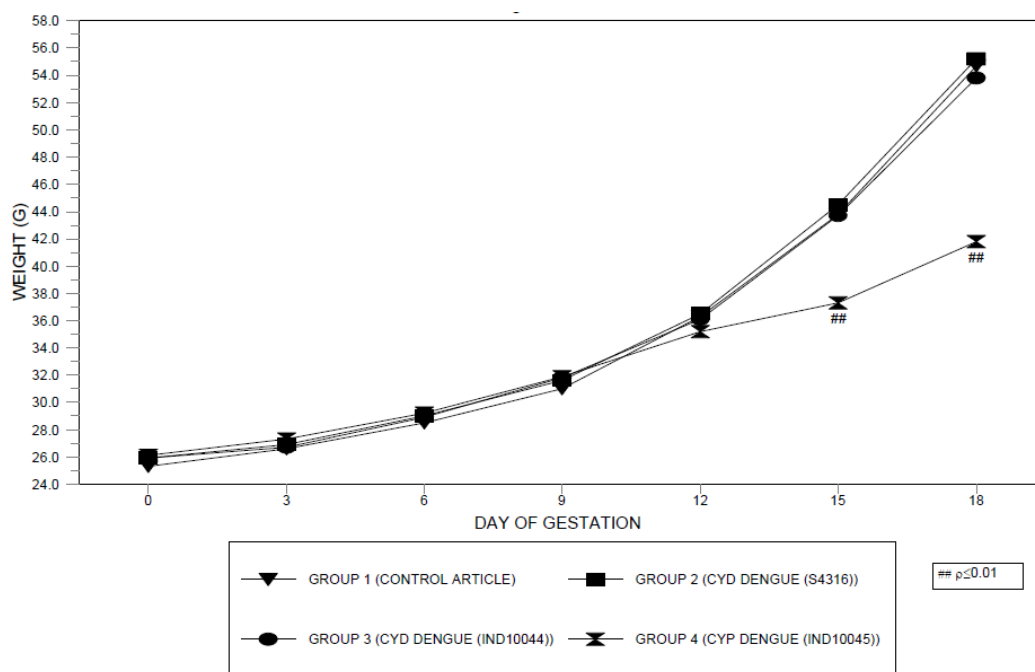


Figure 5: Maternal body weights of cohort B (figure provided by the sponsor)

**Cohort C:** vaccination on DG 12: There was a treatment-related decrease in maternal body weight gains in females given 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 12. The mean maternal body weight gains were very slightly reduced on DGs 12 to 15 and DGs 15 to 18, with reductions reaching statistical significance ( $p \leq 0.05$ ) on DGs 15 to 18, in comparison to the control values. However, the mean maternal body weights in these groups on DG 18 were comparable to the control value. Cohort C Administered CYD Dengue Vaccine on DG 12 Body weights and body weight gains were unaffected by the 5 log<sub>10</sub> CCID<sub>50</sub> dose given on DG 12. Changes in body weights and body weight gains that occurred before DG 12 were considered unrelated to the test article because they occurred before dose administration.

Table 53: Maternal body weights summary cohort C

Day of gestation	Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
		Approx. 5	Approx. 6.5	Approx. 8
0	25.3 ± 1.4	26.0 ± 1.0	26.0 ± 1.3	26.4 ± 1.1**
3	26.6 ± 1.4	27.2 ± 1.2	27.3 ± 1.6	27.3 ± 1.3
6	28.5 ± 1.4	28.8 ± 1.4	28.8 ± 1.8	28.6 ± 1.4
9	31.0 ± 2.0	31.7 ± 1.8	31.7 ± 2.1	31.4 ± 1.6
12	36.3 ± 2.5	37.9 ± 2.1	37.6 ± 2.7	37.6 ± 2.4
15	43.8 ± 3.6	45.2 ± 2.4	44.9 ± 4.4	44.5 ± 3.6
18	54.7 ± 5.6	55.6 ± 3.3	54.4 ± 6.9	53.9 ± 5.0

\*\*Significantly different from the group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 12).

Table 54: Maternal body gains summary cohort C

Day of gestation	Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
		Approx. 5	Approx. 6.5	Approx. 8
0-3	+1.3 ± 0.8	+1.2 ± 0.7	+1.2 ± 0.7	+0.9 ± 0.9
3-6	+2.0 ± 0.5	+1.6 ± 0.5	+1.4 ± 1.0*	+1.4 ± 0.6**
6-9	+2.4 ± 0.8	+2.9 ± 0.9	+3.0 ± 1.0	+2.8 ± 0.7
9-12	+5.3 ± 1.1	+6.2 ± 0.9*	+5.9 ± 1.4	+6.1 ± 1.1*
12-15	+7.5 ± 1.4	+7.2 ± 1.1	+7.3 ± 2.1	+6.9 ± 1.6
15-18	+10.9 ± 2.2	+10.5 ± 1.3	+9.5 ± 2.8*	+9.5 ± 1.8*
6-18	+26.2 ± 4.8	+26.8 ± 3.0	+25.7 ± 6.4	+25.3 ± 4.1
0-18	+29.4 ± 5.1	+29.7 ± 3.2	+28.3 ± 6.4	+27.6 ± 4.6

\* Significantly different from the group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 9). \*\* Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 9).

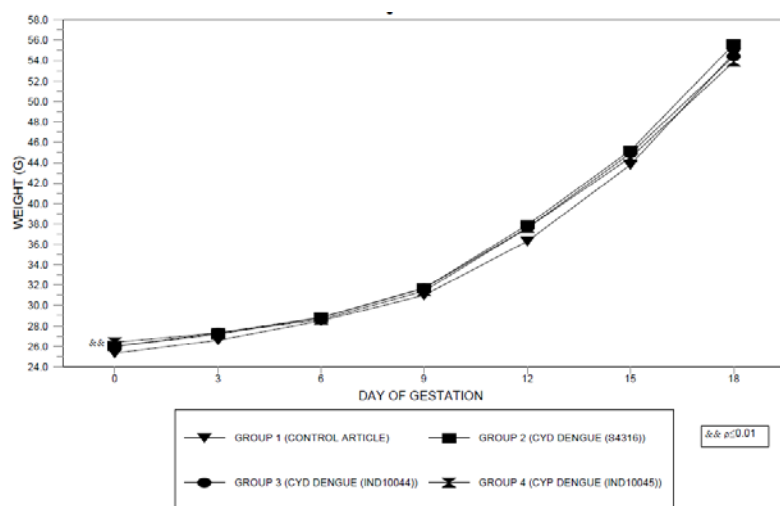


Figure 6: Maternal body weights of cohort C (figure provided by the sponsor)

### Maternal Food Consumption:

Corresponding to reductions in body weight gains, there was a treatment-related decrease in absolute (g/day) and relative (g/kg/day) food consumption following the single dose of CYD Dengue Vaccine at 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub>, as compared to the control group.

**Cohort A:** vaccination on DG 6: A transient treatment-related decrease in food consumption was observed in females given 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6. The mean food consumption on DG 6 to 9 was significantly reduced ( $p \leq 0.05$  or  $p \leq 0.01$ ), as compared to the control group. Thereafter, mean food consumption values were comparable among the dose groups, but cumulative mean food consumption value from DG 6 through DG 18 was significantly reduced ( $p \leq 0.01$ ) at 8 log<sub>10</sub> CCID<sub>50</sub>.

Food consumption was not affected by the by the 5 log<sub>10</sub> CCID<sub>50</sub> dose given on DG 6.

*Table 55: Maternal absolute food consumption cohort A*

Day of gestation	Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
		Approx. 5	Approx. 6.5	Approx. 8
6-9	6.0 ± 0.5	6.0 ± 0.6	5.4 ± 0.9*	4.7 ± 0.8**
9-12	5.9 ± 0.4	5.9 ± 0.6	5.8 ± 0.5	5.8 ± 0.6
12-15	6.6 ± 0.6	6.6 ± 0.7	6.8 ± 0.6	6.6 ± 0.6
15-18	7.3 ± 0.7	7.4 ± 0.5	7.5 ± 0.6	7.1 ± 1.0
6-18	6.5 ± 0.4	6.4 ± 0.4	6.4 ± 0.6	6.0 ± 0.5**

\* Significantly different from the group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 6). \*\*

Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 6).

**Cohort B:** vaccination on DG 9: A treatment-related decrease in food consumption was observed in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9. The mean absolute food consumption values from DG 9 through DG 18 were significantly reduced ( $p \leq 0.01$ ), as compared to the control group. However, the reported differences were small, except for the time period from gestation day 9 to 12. There was also a transient treatment-related decrease in food consumption in females given 6.5 log<sub>10</sub> CCID<sub>50</sub> on DG 9. Food consumption was not affected by the 5 log<sub>10</sub> CCID<sub>50</sub> dose given on DG 9.

*Table 56: Maternal absolute food consumption cohort B*

Day of gestation	Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
		Approx. 5	Approx. 6.5	Approx. 8
6-9	6.0 ± 0.5	6.2 ± 0.8	5.8 ± 1.0	6.0 ± 0.7
9-12	5.9 ± 0.4	5.8 ± 0.6	5.5 ± 1.0	4.6 ± 0.7**
12-15	6.6 ± 0.6	6.7 ± 0.8	6.5 ± 0.6	6.0 ± 0.6**
15-18	7.3 ± 0.7	7.0 ± 0.5	7.1 ± 0.6	6.4 ± 0.6**
6-18	6.5 ± 0.4	6.5 ± 0.6	6.3 ± 0.7	5.8 ± 0.5**

\* Significantly different from the group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 6). \*\*

Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 9).

**Cohort C:** vaccination on DG 12: A slight, but statistically significant treatment-related decrease in food consumption was observed in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 12. The mean food consumption values on DGs 12 to 15 were significantly reduced ( $p \leq 0.01$ ), as compared to the control group. Thereafter, food consumption values were comparable among the dose groups. Food consumption was not affected by the 5 and 6.5 log<sub>10</sub> CCID<sub>50</sub> doses given on DG 12.

*Table 57: Maternal absolute food consumption cohort C*

		CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
Day of gestation	Control	Approx. 5	Approx. 6.5	Approx. 8
6-9	6.0 ± 0.5	6.0 ± 0.8	6.0 ± 0.9	6.0 ± 0.7
9-12	5.9 ± 0.4	6.1 ± 0.8	6.0 ± 0.9	6.0 ± 0.6
12-15	6.6 ± 0.6	6.7 ± 0.6	6.6 ± 0.7	5.8 ± 0.6**
15-18	7.3 ± 0.7	7.4 ± 0.6	7.2 ± 0.7	7.0 ± 0.7
6-18	6.5 ± 0.4	6.6 ± 0.6	6.6 ± 0.6	6.2 ± 0.5

\* Significantly different from the group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 6). \*\* Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 9).

