

Toxicology Review of 20-Valent Pneumococcal Vaccine

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File: BLA 125731/0

Product: 20-valent Pneumococcal Conjugate Vaccine [Diphtheria CRM197 Protein]

Subject: Toxicology study review

Reviewer: Andrew O'Carroll, DVM

Reference: BLA Sections reviewed

- 4.2.3.2 Repeat-Dose Toxicity
- 4.2.3.5 Reproductive and Developmental Toxicity
- 4.2.3.6 Local Tolerance
- 4.2.3.7 Other Toxicity Studies

Sponsor: Wyeth Pharmaceuticals LLC, a Subsidiary of Pfizer, Inc

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EXECUTIVE SUMMARY

20vPnC was evaluated through a series of repeat-dose toxicity studies, one developmental and reproductive toxicity (DART) study and one single-dose subcutaneous local tolerance study, all using (b) (4) rabbits. No toxicological findings were found which would preclude the use of this vaccine in its intended human population at doses up to 4.4 µg per serotype (and 8.8 µg for serotype 6B) up to 5 times intramuscularly. However, histologic evidence of inflammation with degeneration/necrosis of cardiomyocytes was observed at low incidence that was initially deemed test article-related by the study pathologist. There was no clinical correlate for this finding and the relevance and translatability to human subjects should be considered inconclusive at this time. The sponsor included a study where rabbits received saline with varying degrees of stress through procedures which establishes a basic understanding that rabbits may develop stress-related cardiomyopathy, but the results of this study should not be taken into context with the findings of 20vPnC due to the differences in study design and histologic methodology.

In the pivotal repeat-dose toxicity study included in this submission, study rabbits received either saline control, vehicle control, a (b) (4)-valent version of the vaccine and both low (the intended clinical dose) and high doses (2x the clinical dose) of 20vPnC. Aside from the aforementioned histologic finding, there were no treatment-related findings of clinical concern. Treatment-related findings included slightly increased incidence of irritation at injection sites, masses at injection sites, elevations in the acute phase reactants fibrinogen and C-reactive protein, increased germinal center formation in the spleen and injection site-draining lymph nodes, and injection site myofiber chronic-active inflammation with degeneration/necrosis. All of these findings were considered either partially or fully reversible and are considered anticipated sequelae of the intended immune response rather than as a sign of frank toxicity. The single-dose subcutaneous local tolerance study, while limited in design, did not demonstrate any added risk should 20vPnC be administered through this route inadvertently.

In the DART study included in this submission, virgin rabbit does received saline control or 46.2 µg 20vPnC (2.2 µg per serotype except for 4.4 µg serotype 6B, the intended clinical dose) twice prior to mating and twice while pregnant. One subset of does from both groups had terminal caesarean sections on gestation day 29 (near term) to assess uterine effects and fetal development while another subset proceeded to parturition with both the does and F1 kits followed until 35 days postpartum. There was no treatment-related mortality or any treatment-related effects on female fertility and mating performance, fetal development, parturition or postnatal development up until 35 days postpartum. No treatment-related malformations or variations were observed in fetuses or kits in this study. Immunology testing in this study demonstrated evidence of passive transfer of antibodies from vaccinated does to fetuses and kits.

INTRODUCTION

BLA: 125731/0

Sponsor: Wyeth Pharmaceuticals LLC, a Subsidiary of Pfizer, Inc

Product: 20-valent Pneumococcal Conjugate Vaccine [Diphtheria CRM197 Protein]

Proposed use: “Active immunization for the prevention of pneumonia and invasive disease caused by *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F in adults 18 years of age and older”

Introduction: Wyeth Pharmaceuticals has submitted an original biologics licensing application (BLA) for consideration of licensure in the US for their developed vaccine 20-valent Pneumococcal Conjugate Vaccine [Diphtheria CRM197 Protein], which is shortened to “20vPnC” for the purpose of this review. This is a conjugated protein vaccine comprised of capsular polysaccharides from multiple strains of *Streptococcus pneumoniae* conjugated to the nontoxic variant of diphtheria toxin CRM₁₉₇. The purpose of this vaccine is to expand on the *S. pneumoniae* strain coverage of the sponsor’s currently licensed pneumococcal vaccine: Prevnar 13[®] which was based on their previous vaccine Prevnar 7[®]. Despite expanding the vaccine, there was still significant pneumococcal disease burden in the US from non-covered strains. In addition to the polysaccharides found from the strains 1, 3, 4, 5, 6A, 7F, 9V, 12F, 14, 18C, 19A and 23F, 20vPnC also contains the polysaccharides from strains 8, 10A, 11A, 15B, 22F and 33F. 20vPnC contains the same vehicle components as Prevnar 13[®]: (b) (4) succinate buffer, (b) (4) sodium chloride, (b) (4) polysorbate 80 and the adjuvant aluminum phosphate (AlPO₄) at (b) (4) with no preservative.