## **Necropsy:**

### **Maternal necropsy:**

*Table 58: Summary of maternal necropsy findings*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Mice examined	N	25	25	25	25
Mortality	N	0	0	0	0
Appeared normal	N	24	25	25	25
Liver: all lobes numerous tan areas	N	1	0	0	0

**Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):*****Cohort A:*** vaccination on DG 6***Table 59: Summary of ovary, uterine and litter parameters in cohort A***

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )			Historical control data
			Approx. 5	Approx. 6.5	Approx. 8	
Pregnant	N (%)	24 (96.0)	24 (96.0)	24 (96.0)	20 (80.0)	91.2 (60.0-100)
Corpora lutea	Mean ± SD	13.0 ± 2.1	13.0 ± 2.5	13.0 ± 2.1	13.2 ± 2.6	13.4 (11.0-14.5)
Implantations	Mean ± SD	12.8 ± 2.3	12.7 ± 2.4	12.7 ± 2.5	13.0 ± 3.2	13.1 (11.0-14.4)
Litter Size	Mean ± SD	12.1 ± 2.5	12.0 ± 2.5	12.1 ± 2.5	11.0 ± 4.0	-
Live Fetuses	Mean ± SD	12.1 ± 2.5	12.0 ± 2.4	12.1 ± 2.5	11.0 ± 4.0	12.2 (9.7-14.0)
Dead fetuses	Mean ± SD	0	0.1 ± 0.3	0.0 ± 0.2	0.0 ± 0.2	0.3 (0-1.8)
Total Resorptions	Mean ± SD	0.7 ± 0.8	0.7 ± 0.8	0.6 ± 0.7	1.9 ± 2.4	0.9 (0.6-2.1)
Early Resorptions	Mean ± SD	0.6 ± 0.8	0.5 ± 0.7	0.5 ± 0.6	1.7 ± 2.4	0.8 (0.3-1.6)
% Postimplantation loss	Mean ± SD	5.9 ± 6.9	6.4 ± 7.2	4.8 ± 6.1	18.8 ± 27.0	8.3 (5.3-15.5)
Dams with total litter loss	Mean ± SD	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0.2 (0-9.1)
Dams with viable fetuses	N (%)	24 (100.0)	24(100.0)	24(100.0)	19( 95.0)	99.9 (90.6-100)
% Dead or Resorbed Conceptuses/Litter	Mean ± SD	5.9 ± 6.9	6.4 ± 7.2	4.8 ± 6.1	14.6 ± 19.6 <sup>b</sup>	7.2 (4.5-10.4)
Dams with any resorptions	N (%)	12( 50.0)	12( 50.0)	11( 45.8)	14( 70.0)	55.9 (40.0-76.2)
Placenta appeared normal	N (%)	24(100.0)	24(100.0)	24(100.0)	19(100.0)	-
% Live male fetuses/litter	Mean ± SD	50.6 ± 15.6	53.2 ± 14.5	49.9 ± 16.4	43.4 ± 15.4	50.3 (42.4-56.0)
Live fetal body weights	Mean ± SD	1.31 ± 0.07	1.33 ± 0.11	1.30 ± 0.11	1.26 ± 0.09	1.32 (1.20-1.37)
% dead or resorbed conceptuses/litter	Mean ± SD	5.9 ± 6.9	6.4 ± 7.2	4.8 ± 6.1	14.6 ± 19.6	7.2 (4.5-10/4)

*a. Testing facility historical control data compiled between June 2006 and June 2010 in mice based on 31 studies that evaluated 669 pregnant mice on DG 18. ## = Significantly different from the group 1 value ( $p \leq 0.01$ ). b. This value is based on dams with one or more live fetuses.*

There was an increase in the postimplantation loss associated with an increase in the resorptions (total and early) and in the percentage of dead or resorbed conceptuses per litter in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6, in comparison with the control group. Overall, the litter size and number of live fetuses was lower than the respective control values. In addition, one dam had a litter consisting of only resorbed conceptuses. The mean values for the early resorptions, percentage of post implantation loss and percentage of dead or resorbed conceptuses per litter were outside of the historical ranges at the Testing Facility.

Other ovarian and uterine parameters in mice given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6 (i.e., late resorptions, fetal body weights, and percentage of live male fetuses) were comparable to the control values. All placentae appeared normal.

**Cohort B:** vaccination on DG 9**Table 60: Summary of ovary, uterine and litter parameters in cohort B**

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )			Historical control data
			Approx. 5	Approx. 6.5	Approx. 8	
Pregnant	N (%)	24 (96.0)	24 (96.0)	25 (100)	20 (80.0)	91.2 (60.0-100)
Corpora lutea	Mean ± SD	13.0 ± 2.1	13.4 ± 2.4	13.4 ± 2.4	13.2 ± 1.8	13.4 (11.0-14.5)
Implantations	Mean ± SD	12.8 ± 2.3	13.3 ± 2.4	13.2 ± 2.3	13.0 ± 2.2	13.1 (11.0-14.4)
Litter Size	Mean ± SD	12.1 ± 2.5	12.4 ± 2.2	11.6 ± 3.0	3.2 ± 3.9 <sup>##</sup>	12.2 (9.7-14.0)
Live Fetuses	Mean ± SD	12.1 ± 2.5	12.4 ± 2.2	11.5 ± 3.0	3.2 ± 3.8 <sup>##</sup>	12.2 (9.7-14.0)
Total Resorptions	Mean ± SD	0.7 ± 0.8	0.9 ± 0.8	1.6 ± 2.4	9.7 ± 3.9 <sup>##</sup>	0.9 (0.6-2.1)
Early Resorptions	Mean ± SD	0.6 ± 0.8	0.8 ± 0.9	1.5 ± 2.5	9.6 ± 4.0 <sup>##</sup>	0.8 (0.3-1.6)
% Postimplantation loss	Mean ± SD	5.9 ± 6.9	6.5 ± 5.7	12.4 ± 17.4	76.0 ± 27.2 <sup>##</sup>	8.3 (5.3-15.5)
Dams with viable fetuses	N (%)	24(100.0)	24(100.0)	25(100.0)	15( 62.5) <sup>**</sup>	55.9 (40.0-76.2)
Dams with all conceptuses dead or resorbed	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	9 (37.5) <sup>##</sup>	0.2 (0-9.1)
Dams with viable fetuses	N (%)	24 (100.0)	24 (100.0)	25 (100.0)	15 (62.5) <sup>##</sup>	99.9 (90.9-100)
% Dead or Resorbed Conceptuses/Litter	Mean ± SD	5.9 ± 6.9			61.6 ± 25.0 <sup>##</sup>	7.2 (4.5-10.4)
% Live male fetuses/litter	Mean ± SD	50.6 ± 15.6	49.0 ± 14.4		32.5 ± 20.0 <sup>##</sup>	50.3 (42.4-56.0)
Dams with any resorptions	N (%)	12( 50.0)	17( 70.8)	18( 72.0)	24(100.0) <sup>**</sup>	55.9 (40.0-76.2)
Placenta appeared normal	N (%)	24(100.0)	24(100.0)	24( 96.0)	15(100.0)	-
% Live male fetuses/litter	Mean ± SD	50.6 ± 15.6	49.0 ± 14.4	53.0 ± 15.3	32.5 ± 20.0 <sup>**</sup>	1.32 (1.20-1.37)
Live fetal body weights	Mean ± SD	1.31 ± 0.07	1.30 ± 0.08	1.29 ± 0.08	1.20 ± 0.10 <sup>**</sup>	1.32 (1.20-1.37)
% dead or resorbed conceptuses/litter	Mean ± SD	5.9 ± 6.9	6.5 ± 5.7	12.4 ± 17.4	61.6 ± 25.0 <sup>**</sup>	7.2 (4.5-10/4)

a) Testing Facility Historical Control Data compiled between June 2006 and June 2010 in mice based on 31 studies that evaluated 669 pregnant mice on DG 18. <sup>##</sup> = Significantly different from the Group 1 value ( $p \leq 0.01$ ).

There was an increase in post implantation loss associated with an increase in the mean number of resorptions in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9, in comparison with control values. The number of dams with any resorptions, the number of dams with total litter loss and the mean percentage of dead or resorbed conceptuses per litter was increased, in comparison to control values. Consequently, there were significant decreases ( $p \leq 0.01$ ) in mean litter size, mean number of live fetuses and number of dams with viable fetuses, in comparison to control values.

Combined fetal body weights were also significantly reduced ( $p \leq 0.01$ ) in the 8 log<sub>10</sub> CCID<sub>50</sub> group, in comparison to controls (8% less than controls, which reflected a 4% and 8% decrease in male and female fetal weights, respectively). All mean values, except mean combined fetal body weights and number dams with all conceptuses dead or resorbed, were outside the ranges observed historically at the Testing Facility.

Similar increases in the percentage of post implantation loss, mean number of resorptions, number of dams with any resorptions and the mean percentage of dead or resorbed conceptuses per litter were seen in females given 6.5 log<sub>10</sub> CCID<sub>50</sub>, in comparison with control values. Consequently, the mean litter size and the mean number of live fetuses was slightly lower than the control values. However, all mean values remained within the ranges observed historically at the testing facility and there was no dams with total litter loss and no changes in mean combined fetal body weights.

No other ovarian and uterine parameters evaluated in mice intravenously administered CYD Dengue Vaccine on DG 9 were affected by concentrations as high as  $8 \log_{10}$  CCID<sub>50</sub>. All placentae appeared normal. All parameters were unaffected by  $5 \log_{10}$  CCID<sub>50</sub> following the dose on DG 9.

**Cohort C:** vaccination on DG 12

*Table 61: Summary of ovary, uterine and litter parameters in cohort B*

Parameter	Unit	0	CYD Dengue Vaccine ( $\log_{10}$ CCID <sub>50</sub> )			Historical control data
			Approx. 5	Approx. 6.5	Approx. 8	
Pregnant	N (%)	24 (96.0)	24 (96.0)	21 (84.0)	24 (96.0)	91.2 (60.0-100)
Corpora lutea	Mean $\pm$ SD	13.0 $\pm$ 2.1	13.3 $\pm$ 1.8	13.3 $\pm$ 3.2	13.3 $\pm$ 2.0	13.4 (11.0-14.5)
Implantations	Mean $\pm$ SD	12.8 $\pm$ 2.3	13.3 $\pm$ 1.8	13.1 $\pm$ 3.4	13.2 $\pm$ 2.0	13.1 (11.0-14.4)
Litter Size	Mean $\pm$ SD	12.1 $\pm$ 2.5	12.4 $\pm$ 2.1	12.1 $\pm$ 3.6	12.5 $\pm$ 2.4	-
Live Fetuses	Mean $\pm$ SD	12.1 $\pm$ 2.5	12.4 $\pm$ 2.1	12.1 $\pm$ 3.6	12.4 $\pm$ 2.3	12.2 (9.7-14.0)
Total Resorptions	Mean $\pm$ SD	0.7 $\pm$ 0.8	0.8 $\pm$ 0.8	0.8 $\pm$ 1.6	0.7 $\pm$ 0.8	0.9 (0.6-2.1)
Late Resorptions	Mean $\pm$ SD	0.6 $\pm$ 0.8	0.1 $\pm$ 0.3	0.1 $\pm$ 0.5	0.0 $\pm$ 0.0	0.8 (0.3-1.6)
% Postimplantation loss	Mean $\pm$ SD	5.9 $\pm$ 6.9	7.2 $\pm$ 6.5	9.0 $\pm$ 17.1	6.0 $\pm$ 6.5	8.3 (5.3-15.5)
Dams with viable fetuses	N (%)	24(100.0)	24(100.0)	21(100.0)	24(100.0)	55.9 (40.0-76.2)
Dams with any resorptions	N (%)	12( 50.0)	15( 62.5)	10( 47.6)	13( 54.2)	55.9 (40.0-76.2)
Placenta appeared normal	N (%)	24(100.0)	24(100.0)	21(100.0)	24(100.0)	-
% Live male fetuses/litter	Mean $\pm$ SD	50.6 $\pm$ 15.6	56.5 $\pm$ 14.0	51.7 $\pm$ 19.9	48.4 $\pm$ 17.0	50.3 (42.4-56.0)
% dead or resorbed conceptuses/litter	Mean $\pm$ SD	5.9 $\pm$ 6.9	7.2 $\pm$ 6.5	9.0 $\pm$ 17.1	6.0 $\pm$ 6.5	7.2 (4.5-10/4)
Live fetal body weight (g)	Mean $\pm$ SD	1.31 $\pm$ 0.07	1.32 $\pm$ 0.08	1.32 $\pm$ 0.19	1.20 $\pm$ 0.09**	1.32 (1.20-1.37)
Male fetal body weight (g)	Mean $\pm$ SD	1.34 $\pm$ 0.08	1.34 $\pm$ 0.10	1.34 $\pm$ 0.19	1.22 $\pm$ 0.09**	1.34 (1.22-1.40)
Female fetal body weight (g)	Mean $\pm$ SD	1.28 $\pm$ 0.07	1.29 $\pm$ 0.08	1.26 $\pm$ 0.12	1.18 $\pm$ 0.09**	1.29 (1.19-1.34)

a. Testing Facility Historical Control Data compiled between June 2006 and June 2010 in mice based on 31 studies that evaluated 669 pregnant mice on DG 18. \*\* = Significantly different from the Group 1 value ( $p \leq 0.01$ ).

Combined fetal body weights were significantly reduced ( $p \leq 0.01$ ) in the  $8 \log_{10}$  CCID<sub>50</sub> dose group, in comparison to the control group value (8% less than controls), which reflected a 9% decrease ( $p \leq 0.01$ ) in male fetal weights and a 8% decrease ( $p \leq 0.01$ ) in female fetal weights, in comparison to the respective control group values. With the exception of the female fetal body weights, each of these average body weights were within the ranges observed historically at the Testing Facility. There were no changes in mean fetal weights in the 5 and  $6.5 \log_{10}$  CCID<sub>50</sub> dose groups, in comparison to controls.

No other ovarian and uterine parameters evaluated in mice intravenously administered CYD Dengue Vaccine on DG 12 were affected by concentrations as high as  $8 \log_{10}$  CCID<sub>50</sub>. The litter averages for corpora lutea, implantations, the percentage of preimplantation loss, litter sizes, live fetuses, early and late resorptions, the percentage of dead or resorbed conceptuses, the percentage of post implantation loss, and the percentage of live male fetuses were comparable among the 4 dose groups and did not significantly differ the control values. No dam had a litter consisting of only dead or resorbed conceptuses. All placentae appeared normal.

## Results F1 generation

**Fetal Examination:** Fetal abnormalities (alterations) were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

No gross external, soft tissue or skeletal fetal abnormalities (malformations or variations) were attributed to CYD Dengue Vaccine at concentrations as high as 8 log<sub>10</sub> CCID<sub>50</sub> following the single injection on DG 6, 9 or 12. All fetal abnormalities observed in the CYD Dengue vaccine groups generally occurred at low incidence and/or fetuses with similar malformations were found to occur spontaneously in control animals in similar studies conducted at the Testing Facility. There were therefore considered to be incidental.

### Fetal alterations – Caesarean-delivered live fetuses (DG 19)

#### Cohort A

*Table 62: Fetal Alterations - Caesarean-Delivered Live Fetuses Summary – cohort A*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter with fetuses with any alterations observed	N (%)	22( 91.7)	19( 79.2)	21( 87.5)	19(100.0)
Fetuses with any alterations observed	Mean ± SD	49( 16.8)	52( 18.1)	59( 20.3)	55( 25.0)
% fetuses with any alterations/litter	Mean ± SD	17.4 ± 11.1	17.5 ± 13.9	19.9 ± 14.3	25.4 ± 10.4

\*Significantly different from the Group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 12);

\*\*Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to garoups 2 through 4 (dosing day 12).

#### Cohort B

*Table 63: Fetal Alterations - Caesarean-Delivered Live Fetuses Summary – cohort B*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter with fetuses with any alterations observed	N (%)	22( 91.7)	16( 66.7)#	18( 72.0)	6( 40.0)**
Fetuses with any alterations observed	Mean ± SD	49( 16.8)	29( 9.7)	35( 12.2)	10( 13.0)
% fetuses with any alterations/litter	Mean ± SD	17.4 ± 11.1	9.8 ± 8.8	13.5 ± 12.9	11.1 ± 16.5

\*Significantly different from the Group 1 value ( $p \leq 0.05$ ); analyses restricted to Groups 2 through 4 (Dosing Day 12); \*\*Significantly different from the Group 1 value ( $p \leq 0.01$ ); analyses restricted to Groups 2 through 4 (Dosing Day 12).

#### Cohort C

*Table 64: Fetal Alterations - Caesarean-Delivered Live Fetuses Summary – cohort C*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter with fetuses with any alterations observed	N (%)	22( 91.7)	16( 66.7)*	16( 76.2)	12( 50.0)**
Fetuses with any alterations observed	Mean ± SD	49( 16.8)	36( 12.1)*	38( 14.9)	24( 8.0)**
% fetuses with any alterations/litter	Mean ± SD	17.4 ± 11.1	12.1 ± 10.8	19.2 ± 22.8	8.1 ± 11.1**

\*Significantly different from the Group 1 value ( $p \leq 0.05$ ); analyses restricted to groups 2 through 4 (dosing day 12);

\*\*Significantly different from the group 1 value ( $p \leq 0.01$ ); analyses restricted to groups 2 through 4 (dosing day 12).

**Fetal alterations: F<sub>1</sub> generation****Dosing day 6 (Cohort A)***Table 65: Fetal Alterations: Caesarean-delivered live fetuses (day 18 of gestation) -cohort A-summary*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter evaluated	N	24	14	24	19
Fetuses evaluated (live)	N	291 (291)	289	291	221
Litters with any alteration observed	N (%)	22 (91.7)	19 (79.2)	21 (87.5)	19 (100)
Fetuses with any alteration observed	N (%)	49 (16.8)	52 (18.1)	59 (20.3)	55 (25.0)
% Fetuses with any alterations/litter	Mean ± SD	17.4 ± 11.1	17.5 ± 13.9	19.9 ± 14.3	25.4 ± 10.4

There were no treatment-related differences in the overall litter and fetal incidence of abnormalities at any concentration in litters of females given CYD Dengue Vaccine on DG 6. In Groups 1 through 4, litters with fetuses with alterations numbered 22 (91.7%), 19 (79.2%), 21 (87.5%) and 19 (100%), respectively. The numbers of fetuses with any alteration observed were 49 (16.8%), 52 (18.1%), 59 (20.3%) and 55 (25.0%) and the percentages of fetuses with any alteration per litter were 17.4%, 17.5%, 19.9% and 25.4% in these same respective dose groups.

**Dosing day 9 (Cohort B)***Table 66: Fetal Alterations: Caesarean-delivered live fetuses (day 18 of gestation) -cohort B-summary*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter evaluated	N	24	24	25	15
Fetuses evaluated (live)	N	291 (291)	299	289	78
Litters with any alteration observed	N (%)	22 (91.7)	16 (66.7)	18 (72.0)	6 (40.0)**
Fetuses with any alteration observed	N (%)	49 (16.8)	29(9.7)	35(12.2)	10(13.0)
% Fetuses with any alterations/litter	Mean ± SD	17.4 ± 11.1	9.8 ± 8.8	13.5 ± 12.9	11.1 ± 16.5

There were fewer litters with alterations in the 5 and 8 log<sub>10</sub> CCID<sub>50</sub> dose groups, in comparison to the control value. In Groups 1 through 4, litters with fetuses with alterations numbered 22 (91.7%), 16 (66.7%), 18 (72.0%) and 6 (40.0%), respectively. The numbers of fetuses with any alteration observed were 49 (16.8%), 29 (9.7%), 35 (12.2%) and 10 (13.0%) and the percentages of fetuses with any alteration per litter were 17.4%, 9.8%, 13.5% and 11.1% in these same respective dose groups. These statistically significant reductions ( $p \leq 0.05$  and  $p \leq 0.01$ , respectively) at 5 and 8 log<sub>10</sub> CCID<sub>50</sub> were not attributed the test article because: 1) the reductions were not dose-dependent; and 2) an increase in these parameters, rather than a decrease, would be the expected effect of a developmental toxicant

**Dosing day 12 (Cohort C)***Table 67: Fetal Alterations: Caesarean-delivered live fetuses (day 18 of gestation) -cohort C-summary*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter evaluated	N	24	24	21	24
Fetuses evaluated (live)	N	291 (291)	298 (298)	255 (255)	300 (299)
Litters with any alteration observed	N (%)	22 (91.7)	16 (66.7)*	16(76.2)	12 (50.0)**
Fetuses with any alteration observed	N (%)	49 (16.8)	36 (12.1)*	38 (14.9)	24 (8.0)**
% Fetuses with any alterations/litter	Mean ± SD	17.4 ± 11.1	12.1 ± 10.8	19.2 ± 22.8	8.1 ± 11.1**

\*Significantly different from the Group 1 value (p≤0.05); \*\*Significantly different from the Group 1 value (p≤0.01);

There were fewer litters and fetuses (p≤0.05 or p≤0.01) with alterations in the 5 and 8 log<sub>10</sub> CCID<sub>50</sub> dose groups, in comparison to the control values. In addition, the percentage of fetuses with any alterations per litter was significantly reduced (p≤0.01) in the 8 log<sub>10</sub> CCID<sub>50</sub> dose group, in comparison to the control value. In Groups 1 through 4, litters with fetuses with alterations numbered 22 (91.7%), 16 (66.7%), 16 (76.2%) and 12 (50.0%), respectively. The numbers of fetuses with any alteration observed were 49 (16.8%), 36 (12.1%), 38 (14.9%) and 24 (8.0%) and the percentages of fetuses with any alteration per litter were 17.4%, 12.1%, 19.2% and 8.1% in these same respective dose groups. These statistically significant reductions (p≤0.05 or p≤0.01) at 5 and 8 log<sub>10</sub> CCID<sub>50</sub> were not attributed the test article because an increase in these parameters, rather than a decrease, would be the expected effect of a developmental toxicant.