The intended clinical dosing regimen of 20vPnC is for recipients to receive a single 0.5 mL intramuscular injection with a total dose of 46.2 µg with (b) (4) aluminum phosphate adjuvant.

The nonclinical toxicology program for this BLA submission includes one pivotal repeat-dose toxicology study, six supportive repeat-dose toxicology studies, one single-dose local tolerance study and one developmental and reproductive toxicology (DART) study. Because of some concerning findings observed during the post-mortem examinations of the rabbits in the pivotal toxicology study, the submission includes one pathology working group report and a white paper further discussing these changes. The other nonclinical studies submitted under sections 4.2.1 (pharmacology) are not included in this review.

Overview of the toxicology testing program:

<i>Study^a</i>	<i>Study (Sponsor) Number</i>	<i>Dose Group</i>	<i>Total Volume (mL)^b</i>
Repeat-Dose Toxicity 59-Day, 5-Dose (1 dose/2 weeks) IM toxicity Study with (b) (4) and 20vPnC Vaccines in (b) (4) Rabbits with a 4-Week Recovery Period	12GR385	Saline control Vehicle (b) (4) 20vPnC ^d 20vPnC ^d	0.5 0.5 1.0 0.5 1.0
59-Day, 5-Dose (1 dose/2 weeks) IM Toxicity Study with 20vPnC Vaccine in (b) (4) Rabbits	13GR165	Saline control 20vPnC ^d	1.0 1.0
(b) (4)			
59-Day, 5-Dose (1 dose/2 weeks) IM Toxicity Study with (b) (4) and 20vPnC Vaccines in (b) (4) Rabbits	8294056 (13GR370)	Saline control 20vPnC ^d (b) (4)	1.0 1.0 1.0 1.0 1.0
(b) (4)			
(b) (4)			
59-Day, 5-Dose (1 dose/2 weeks) IM Study of Study Procedures in Female (b) (4) Rabbits	15GR382	Saline	1.0
Local Tolerance Single Dose Subcutaneous Local Tolerance Study of PF-06482077 in Female (b) (4) Rabbits	19GR379	Saline 20vPnC	0.5 0.5
Developmental and Reproductive Toxicity A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations of PF-0648077 Vaccine by Intramuscular Administration in the Rabbit	18GR287	Saline 20vPnC	0.5 0.5

Table 1: Overview of the nonclinical toxicology program – Al = aluminum; AlPO₄ = aluminum phosphate; CRM197 = Cross reactive material 197; IM = Intramuscular; (b) (4) ; PnC – Pneumococcal conjugate; v = valent

a. All in vivo studies were conducted with male and female animals with the exception of 15GR382 which utilized females only.

b. Each vaccine was formulated in a suspension containing (b) (4) succinate buffer, (b) (4) NaCl at (b) (4)

(b) (4) polysorbate 80, (b) (4) Al as AlPO_4 . This suspension was also used to dose animals that received the vehicle alone. Doses were administered as one or two 0.5 mL injections/dosing day (1 or 2x the anticipated human dose and/or dose volume) into the left and/or right quadriceps muscles.

c. Contains serotypes (b) (4) each conjugated to CRM₁₉₇. The vaccine contains (b) (4) µg/mL of each of the polysaccharides except serotype 6B which is supplied at (b) (4) µg/mL.

d. Contains serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 15B, 22F, 33F, 8, 10A, 11A, and 12F each conjugated to CRM₁₉₇. The vaccine contains (b) (4) µg/mL of each of the polysaccharides except serotype 6B which is supplied at (b) (4) µg/mL.

e. (b) (4)