**Fetal alterations: fetal gross external alterations (Caesarean delivery live fetuses, day 18 of gestation):***Table 68: Fetal gross external alterations summary*

Parameter			Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
Cohort A (Dosing Day 6)						
Litters evaluated		N (%)	24	24	24	19
Litter with live fetus		N (%)	24	24	24	19
Fetuses evaluated (live)		N (%)	291	287	290	220
HEAD: EXENCEPHALY	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.3)	0( 0.0)	0( 0.0)	0( 0.0)
EYE: LID(S) OPEN	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.3)	0( 0.0)	0( 0.0)	0( 0.0)
FORE AND/OR HINDLIMBS: ROTATED	Litter incidence	N (%)	2( 8.3)	0( 0.0)	1( 4.2)	2( 10.5)
	Fetal incidence	N (%)	2( 0.7)	0( 0.0)	1( 0.3)	2( 0.9)
PALATE: CLEFT	Litter incidence	N (%)	0( 0.0)	3( 12.5)	1( 4.2)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	3( 1.0)	1( 0.3)	0( 0.0)
Cohort B (Dosing Day 9)						
Litters evaluated		N (%)	24	24	25	15
Litter with live fetus		N (%)	24	24	25	15
Fetuses evaluated (live)		N (%)	291	299	288	77
HEAD: EXENCEPHALY	Litter incidence	N (%)	1( 4.2)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.3)	1( 0.3)	0( 0.0)	0( 0.0)
EYE: LID(S) OPEN	Litter incidence	N (%)	1( 4.2)	3( 12.5)	3( 12.0)	0( 0.0)

Parameter			Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
	Fetal incidence	N (%)	1( 0.3)	4( 1.3)	3( 1.0)	0( 0.0)
FORE AND/OR HINDLIMBS: ROTATED	Litter incidence	N (%)	2( 8.3)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	2( 0.7)	0( 0.0)	0( 0.0)	0( 0.0)
PALATE: CLEFT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 6.7)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 1.3)
<b>Cohort C (Dosing Day 12)</b>						
Litters evaluated		N (%)	24	24	21	24
Litter with live fetus		N (%)	24	24	21	24
Fetuses evaluated (live)		N (%)	291	268	255	299
TAIL: BENT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 4.2)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.3)
EYE: LID(S) OPEN	Litter incidence	N (%)	1( 4.2)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.3)	2( 0.7)	0( 0.0)	0( 0.0)
FORE AND/OR HINDLIMBS: ROTATED	Litter incidence	N (%)	2( 8.3)	1( 4.2)	4( 19.0)	2( 8.3)
	Fetal incidence	N (%)	2( 0.7)	1( 0.3)	5( 2.0)	2( 0.7)
<i>PALATE: CLEFT</i>	<i>Litter incidence</i>	<i>N (%)</i>	<i>0( 0.0)</i>	<i>0( 0.0)</i>	<i>3( 14.3)</i>	<i>1( 4.2)</i>
	<i>Fetal incidence</i>	<i>N (%)</i>	<i>0( 0.0)</i>	<i>0( 0.0)</i>	<i>3( 1.2)</i>	<i>1( 0.3)</i>

(Sponsor's reference number: SP0056 DV1014), caesarean-delivered live fetuses (day 18 of gestation)

### Fetal alterations: soft tissue alterations: F<sub>1</sub> generation

Table 69: Fetal soft tissue alterations summary

Parameter			Control	CYD Dengue Vaccine (log10 CCID50)		
				Approx. 5	Approx. 6.5	Approx. 8
Cohort A (Dosing Day 6)						
Litters evaluated		N (%)	24	24	24	19
Litter with live fetus		N (%)	24	24	24	19
Fetuses evaluated (live)		N (%)	139	139	139	105
PALATE: CLEFT	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
EYES: RETINA FOLDED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
SITUS INVERSUS	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 1.0)
Cohort B (Dosing Day 9)						
Litters evaluated		N (%)	24	24	25	15
Litter with live fetus		N (%)	24	24	25	13
Fetuses evaluated (live)		N (%)	139	142	141	35
PALATE: CLEFT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 7.7)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 2.8)*
BRAIN: IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
VESSELS: PRONOUNCED DUCTUS ARTERIOSUS	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
VESSELS: PULMONARY PASSES DORSAL TO TRACHEA AND ESOPHAGUS	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	0( 0.0)

Parameter			Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
KIDNEYS: ABSENT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	2( 8.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	2( 1.4)	0( 0.0)
URETER: ABSENT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	2( 8.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	2( 1.4)	0( 0.0)
<b>Cohort C (Dosing Day 12)</b>						
Litters evaluated		N (%)	24	24	21	24
Litter with live fetus		N (%)	24	24	21	24
Fetuses evaluated (live)		N (%)	139	141	123	143
PALATE: CLEFT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	2( 10.0)	1( 4.2)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	3( 2.4)	1( 0.7)
EYES: MICROPHTHALMIA	Litter incidence	N (%)	0( 0.0)	1( 4.2)	1( 5.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	1( 0.8)	0( 0.0)
EYES: LENS, MALPOSITIONED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 5.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.8)	0( 0.0)
EYES: RETINA FOLDED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	2( 8.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	2( 1.4)
EYES: LIDS OPEN	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	2( 1.4)	0( 0.0)	0( 0.0)

(Sponsor's reference number: SP0056 DV1014), caesarean-delivered live fetuses (day 18 of gestation); \*Significantly different from the Group 1 value  $p \leq 0.01$

### Fetal alterations: fetal skeletal alterations: F<sub>1</sub> generation

#### Dosing day 6 (Cohort A):

The only statistically significant findings occurred at skeletal examination and included an increase ( $p \leq 0.01$ ) in the litter and fetal incidence of an incompletely ossified palate at 5 log<sub>10</sub> CCID<sub>50</sub> (3 fetuses from 3 litters vs. none in the controls) and in the fetal incidence of interfrontals at 8 log<sub>10</sub> CCID<sub>50</sub> (22 fetuses from 12 litters vs. 16 fetuses from 8 litters in controls). These statistically significant increases in skeletal abnormalities were considered unrelated to CYD Dengue Vaccine because: 1) the increases were not dose-dependent; 2) the litter and/or fetal incidences were within the historical range of the Testing Facility and/or 3) the litter incidence, did not differ significantly from the control value.

Table 70: Fetal skeletal alterations

Parameter	Unit		Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
Cohort A (Dosing Day 6)						
Litters evaluated		N (%)	24	24	24	19
Litter with live fetus		N (%)	24	24	24	19
Fetuses evaluated		N (%)	152	150	152	115
Fetuses evaluated live		N (%)	152	149	151	115
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL	Litter incidence	N (%)	8( 33.3)	7( 29.2)	8( 33.3)	12( 63.2)
	Fetal incidence	N (%)	16( 10.5)	10( 6.7)	11( 7.3)	22(19.1)*
SKULL: FRONTAL, INCOMPLETELY OSSIFIED	Litter incidence	N (%)	1( 4.2)	3( 12.5)*	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	3( 2.0)*	0( 0.0)	0( 0.0)
SKULL: PARIETAL, NOT OSSIFIED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	0( 0.0)	0( 0.0)

Parameter	Unit		Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
SKULL: INTERPARIETALS, NOT OSSIFIED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	0( 0.0)	0( 0.0)
SKULL: SUPRAOCCIPITAL, INCOMPLETELY OSSIFIED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	0( 0.0)	0( 0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA	Litter incidence	N (%)	16( 66.7)	15( 62.5)	18( 75.0)	14( 73.7)
	Fetal incidence	N (%)	32( 21.0)	32( 21.5)	43( 28.5)	32( 27.8)
CERVICAL VERTEBRAE: ARCHES, FUSED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	1( 4.2)	2( 10.5)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	1( 0.7)	2( 1.7)
CERVICAL VERTEBRAE: HEMIVERTEBRA	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.9)
CERVICAL VERTEBRAE: ARCH, IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.9)
RIBS: THICKENED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	0( 0.0)	0( 0.0)
THORACIC VERTEBRAE: HEMIVERTEBRA	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.9)
THORACIC VERTEBRAE: CENTRA, FUSED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	2( 10.5)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	2( 1.7)
THORACIC VERTEBRAE: ARCH, SMALL	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.9)
THORACIC VERTEBRAE: ARCH, IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.9)
THORACIC VERTEBRAE: ARCHES, FUSED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	1( 0.9)
THORACIC VERTEBRAE: ARCH, INCOMPLETELY OSSIFIED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	1( 0.9)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	1( 0.9)
THORACIC VERTEBRAE: CENTRUM, BIFID	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	0( 0.0)
RIBS: FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	2( 8.3)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	2( 1.3)	1( 0.9)
RIBS: IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	1( 0.9)
RIBS: TWO SEGMENTS	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	0( 0.0)
RIBS: SHORT	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	0( 0.0)
MANUBRIUM: FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	2( 8.3)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	2( 1.3)	0( 0.0)
MANUBRIUM: IRREGULARLY SHAPED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	0( 0.0)	0( 0.0)
STERNAL CENTRA: SUMMARIZATION	Litter incidence	N (%)	3( 12.5)	7( 29.2)	5( 20.8)	4( 21.0)
	Fetal incidence	N (%)	3( 2.0)	7( 4.7)	6( 4.0)	4( 3.5)
STERNAL CENTRA: ASYMMETRIC	Litter incidence	N (%)	3( 12.5)	7( 29.2)	3( 12.5)	3( 15.8)
	Fetal incidence	N (%)	3( 2.0)	7( 4.7)	4( 2.6)	3( 2.6)
STERNAL CENTRA: FUSED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	2( 8.3)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	2( 1.3)	0( 0.0)
	Litter incidence	N (%)	1( 4.2)	0( 0.0)	1( 4.2)	0( 0.0)