SECTION 4.2.3.2 REPEAT DOSE TOXICITY

59-DAY INTRAMUSCULAR TOXICITY STUDY OF PF-(b) (4) AND PF-06482077 IN RABBITS WITH A 30-DAY RECOVERY PERIOD

Study number: 12GR385

Performing laboratory: Pfizer (b) (4)

Study initiation date: December 17th, 2012

Final Report date: March 4th, 2014

Test article batch/lot:

- (b) (4) -valent test article lot (b) (4) expiration date April 23rd, 2013
- PF-06482077: 20-valent test article lot 127931-089, expiration date April 17th, 2013
- PF-06414248: Adjuvant/vehicle control lot (b) (4), expiration date April 24th, 2013; contains (b) (4) succinate buffer and (b) (4) NaCl, Polysorbate 80 and (b) (4) aluminum phosphate as AlPO_4
- *Sterile saline for injection*: saline control lot (b) (4) expiration date August 31st, 2014

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

Breeder/supplier: (b) (4)

Number of animals per group and sex: 10

Age: 7 months

Body weight range: Males – 2.7-3.5 kg; Females – 2.7-3.7 kg

Means of administration: Intramuscular injection via needle and syringe

Site of administration: Left and right quadriceps (vastus lateralis muscle)

Volume of injection: 0.5 mL

Frequency of administration and study duration: a total of 5 injections separated by 2 weeks for a treatment phase of 59 days followed by a 30-day recovery period

Dose: 2.2 – (b) (4) µg/serotype except for 6B which was dosed at 4.4–(b) (4) µg

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

Report status: Final

Experimental design: Animals were randomized, acclimated for a minimum of 26 days then assigned to one of five groups according to table 1 below. Administrations of test and

control articles occurred on study days 1, 15, 29, 43 and 57. One half of the study animals were humanely euthanized at the end of the study phase on day 59 with the other half being euthanized after the recovery period on study day 87.

<i>Group</i>	<i>Treatment</i>	<i>Dose (μg)*</i>	<i>Dose Volume**</i>	<i>Treatment Phase Animals (No.)</i>	<i>Recovery Phase Animals (No.)</i>
1	Saline	0	0.5	5	5
2	Vehicle/AlPO ₄	0	0.5	5	5
3	(b) (4)	(b) (4)	1.0	5	5
4	20vPnC	2.2 (4.4)	0.5	5	5
5	20vPnC	4.4 (8.8)	1.0	5	5

Table 2: Group assignments – *dose presented for all serotypes except for 6B which is presented in parentheses; **groups 1, 2 and 4 received one injection in the left vastus lateralis muscle per administration while group 3 and 5 animals received two injections

Methods for blood collection: Fasted blood samples were collected, but no details regarding collection site or analyzers used were provided

Randomization procedure: Yes, computer-assisted procedure based on body weights

Statistical analysis plan: Yes, one-way analysis of variance (ANOVA) and Dunnett's test.

- All statistically analyses in this study were conducted comparing the vaccinated groups (b) (4) and 20vPnC) to the **adjuvant** control group.

The following parameters were evaluated:

<i>Parameters</i>	<i>Frequency of Testing</i>
Clinical signs ¹	Twice daily
Clinical examinations	Not collected
Body weight	SD 1, 4, 8, 15, 18, 22, 29, 32, 36, 43, 46, 50, 57, 60, 67, 74, 81 and 86
Food consumption	Daily
Body temperature	Pre-dose and 4 and 24 hours post dose
Ophthalmologic exam	Pretest and SD 52
Clinical chemistry*	Pretest, SD 3, 15, 29 and 57
Hematology*	Pretest, SD 3, 15, 29 and 57
Coagulation*	Pretest, SD 3, 15, 29 and 57
Immunological response	Pretest, SD 15, 29, 57, 59 and 87
Evaluation of site of inoculation (Dermal Draize scoring method)	Pre-dose and approximately 4 and 24 hours after dosing, plus 48 and 72 hours after dosing if score is 2 or greater
Post-mortem examinations (necropsy, organ weights and histopathology)	Treatment phase: SD 59 Recovery phase: SD 87

Table 3: Experimental design – *details on blood collection sites not provided; SD = study day

¹ Details regarding clinical signs observed or examined were not provided.

Postmortem procedures: The following tissues were collected at necropsy.