Parameter	Unit		Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
STERNAL CENTRA: INCOMPLETELY OSSIFIED	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	1( 0.7)	0( 0.0)
STERNAL CENTRA: IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	0( 0.0)	1( 0.9)
XIPHOID: IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.2)	1( 5.3)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.7)	1( 0.9)
<b>Cohort B (Dosing Day 9)</b>						
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL	Litter incidence	N (%)	8( 33.3)	6( 25.0)	8( 32.0)	4( 26.7)
	Fetal incidence	N (%)	16( 10.5)	12( 7.6)	15( 10.2)	6( 14.3)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA	Litter incidence	N (%)	16( 66.7)	7( 29.2)*	8( 32.0)*	1(6.7)*
	Fetal incidence	N (%)	32( 21.0)	8( 5.1)*	14( 9.5)**	2(4.8)*
THORACIC VERTEBRAE: HEMIVERTEBRA	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
RIBS: SPLIT	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
MANUBRIUM: FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
MANUBRIUM: IRREGULARLY SHAPED	Litter incidence	N (%)	1( 4.2)	0( 0.0)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	1( 0.6)	0( 0.0)	0( 0.0)	0( 0.0)
STERNAL CENTRA: SUMMARIZATION	Litter incidence	N (%)	3( 12.5)	3( 12.5)	1( 4.0)	1( 6.7)
	Fetal incidence	N (%)	3( 2.0)	3( 1.9)	1( 0.7)	1( 2.4)
STERNAL CENTRA: ASYMMETRIC	Litter incidence	N (%)	3( 12.5)	2( 8.3)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	3( 2.0)	2( 1.3)	0( 0.0)	0( 0.0)
STERNAL CENTRA: FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	1( 4.0)	1( 6.7)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	1( 0.7)	1( 2.4)
STERNAL CENTRA: IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
XIPHOID: IRREGULARLY SHAPED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.7)	0( 0.0)	0( 0.0)
<b>Cohort B (Dosing Day 12)</b>						
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL	Litter incidence	N (%)	8( 33.3)	7( 29.2)	8( 38.1)	4( 16.7)
	Fetal incidence	N (%)	16( 10.5)	10( 6.4)	12( 9.1)	8( 5.1)
SKULL: MAXILLA, SHORT	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
SKULL: NASAL, SHORT	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
SKULL: PREMAXILLA, SHORT	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
SKULL: FRONTALS, SUTURE LARGE	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.8)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.8)	0( 0.0)
SKULL: PALATE, INCOMPLETELY OSSIFIED	Litter incidence	N (%)	0( 0.0)	0( 0.0)	1( 4.8)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	0( 0.0)	1( 0.8)	0( 0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA	Litter incidence	N (%)	16( 66.7)	11( 45.8)	8( 38.1)	7( 29.2)
	Fetal incidence	N (%)	32( 21.0)	22 ( 14.0)**	14( 10.6)*	11( 7.0)*
LUMBAR VERTEBRAE: ARCH, SMALL	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
LUMBAR VERTEBRAE: CENTRA, FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
LUMBAR VERTEBRAE: ARCHES, FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)

Parameter	Unit		Control	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
				Approx. 5	Approx. 6.5	Approx. 8
SACRAL VERTEBRAE: ARCHES, FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
CAUDAL VERTEBRAE: ARCH, NOT OSSIFIED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
RIBS: THICKENED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)
STERNAL CENTRA: SUMMARIZATION	Litter incidence	N (%)	3( 12.5)	1( 4.2)	2( 9.5)	0( 0.0)
	Fetal incidence	N (%)	3( 2.0)	1( 0.6)	3( 2.3)	0( 0.0)
STERNAL CENTRA: ASYMMETRIC	Litter incidence	N (%)	3( 12.5)	1( 4.2)	2( 9.5)	0( 0.0)
	Fetal incidence	N (%)	3( 2.0)	1( 0.6)	3( 2.3)	0( 0.0)
STERNAL CENTRA: FUSED	Litter incidence	N (%)	0( 0.0)	1( 4.2)	0( 0.0)	0( 0.0)
	Fetal incidence	N (%)	0( 0.0)	1( 0.6)	0( 0.0)	0( 0.0)

(Sponsor's reference number: SP0056 DV1014), caesarean-delivered live fetuses (day 18 of gestation); \*Significantly different from the Group 1 value  $p \leq 0.01$ ; \*\* Significantly different from the Group 1 value  $p \leq 0.05$

#### Cohort A Administered CYD Dengue Vaccine on DG 6:

Malformations that were observed included: cleft palate (gross external); cleft palate and situs inversus (soft tissue); an incompletely ossified palate, fused arches in at least one cervical or thoracic vertebra, a hemivertebra present as a cervical or a thoracic vertebra, fused centra in at least one thoracic vertebra, a small arch in at least one thoracic vertebra, fused or short ribs (skeletal). Variations that were observed included: medially rotated hindlimb(s) (gross external); a folded retina in one eye (soft tissue); an extra site of ossification between the frontal bones (an interfrontal), cervical ribs, irregularly shaped arches in at least one cervical or thoracic vertebra, bifid or unilaterally ossified centra in at least one thoracic vertebra, an incompletely ossified arch in at least on thoracic vertebra, irregularly shaped ribs, ribs in two segments, a fused manubrium, asymmetric, fused, incompletely ossified and/or irregularly shaped sternal centra, and an irregularly shaped xiphoid process (skeletal).

The only statistically significant findings occurred at skeletal examination and included an increase ( $p \leq 0.01$ ) in the litter and fetal incidence of an incompletely ossified palate at 5 log<sub>10</sub> CCID<sub>50</sub> (3 fetuses from 3 litters vs. none in the controls) and in the fetal incidence of interfrontals at 8 log<sub>10</sub> CCID<sub>50</sub> (22 fetuses from 12 litters vs. 16 fetuses from 8 litters in controls). These statistically significant increases in skeletal abnormalities were considered unrelated to CYD Dengue Vaccine because: 1) the increases were not dose-dependent; 2) the litter and/or fetal incidences were within the historical range of the Testing Facility and/or 3) the litter incidence did not differ significantly from the control value.

#### Cohort B Administered CYD Dengue Vaccine on DG 9:

Malformations that were observed included: open eyelids, exencephaly and cleft palate (gross external); cleft palate, an irregularly shaped brain, a pronounced ductus arteriosus, and an absent kidney and ureter (soft tissue); and a hemivertebra present as a thoracic vertebra and split ribs (skeletal). Variations that were observed included: a pulmonary artery that passed dorsal to the trachea and esophagus (soft tissue); and an extra site of ossification between the frontal bones

(an interfrontal), cervical ribs, a fused manubrium, irregularly shaped, fused or asymmetric sternal centra, and a large xiphoid process (skeletal).

The only statistically significant findings observed at gross external, soft tissue or skeletal examination and included an increase ( $p \leq 0.01$ ) in the fetal incidence of cleft palate at 8 log<sub>10</sub> CCID<sub>50</sub> (1 fetus from 1 litter) and a decrease ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the litter and fetal incidence of cervical ribs at 5, 6.5, and 8 log<sub>10</sub> CCID<sub>50</sub> (8, 14 and 2 fetuses from 7, 8 and 1 litters, respectively, compared to 32 fetuses from 16 control litters). These statistically significant changes in soft tissue or skeletal abnormalities were considered unrelated to CYD Dengue Vaccine because: 1) the litter and/or fetal incidences were within the historical range of the Testing Facility and/or 2) the litter incidence did not differ significantly from the control value.

#### Cohort C Administered CYD Dengue Vaccine on DG 12:

Malformations that were observed included: a bent tail, cleft palate and open eyelids (gross external); cleft palate, microphthalmia, a malpositioned lens in one or both eyes and open eyelids, (soft tissue); short maxillae and premaxillae, short nasal bones, an incompletely ossified palate, a small or fused arch in at least one lumbar and/or sacral vertebra, a fused centrum in at least one lumbar vertebra (skeletal). Variations that were observed included: medially rotated limb(s) (gross external); a folded retina in one or both eyes (soft tissue); an extra site of ossification between the frontal bones (an interfrontal), a large frontal suture, cervical ribs, an arch that was not ossified in at least one caudal vertebra, thickened ribs, asymmetric or fused sternal centra (skeletal).

The only statistically significant findings were observed at skeletal examination and included a decrease ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the fetal incidence of cervical ribs at 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> (22, 14 and 11 fetuses from 11, 8 and 7 litters, respectively, compared to 32 fetuses from 16 control litters). These statistically significant changes in skeletal abnormalities were considered unrelated to CYD Dengue Vaccine because: 1) the increases were not dose dependent; 2) the litter and/or fetal incidences were within the historical range of the Testing Facility; and/or 3) the litter incidence did not differ significantly from the control value.

#### **Fetal Ossification Site Averages:**

The most pronounced differences of fetal skeletal ossification were observed in litters assigned to Cohort B (Dosing Day DG 9) and were observed in each CYD Dengue Vaccine treated-group. The changes in litters of females given 8 log<sub>10</sub> CCID<sub>50</sub> occurred at a concentration where reductions in fetal body weights were observed, but in the absence of fetal abnormalities. The changes in litters of females given 6.5 log<sub>10</sub> CCID<sub>50</sub> occurred at a concentration where effects on maternal body weight or food consumption were observed, but in the absence of reduced fetal body weights, fetal abnormalities and will most likely resolve itself with further growth and development. The changes in litters of females given 5 log<sub>10</sub> CCID<sub>50</sub> were considered of no toxicological significance because they occurred in the absence of reduced fetal body weights, fetal abnormalities, effects on maternal body weight or food consumption and will most likely resolve itself with further growth and development.

*Table 71: Summary of CYD Dengue Vaccine-related Fetal Ossification Sites (mean) (submitted by the sponsor).*

Ossification Parameter	0	Cohort A (Dosing Day DG 6)			Cohort B (Dosing Day DG 9)			Cohort C (Dosing Day DG 12)			Historical Control Data
		5 log <sub>10</sub>	6.5 log <sub>10</sub>	8 log <sub>10</sub>	5 log <sub>10</sub>	6.5 log <sub>10</sub>	8 log <sub>10</sub>	5 log <sub>10</sub>	6.5 log <sub>10</sub>	8 log <sub>10</sub>	
Thoracic Vertebrae	13.41	13.36	13.42	13.64*	13.40	13.22 <sup>#</sup>	13.15 <sup>##</sup>	13.48	13.37	13.46	13.37 (13.25-13.48)
Lumbar Vertebrae	5.58	5.64	5.57	5.34**	5.59	5.78 <sup>#</sup>	5.81 <sup>#</sup>	5.50	5.61	5.54	5.63 (5.51-5.74)
Caudal Vertebrae	7.82	8.23	7.58	7.52	7.49	7.25	6.68 <sup>##</sup>	7.71	7.48	6.53 <sup>&amp;&amp;</sup>	8.33 (7.62-8.82)
Ribs (Pairs)	13.34	13.29	13.37	13.59**	13.33	13.19 <sup>#</sup>	13.1 <sup>1##</sup>	13.42	13.27	13.38	13.31 (13.20-13.42)
Forelimb Phalanges	11.97	12.10	11.75	12.04	11.39 <sup>#</sup>	11.54 <sup>#</sup>	11.44	11.64	11.64	11.31 <sup>&amp;&amp;</sup>	11.80 (11.13-12.03)
Hindlimb Tarsals	0.70	0.78	0.64	0.48	0.53	0.56	0.39	0.72	0.66	0.38 <sup>&amp;&amp;</sup>	0.85 (0.64-1.03)
Hindlimb Phalanges	11.68	11.87	11.21	11.26	10.31 <sup>##</sup>	10.28 <sup>##</sup>	10.21 <sup>##</sup>	10.46 <sup>&amp;&amp;</sup>	10.42 <sup>&amp;&amp;</sup>	9.87 <sup>&amp;&amp;</sup>	11.25 (10.30-11.88)

a. Testing Facility Historical Control Data compiled between June 2006 and June 2010 in mice based on 17 studies that evaluated 356 litters and 2273 fetuses; \* Significantly different from the Group 1 value ( $p \leq 0.05$ ); \*\* Significantly different from the Group 1 value ( $p \leq 0.01$ ); <sup>#</sup> Significantly different from the Group 1 value ( $p \leq 0.05$ ); <sup>##</sup> Significantly different from the Group 1 value ( $p \leq 0.01$ ). && Significantly different from the Group 1 value ( $p \leq 0.01$ ).

#### Cohort A: administered CYD Dengue Vaccine on DG 6:

There was a slight significant increase ( $p \leq 0.01$ ) in the incidence of supernumerary thoracic ribs with significant increases and decreases ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the numbers of thoracic and lumbar vertebrae, respectively, in the 8 log<sub>10</sub> CCID<sub>50</sub> group. The sponsor points out that these findings are common variations observed at maternally toxic doses. The average number of ossified hindlimb tarsals was decreased in the 8 log<sub>10</sub> CCID<sub>50</sub> group, in comparison to the control value. There were no changes seen in the 5 and 6.5 log<sub>10</sub> CCID<sub>50</sub> dose groups, in comparison to controls.

#### Cohort B: administered CYD Dengue Vaccine on DG 9:

There were significant decreases ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the average number of ossified thoracic vertebrae and corresponding rib pairs, and a significant increase ( $p \leq 0.05$ ) in the average number of ossified lumbar vertebrae in the 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> groups, in comparison to the control values. The average number of ossified caudal vertebrae was decreased in the 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> dose groups, which reached statistical significance ( $p \leq 0.01$ ) at 8 log<sub>10</sub> CCID<sub>50</sub>, in comparison to the control value. Each of these average values at 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> was outside of the ranges observed historically at the Testing Facility, but the delays at 8 log<sub>10</sub> CCID<sub>50</sub> occurred at a concentration where reductions in the fetal body weights were observed.

In addition, the average number of ossified hindlimb tarsals and phalanges was decreased in each CYD Dengue Vaccine-treated group, but only the average values for the hindlimb phalanges were significantly decreased ( $p \leq 0.01$ ) relative to the control value. The delays in ossification at 8 log<sub>10</sub> CCID<sub>50</sub> occurred at a concentration where reductions in the combined fetal body weights were observed and the changes were therefore considered of toxicological significance.

A similar trend of slight decreases in ossified hindlimb tarsals and phalanges was observed in the group given 5 or 6.5 log<sub>10</sub> CCID<sub>50</sub>, in comparison to controls. These ossification changes were minimal, limited to two skeletal structures only, which ossified in the last days of pregnancy and the first days of postnatal life and/or considered likely related to the individual variability of the maturity of the fetus which would resolve itself with further growth and development. In the absence of other changes at this dose level including any fetal abnormalities and/or maternal toxicity, the changes were considered of no toxicological significance.

Cohort C: administered CYD Dengue Vaccine on DG 12:

The average number of ossified caudal vertebrae and forelimb phalanges was significantly decreased ( $p \leq 0.01$ ) in the 8 log<sub>10</sub> CCID<sub>50</sub> group, in comparison to the control values. In addition, the average number of ossified hindlimb tarsals at 8 log<sub>10</sub> CCID<sub>50</sub> and average number of ossified hindlimb phalanges in each CYD Dengue Vaccine-treated group was significantly decreased ( $p \leq 0.01$ ), in comparison to the respective control values. Only the average values for the number of ossified caudal vertebrae, hindlimb tarsals and hindlimb phalanges at 8 log<sub>10</sub> CCID<sub>50</sub> were below the range observed historically at the Testing Facility. The delays in ossification at 8 log<sub>10</sub> CCID<sub>50</sub> occurred at a concentration where reductions in the fetal body weights were observed.

Differences seen in the 5 and 6.5 log<sub>10</sub> CCID<sub>50</sub> dose groups, in comparison to controls, were considered of no toxicological significance since they were minimal and within the historical range at the Testing Facility.

**Viral Exposure by qRT-PCR:**

CYD Dengue Vaccine was detected by qRT-PCR detection of YFNS5 RNA in 12/13 dams given 8 log<sub>10</sub> CCID<sub>50</sub>, but not in dams given lower doses. YFNS5 RNA was not detected in the embryos of mice in any group.

**Serology:**

There was a dose-related increase in the incidence of positive maternal serum samples with CYD dengue antibodies and related transfer to fetuses. In addition, there was a lower incidence of positive maternal and fetal samples when CYD Dengue Vaccine was administered to the dams later during the gestation.

*Table 72: Correlation of seroconversion and fetal resorption*

Group (dams)	Number of animals tested	Seropositive* with resorption (% of animals tested)	Seronegative with resorption (% of animals tested)	Seropositive* without resorption (% of animals tested)	Seronegative without resorption (% of animals tested)
control	10	1 (10%)	2 (20%)	3 (30%)	4 (40%)

5 log <sub>10</sub> DG6	10	5 (50%)	0 (0%)	5 (50%)	1 (10%)
5 log <sub>10</sub> DG9	10	5 (50%)	3 (30%)	0 (0%)	2 (20%)
5 log <sub>10</sub> DG12	10	1 (10%)	4 (40%)	2 (20%)	3 (30%)
6.5 log <sub>10</sub> DG 6	10	2 (20%)	0 (0%)	8 (80%)	0 (0%)
6.5 log <sub>10</sub> DG 9	10	8 (80%)	0 (0%)	2 (20%)	0 (0%)
6.5 log <sub>10</sub> DG 12	10	2 (20%)	3 (30%)	4 (40%)	1 (10%)
8 log <sub>10</sub> DG 6	10	7 (70%)	0 (0%)	3 (30%)	0 (0%)
8 log <sub>10</sub> DG 9	10	10 (100%)	0 (0%)	0 (0%)	0 (0%)
8 log <sub>10</sub> DG 12	10	5 (50%)	0 (0%)	5 (50%)	0 (0%)

\*Dams that were seropositive for at least one serotype

## CONCLUSIONS:

Under the conditions of the study, one intravenous injection of CYD Dengue Vaccine at 5, 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6, 9 or 12 did not result in any mortality, any adverse clinical signs or gross lesions at any concentration. The doses of 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> induced reductions in female body weight gains and food consumption and increases in postimplantation loss. The most pronounced effects occurred in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 and were associated with reduced fetal body weights in litters of females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 or 12. Changes at the fetal examination were limited to delays in skeletal ossification at 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> of CYD Dengue Vaccine where reductions in the fetal body weights and maternal toxicity occurred, but no fetal abnormalities. At 5 log<sub>10</sub> CCID<sub>50</sub> CYD Dengue Vaccine, there were no changes of toxicological significance.

Based on the results of this study, the maternal and developmental no-observed-adverse-effect level (NOAEL) for CYD Dengue Vaccine is 5 log<sub>10</sub> CCID<sub>50</sub>.

## Lactation Study:

### Lactation Study in (b) (4) Mice Following One Intravenous Administration (SP0056 DV1109)

*Reviewer: Claudia Wrzesinski*

#### SUMMARY:

(b) (4) mice were administered once on day 14 of lactation (DL 14) via an intravenous bolus injection of either the control article or the test article formulations at approximately 5, 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> of each CYD Dengue virus.

There was a transient dose-related body weight loss following the single dose of CYD Dengue Vaccine on DL 14 in groups given 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub>. There were no treatment-related clinical signs observed in the pups at any concentration of CYD Dengue Vaccine and no treatment-related differences in any litter parameter.

CYD Dengue Vaccine RNA (YFNS5 RNA) was detected in the serum of 6/15 dams given 8 log<sub>10</sub> CCID<sub>50</sub>, with no evidence of viral transfer to pups in any groups. Slight seroconversion and some anti-CYD antibody transfer from dams to pups was shown by an increase in incidence of anti-CYD antibody positive serum samples from treated dams and their pups.