<i>Organ/Tissue</i>	<i>Collected</i>	<i>Not collected</i>
Adrenal glands	!*	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum)	!	
Brain (layers not specified)	!*	
Cecum	!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes	!	
Fallopian tubes (oviduct)	!	
Gall bladder	!	
Gross lesions (if any)	!	
Gut-Associated Lymphoid Tissue (GALT)	!	
Harderian gland (if applicable)		NC
Heart	!	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		NC
Larynx	!	
Liver	!*	
Lung	!	
Lymph nodes (iliac)	!	
Lymph nodes (inguinal)	!	
Lymph nodes (mandibular)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		NC
Optic nerve	!	
Ovaries	!*	
Pancreas	!	
Parathyroid	!	
Pituitary gland	!*	
Prostate	!*	
Rectum		NC

<i>Organ/Tissue</i>	<i>Collected</i>	<i>Not collected</i>
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicle	!	
Skeletal muscle	!	
Skin and adnexa	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach	!	
Testes	!*	
Thymus	!*	
Thyroid	!*	
Tongue	!	
Trachea	!	
Ureters	!	
Urinary bladder	!	
Uterus	!	
Vagina	!	
Zymbal's gland		NA

Table 4: Histology – tissues examined for histology are marked with an “!”; tissues marked with an asterisk were weighed; NC = not collected; NA = not applicable

Tissues were first examined macroscopically for gross findings, then embedded in paraffin wax, sectioned at and stained with hematoxylin and eosin. All of the above tissues were in examined in all animals of both sexes from all five study groups.

RESULTS

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

Clinical signs: An increased incidence of transient, self-resolving “masses” was observed at a higher incidence in treated animals compared to controls. See figure 2 below. These resolved prior to necropsy and no microscopic correlation was found. Otherwise, there were no treatment-related clinical observations of toxicologic concern with the remainder of the abnormal observations being those commonly observed in laboratory rabbits, such as abrasions for example.

<i>Group</i>	<i>Animal (Sex)</i>	<i>Clinical Sign</i>	<i>Total No. of Days (Study Days of Occurrence)</i>
3	22(M)	Mass, injection site #2	2 (Day 44-45)
3	26(M)	Mass, injection site #2	20 (Day 44-47, 49-53, 56-66)
3	30(M)	Mass, injection site #1	11 (Day 43-53)
3	72(F)	Mass, injection site #2	10 (Day 31-40)
4	32(M)	Mass, injection site #1	6 (Day 44-49)

<i>Group</i>	<i>Animal (Sex)</i>	<i>Clinical Sign</i>	<i>Total No. of Days (Study Days of Occurrence)</i>
4	36(M)	Mass, injection site #1	4 (Day 59-62)
4	38(M)	Mass, injection site #1	8 (Day 59-66)
5	46(M)	Mass, injection site #1	11 (Day 59-69)
5	47(M)	Mass, injection site #2	13 (Day 31-43)
5	49(M)	Mass, injection site #2	4 (Day 31-64)

Table 5: Incidence of injection site masses – M = male; F = female

Ophthalmologic examinations: There were no treatment-related effects on ophthalmologic observations during the course of treatment phase.

Body temperature:

Group	Males	Females
1 (Control)	0	0
2	1	0
3	0	0
4	1	0
5	0	0

Table 6: Body temperature – values above represent occurrences for body temperature $\geq 40^{\circ}$ C

Body weight change:

<i>Study Days</i>	<i>N</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
1-4	10	-17.5	-19.8	-9.4	-15.1	-15.5
4-8	10	57.2	40.6	47.0	61.2*	62.0*
8-15	10	-41.9	-19.4	-34.9	-52.8	-66.9
15-18	10	89.7	85.4	84.6	112.0	112.7
18-22	10	38.0	37.7	39.4	38.9	42.5
22-29	10	-25.1	-23.5	-37.5	-28.3	-65.3
29-32	10	106.1	87.4	99.8	96.4	121.5*
32-36	10	19.0	23.7	18.9	15.5	37.5
36-43	10	82.7	82.8	62.2	57.8	56.8
43-46	10	-3.5	-20.6	-14.9	-4.6	-2.0
46-50	10	36.1	41.6	47.5	45.4	34.9
50-57	10	-31.2	-54.8	-55.8	-38.3	-55.2
60-67	5	78.4	73.8	50.7	75.0	60.7
67-74	5	52.6	37.1	20.0	53.0	43.8
74-81	5	17.2	9.6	34.4	27.6	35.5
81-86	5	29.2	31.7	41.1	31.6	33.8
1-57	10	309.7	261.1	247.0	288.1	263.0
60-86	5	177.4	152.2	146.1	1887.3	173.7