**Study no.:** Sponsor Reference No. SP0056 DV1109; Testing Facility Study No. 20016586

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

**Experimental Start Date:** 20 September 2011 (Protocol)

**GLP compliance:** yes

**QA reports:** yes

**Drug, lot #, and % purity:** CYD Dengue Vaccine (Tetravalent Dengue Chimeric Vaccine):

Concentration approximately 5 log<sub>10</sub> CCID<sub>50</sub>/dose: S4316, purity: 100%

Concentration approximately 6.5 log<sub>10</sub> CCID<sub>50</sub>/mL: IND10044, purity: 100%

Concentration approximately 8 log<sub>10</sub> CCID<sub>50</sub>/mL: IND10045; purity: 100%

**Doses:** Dose selection was based on the response elicited by administration of CYD Dengue Vaccine in previous investigative and preliminary embryo-fetal studies. The dose level of approximately 5 log<sub>10</sub> CCID<sub>50</sub> of each CYD Dengue virus serotype corresponds to the human dose of vaccine at which no effects to dams and fetuses, with no antibody or virus detection were seen. The dose of approximately 6.5 log<sub>10</sub> CCID<sub>50</sub> was an intermediate dose at which no effects to dams and fetuses, with limited antibody response and transfer, and no virus detection. The dose of approximately 8 log<sub>10</sub> CCID<sub>50</sub> was the maximum feasible dose. At this dose level, virus was detected in fetuses with limited antibody response and minimal maternal toxicity and little or no developmental toxicity.

**Species/strain:** (b) (4) mice

**Number/sex/group:** 5 controls and 25 treated female mice per timepoint

**Route, formulation, volume, and infusion rate:** Intravenous

**Study design:**

One hundred timed-mated (b) (4) mice were randomly assigned to four dose groups (25 mice per main study dose group). An additional fifty satellite mice were randomly assigned to four dose groups (5 control mice and 15 mice per treated group). Female mice were administered once on day 14 of lactation (DL 14) via an intravenous bolus injection of either the control article (0.9% sodium chloride) or the test article formulations at approximately 5, 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> of each CYD Dengue virus.

Table 73: Study design (provided by the sponsor)

Group No.	Test Material	Concentration (log <sub>10</sub> CCID <sub>50</sub> per Dengue Virus Serotype)	Dose Volume (mL)	No. of Main Study Mice	No. of Satellite Mice <sup>c</sup>
1	Control Article	0 <sup>a</sup>	0.5	25	5
2	CYD Dengue (S4316)	approximately 5	0.5 <sup>b</sup>	25	15
3	CYD Dengue (IND10044)	approximately 6.5	0.5	25	15
4	CYD Dengue (IND10045)	approximately 8	0.5	25	15

<sup>a</sup> 0.9% Sodium Chloride Injection, (b) (4)

<sup>b</sup> For Group 2, the dose volume was based on the fill volume of the vial (contents of 1 vial/mouse; approximately 500 µL).

<sup>c</sup> Satellite mice used for viral exposure and transfer (qRT-PCR analysis).

**METHODS:****Parameters and endpoints evaluated:****In-life Procedures, Observations, and Measurements - F0 Generation:**

**Viability Checks:** twice daily

**Clinical Observations:** once during the acclimation period, on the day of delivery (Day 1 *postpartum*), and once daily until scheduled euthanasia. On the day of dose administration, postdose observations were recorded at approximately hourly intervals for the first 4 hours and at the end of the normal working day. On the day of dose administration, postdose observations were recorded at approximately hourly intervals for the first 4 hours and at the end of the normal working day.

**Body Weights:** Body weights were recorded once weekly during the gestation period, on day of delivery (Day 1 *postpartum*), and Days 4, 7, 14 (before dosing), 15, 18 and/or 21 *postpartum*

**Natural Delivery Observations:** Female mice were evaluated for adverse clinical signs, litter signs (defined as all pups delivered) and pup viability at birth.

**In-life Procedures, Observations, and Measurements - F1 Generation:****Viability Checks:** twice daily**Clinical Observations:** once daily**Body Weights:** Days 1 (birth), 4, 7, 14 (before dosing of dams), 15, 18 and/or 21 *postpartum***Developmental Landmarks:**Hair growth: initiated day 7 *postpartum*; incisor eruption: initiated day 9 *postpartum*; eye opening: day 13 *postpartum*.**Viral Exposure by qRT-PCR:** maternal milk samples: day 15 *post-partum*, 24h after administration; Maternal Blood Sample Collection: Day 15 *postpartum*; Pup Blood Sample Collection: Day 15 *postpartum***Immunogenicity:** Maternal Blood Samples: Day 21 *postpartum*; Pup Blood Samples: Day 21 *postpartum***RESULTS:****F0 Generation****Mortality:** There was no treatment-related mortality.**Maternal Clinical Observations:** There were no adverse clinical observations in dams given any concentration of CYD Dengue Vaccine.**Maternal Body Weights and Body Weight Changes:** A transient statistically significant treatment-related body weight loss was observed following the single dose of CYD Dengue vaccine on DL 14 in groups given 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> on DL 15. Body weights and body weight gains were unaffected by the 5 log<sub>10</sub> CCID<sub>50</sub> concentrations of CYD Dengue Vaccine.  
*Table 74: Summary of Body Weights and Body Weight Changes*

Interval	Control	5 log <sub>10</sub> CCID <sub>50</sub>	6.5 log <sub>10</sub> CCID <sub>50</sub>	8 log <sub>10</sub> CCID <sub>50</sub>
DL 14 to 15	-0.3 ± 2.4	-0.8 ± 1.9	-2.6 ± 2.2**	-3.6 ± 2.4**
DL 15 to 18	-0.9 ± 2.4	-1.5 ± 2.4	-0.1 ± 1.7	2.1 ± 1.7**
DL 18 to 21	-2.7 ± 2.6	-1.8 ± 3.2	-1.1 ± 3.6	-0.5 ± 3.6
DL 14 to 21	-3.9 ± 4.1	-4.1 ± 2.8	-3.9 ± 3.6	-2.0 ± 3.8
DL 14	48.4 ± 2.7	49.1 ± 3.1	47.9 ± 3.4	47.0 ± 2.7
DL 15	48.1 ± 2.7	48.2 ± 3.4	45.2 ± 3.0**	43.3 ± 2.7**
DL 18	47.2 ± 3.1	46.8 ± 3.2	45.1 ± 3.2	45.4 ± 2.8
DL 21	44.4 ± 4.2	44.9 ± 3.8	44.0 ± 3.7	44.9 ± 4.2

\*\*: Significantly different from the control group value (p≤0.01).

**Natural Delivery Observations:** Before dosing, all mice were pregnant and delivered litters. Values for the numbers of dams delivering litters, averages for implantation sites per delivered litter, the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, and the percentage of male pups per number of pups sexed per litter were comparable among the four groups.

**Litter Observations:** There were no treatment-related changes in any CYD Dengue Vaccine dose group in litter endpoints including the lactation index, surviving pups per litter through DL 14 to DL 21, live litter size at weighing and pup weight per litter.

There were 1, 4, 2 and 8 pups in the 0, 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> groups, respectively, that were found dead between DL 1 and DL 20 and had a complete necropsy performed. The increased incidence of mortality in the 8 log<sub>10</sub> CCID<sub>50</sub> group was not attributed to CYD Dengue Vaccine because a majority of the deaths occurred prior to dose administration on DL 14.

**Maternal Necropsy:** All F0 generation mice appeared normal at necropsy examination.

### **F1 Generation Pups**

**Clinical Observations:** There were no treatment-related adverse clinical signs in any CYD Dengue Vaccine dose group.

**Reflex and Physical Development:** There were no biologically important differences among the 3 CYD Dengue Vaccine groups in the measures of reflex and physical development (i.e., hair growth, tooth eruption and eye opening). All statistically significant changes ( $p \leq 0.05$  or  $p \leq 0.01$ ) in reflex and physical development parameters were considered unrelated to the intravenous administration of CYD Dengue Vaccine because the endpoints were measure before dosing and/or the changes occurred prior to dose administration. Most evaluated developmental endpoints with the exception of the “eye opening” were observed before administering the vaccine on DL 14, therefore the effect of the vaccine can not be determined on these endpoints.

*Table 75: Reflex and physical development – summary- F1 generation*

Parameter	Unit	0	CYD Dengue Vaccine (log <sub>10</sub> CCID <sub>50</sub> )		
			Approx. 5	Approx. 6.5	Approx. 8
Litter tested	N	25	25	25	25
Hair growth (day 7)	Mean ± SD	99.7 ± 1.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Hair growth (day 8)	Mean ± SD	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Tooth eruption (day 9)	Mean ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 1.6
Tooth eruption (day 10)	Mean ± SD	2.0 ± 5.2	5.5 ± 12.1	5.0 ± 16.7	0.7 ± 2.4
Tooth eruption (day 11)	Mean ± SD	35.1 ± 30.6	55.5 ± 36.8*	66.1 ± 26.9**	53.2 ± 29.5
Tooth eruption (day 12)	Mean ± SD	90.8 ± 25.1	95.2 ± 18.6	94.9 ± 17.7	96.4 ± 16.9
Tooth eruption (day 13)	Mean ± SD	100.0 ± 0.0	99.5 ± 2.3	99.8 ± 1.2	98.8 ± 6.2
Tooth eruption (day 14)	Mean ± SD	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Eye opening (day 13)	Mean ± SD	11.6 ± 19.0	9.5 ± 20.7	17.0 ± 30.2	13.3 ± 19.5
Eye opening (day 14)	Mean ± SD	53.3 ± 33.4	61.9 ± 33.1	63.7 ± 31.2	66.7 ± 34.9
Eye opening (day 15)	Mean ± SD	91.2 ± 17.9	90.3 ± 19.7	92.5 ± 15.2	95.9 ± 9.9
Eye opening (day 16)	Mean ± SD	98.9 ± 4.2	100.0 ± 0.0	98.6 ± 4.2	99.7 ± 1.5
Eye opening (day 17)	Mean ± SD	100.0 ± 0.0	100.0 ± 0.0	99.7 ± 1.4	100.0 ± 0.0
Eye opening (day 18)	Mean ± SD	100.0 ± 0.0	100.0 ± 0.0	99.7 ± 1.4	100.0 ± 0.0
Eye opening (day 19)	Mean ± SD	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

**Viral Exposure by qRT-PCR:** CYD Dengue Vaccine RNA (i.e., YFNS5 RNA) was detected in the serum of 6/15 dams given 8 log<sub>10</sub> CCID<sub>50</sub>, but not in the serum of dams given lower doses. YFNS5 RNA was not detected in the milk of lactating mice (milk sample was taken 24 hours after vaccine administration) or in the serum of pups (serum sample was taken 24 hours after vaccine administration) in any groups.

**Immunogenicity:** Slight seroconversion and some antibody transfer from dams to pups was shown by a dose-related increased incidence of anti-CYD antibody positive samples in dams and pups following the intravenous injection of CYD Dengue Vaccine given to lactating dams.

**CONCLUSION:**

One intravenous injection of CYD Dengue Vaccine at 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> in lactating mice was well tolerated with treatment-related effects limited to a transient body weight loss on the day after injection in females given 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> and no treatment-related changes in litter parameters at any dose. However, the only developmental endpoint that can be evaluated in this study is the eye-opening endpoint. All other developmental endpoints were observed before the day of the vaccine administration on DL 14 and therefore the effect of the vaccine administration on these endpoints cannot be evaluated. Exposure to CYD Dengue virus was shown by detection of the virus in approximately one third of the mice given 8 log<sub>10</sub> CCID<sub>50</sub> with no evidence of viral transfer to pups. There was slight seroconversion and anti-CYD antibody transfer from dams to pups following the intravenous injection of CYD Dengue Vaccine given to lactating mice.

## **LABELING:**

### **8. USE IN SPECIFIC POPULATIONS**

#### **8.1 Pregnancy**

##### Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to DENG VAXIA during pregnancy. Women who receive DENG VAXIA during pregnancy are encouraged to contact directly, or have their healthcare professional contact, Sanofi Pasteur Inc. at 1-800-822-2463 (1-800-VACCINE) to enroll in or obtain information about the registry.

##### Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

No specific studies of DENG VAXIA have been performed among pregnant women. A limited number of cases of inadvertent exposure during pregnancy were reported during clinical studies. Isolated adverse pregnancy outcomes (e.g., stillbirth, intrauterine death, spontaneous abortion, blighted ovum) have been observed for these exposed pregnancies, with similar frequency and nature in the vaccinated individuals compared to the control group, and with risk factors identified for all cases. Data are not sufficient to determine the effects of DENG VAXIA on pregnancy, embryo-fetal development, parturition and post-natal development.

In two developmental toxicity studies, the effect of DENG VAXIA on embryo-fetal and post-natal development was evaluated in pregnant rabbits and mice. A developmental toxicity study was performed in female rabbits given a 5 log<sub>10</sub> 50% cell culture infectious dose (CCID<sub>50</sub>) of DENG VAXIA (full human dose ranging from 4.5 log<sub>10</sub> to 6.0 log<sub>10</sub>) by intravenous injection prior to mating and during gestation. The study revealed no evidence of harm to the fetus due to DENG VAXIA. In another study, female mice were administered a single dose of 5 log<sub>10</sub> CCID<sub>50</sub>, 6.5 log<sub>10</sub> CCID<sub>50</sub> (about 3 times the highest human dose) or 8 log<sub>10</sub> CCID<sub>50</sub> (about 100 times the highest human dose) of DENG VAXIA by intravenous injection during gestation. Fetal toxicities were observed at maternally toxic doses. [*See Animal Data (8.1).*]

##### Animal Data

In two developmental toxicity studies, the effect of DENG VAXIA on embryo-fetal and post-natal development was evaluated in pregnant rabbits and mice.

Rabbits were administered a full human dose [0.5 mL (5 log<sub>10</sub> CCID<sub>50</sub>/animal/occasion)] of DENG VAXIA by intravenous injection 30 and 10 days before mating and on Days 6, 12 and 27

during gestation. No vaccine-related fetal malformation or variations and adverse effects on female fertility or pre-weaning development were reported in this study. Pregnant mice were administered a single dose of either 5 log<sub>10</sub> CCID<sub>50</sub> (full human dose ranging from 4.5 log<sub>10</sub> to 6.0 log<sub>10</sub>), 6.5 log<sub>10</sub> CCID<sub>50</sub> (about 3 times the highest human dose) or 8 log<sub>10</sub> CCID<sub>50</sub> (about 100 times the highest human dose) of DENG VAXIA by intravenous injection on Day 6, 9 or 12 of gestation. At doses of 6.5 log<sub>10</sub> CCID<sub>50</sub> or 8 log<sub>10</sub> CCID<sub>50</sub> of DENG VAXIA, maternal toxicity was observed which was associated with increased postimplantation loss and at doses of 8 log<sub>10</sub> CCID<sub>50</sub> with reduced fetal body weight. The significance of this observation for humans is unknown, especially considering the different route of administration (the human route of administration is subcutaneous) and dose levels which exceeded the intended human dose. There were no vaccine related fetal malformations or other evidence of teratogenesis noted in this study.

## 8.2 Lactation

### Risk Summary

Human data are not available to assess the impact of DENG VAXIA on milk production, its presence in breast milk, or its effects on the breastfed child. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for DENG VAXIA and any potential adverse effects on the breastfed child from DENG VAXIA or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine. A lactation study was performed in mice which received a single vaccine administration on day 14 of lactation and did not show DENG VAXIA in the milk.

### Animal Data

A developmental toxicity study in which female mice were administered a single injection of 5 log<sub>10</sub> CCID<sub>50</sub> (full human dose ranging from 4.5 log<sub>10</sub> to 6.0 log<sub>10</sub>), 6.5 log<sub>10</sub> CCID<sub>50</sub> or 8 log<sub>10</sub> CCID<sub>50</sub> of DENG VAXIA by intravenous injection on Day 14 of lactation showed no presence of DENG VAXIA in breast milk in mice when measured 24 hours after vaccine administration.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

DENG VAXIA has not been evaluated for carcinogenic or mutagenic potential or impairment of male fertility. Exposure of female rabbits to DENG VAXIA prior to and during gestation did not impair fertility. [See *Use in Specific Populations* (8.1).]

## **OVERALL SUMMARY:**

### **General toxicology:**

CYD dengue vaccine was evaluated in a repeat-dose toxicity study in the monkey, animals were given three SC injections at days 1, 27 and 55 of either control or one full human dose.

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, dermal scores, body weight (gain), food consumption, urinalysis, ophthalmoscopic parameters, gross pathology, or microscopic anatomy were reported.

Changes in liver enzymes and hematology parameters (lymphocyte, WBC, monocyte, eosinophils, and basophils) were reported and were not considered of toxicological importance. Changes in the weights of the adrenal, epididymides, spleen, thymus, pituitary, and the heart were not associated with any macroscopic and/or microscopic findings and were not considered toxicologically important. Even though no microscopic findings associated with the increase in testis and ovary weight, the changes were significant (at study days 65 and 76, testis weight was increased 328% and 162% and ovary weight was decreased 26% and 61%, respectively) and might be related to the test article treatment. Immune responses due to test article treatment were reported.

Based on the overall findings in this study, it can be concluded that in (b) (4) monkeys, administration of dengue tetravalent vaccine (3 or 2 doses) had no adverse effects in terms of systemic toxicity.

### **Reproductive toxicology:**

The sponsor submitted two immunogenicity and viremia studies in non-pregnant female rabbits and mice (SP0056 IS0906 and SP0056 IS0907) and two preliminary dose-range DART studies in pregnant female rabbit and mice (SP0056 PS1002 and SP0056 PS1003, respectively) to support the species selection and to determine the study designs and dose levels for the pivotal DART studies in rabbits (SP0056 DV1013) and mice (SP0056 DV1014). The sponsor also submitted a lactation study in mice (SP0056 DV1109). All pivotal studies used the intravenous route of administration since a stronger immunogenicity and viremia was seen which was more comparable to the human situation.

In the immunogenicity studies, immunogenicity and viremia analysis were used as endpoints comparing the IV and SC route of administration. In rabbits seroconversion to all serotypes was seen independent of the route of injection and the dose level. The titers were higher and detected earlier when animals were treated by the IV route compared to the SC route and at the high dose. In rabbits viremia was detected at a low level only on the day after the injection when given at a high dose via the IV route. In mice, viremia was detected on the day after the injection as well as the following two days given by IV, it was only detected in the high dose group but not in lower dose groups. Seroconversion was limited to occasional animals and/or to serotypes 1 and 3 in all groups. The mouse was therefore selected for the evaluation of the effects of the viremia, but not the antibody response. In humans viremia was observed in up to 16% of the vaccinated

volunteers with an overall rate of 3.3% (16% of infants/toddlers, 2% of children, 0.7% of adolescents and 15% of adult).

Based on investigative and preliminary dose-range data, the rabbit and the mouse were considered acceptable as models for DART studies with a robust antibody response in the rabbit and detectable viremia in the mouse. The rabbit was therefore selected for the evaluation of the effects of the antibody response, but not the viremia. The mouse was therefore selected for the evaluation of the effects of the viremia, but not the antibody response.

Rabbits received a full human on day -30 and -10 as well as on gestation day 6, 12 and 27 by IV administration. No indication of maternal systemic toxicity were reported. No test article-effects on mating performance and fertility, and no indication of teratogenic potential of the test vaccine as well as no effect on pre and post-natal development of the pups were reported. A robust specific antibody response against each CYD Dengue virus serotypes were reported in all females. And, an efficient transfer of the vaccine specific maternal antibodies to the fetuses and the pups was reported.

The mouse was selected to investigate the exposure to the virus after one IV injection at a dose of 5, 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6, 9 or 12. One intravenous injection of CYD Dengue Vaccine at 5, 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> on DG 6, 9 or 12 did not result in any mortality, any adverse clinical signs or gross lesions at any concentration. The doses of 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> induced reductions in female body weight gains and food consumption and increases in post implantation loss. The most pronounced effects occurred in females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 and were associated with reduced fetal body weights in litters of females given 8 log<sub>10</sub> CCID<sub>50</sub> on DG 9 or 12. Changes at the fetal examination were limited to delays in skeletal ossification at 6.5 or 8 log<sub>10</sub> CCID<sub>50</sub> of CYD Dengue Vaccine where reductions in the fetal body weights and maternal toxicity occurred, but no fetal abnormalities were observed. At 5 log<sub>10</sub> CCID<sub>50</sub> CYD Dengue Vaccine, there were no changes of toxicological significance. Based on the results of this study, the maternal and developmental no-observed-adverse-effect level (NOAEL) for CYD Dengue Vaccine is 5 log<sub>10</sub> CCID<sub>50</sub>.

One intravenous injection of CYD Dengue Vaccine at 5, 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> in lactating mice was generally tolerated with treatment-related effects limited to a transient body weight loss on the day after injection in females given 6.5 and 8 log<sub>10</sub> CCID<sub>50</sub> and no treatment-related changes in litter parameters at any dose. However, the only developmental endpoint that can be evaluated in this study is the eye-opening endpoint. All other developmental endpoints were observed before the day of the vaccine administration on DL 14 and therefore the effect of the vaccine administration on these endpoints cannot be evaluated. Exposure to CYD Dengue virus was shown by detection of the virus in approximately one third of the mice given 8 log<sub>10</sub> CCID<sub>50</sub> with no evidence of viral transfer to pups. There was slight seroconversion and anti-CYD antibody transfer from dams to pups following the intravenous injection of CYD Dengue Vaccine given to lactating mice.

**Concurrence:** Martin D. Green