Table 7: Male body weight change – values represent changes in mean body weight in grams between listed study days; administrations occurred on study days 1, 15, 29, 43 and 57; * $p < 0.05$

<i>Study Days</i>	<i>N</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
1-4	10	1.2	-33.7	-9.3	-4.4	-11.4
4-8	10	69.2	72.8	74.6	66.2	67.0
8-15	10	24.4	-0.2	-2.2	-30.4	-4.4
15-18	10	55.7	60.4	64.2	83.6	51.1
18-22	10	66.5	65.0	69.4	58.6	55.6
22-29	10	-16.9	7.2	2.1	-11.5	-33.2*
29-32	10	84.3	79.3	61.2	79.5	91.4
32-36	10	49.4	39.8	59.9	49.7	20.8
36-43	10	80.8	90.1	72.6	44.0	79.9
43-46	10	16.5	2.3	12.4	30.7	5.1
46-50	10	41.1	32.1	29.9	40.1	33.3
50-57	10	-19.5	-29.9	-21.8	-53.9	-24.6
60-67	5	77.1	100.1	103.9	75.1	55.9
67-74	5	21.2	87.6	76.6	87.9	45.4
74-81	5	79.5	59.1	58.1	49.4	55.8
81-86	5	NA	NA	NA	NA	NA
1-57	10	452.7	385.2	417.4	352.2	330.5
60-86	5	177.8	246.9	238.6	212.5	157.2

Table 8: Female body weight change – values represent changes in mean body weight in grams between listed study days; administrations occurred on study days 1, 15, 29, 43 and 57; *p<0.05; NA = not available

No body weight change calculations were provided between study days 81 and 86 for female animal groups.

Food consumption:

<i>Study Days</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
1-2	144.77	141.52	150.00	141.86	146.77
3-4	150.00	144.91	146.33	145.39	150.00
15-16	147.76	145.27	146.65	144.32	150.00
16-17	145.30	150.00	150.00	148.34	147.48
17-18	142.47	139.86	145.79	150.00	145.81
29-30	142.24	143.43	141.72	145.27	142.98
30-31	144.64	140.87	147.66	147.82	148.13
31-32	146.90	142.94	143.73	150.00	150.00
43-44	146.08	144.36	143.70	141.35	142.18
44-45	147.30	140.43	137.91	136.65	146.89
45-46	147.21	144.84	142.20	140.61	142.69
57-58	140.75	137.55	132.81	133.61	139.20

Table 9: Male food consumption – values represent changes in mean body weight in grams between listed study days; administrations occurred on study days 1, 15, 29, 43 and 57; study days selected followed administrations

<i>Study Days</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
1-2	146.76	142.44	150.00	148.21	143.11
3-4	148.18	141.61	150.00	150.00	144.25
15-16	150.00	145.39	150.00	148.02	143.52
16-17	147.62	148.44	147.99	150.00	144.96
17-18	150.00	145.18	147.74	147.68	143.62
29-30	143.57	135.60	143.01	148.02	138.43
30-31	148.37	147.63	145.30	148.77	145.88
31-32	147.21	147.69	147.11	150.00	143.81
43-44	146.37	139.58	141.68	137.30	132.57
44-45	148.04	146.16	147.63	148.25	145.22
45-46	150.00	150.00	143.51	150.00	138.64
57-58	143.48	134.19	139.42	137.62	138.70

Table 10: Female food consumption – values represent changes in mean body weight in grams between listed study days; administrations occurred on study days 1, 15, 29, 43 and 57; study days selected followed administrations

Despite how the study design called for food consumption to be measured daily, there were days where mean food consumption data was not provided: e.g. 2-3, 58-59 and 59-60. Additionally, in the food consumption data summary results which were statistically analyzed, the data does not start until study day 4. Therefore, the above data is taken from the individual data and not statistically analyzed.

Clinical chemistry:

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5, i.e. \uparrow 1.6 or \downarrow 1.6)</i>	<i>NOT OF NOTE</i>
ELECTROLYTE BALANCE		Calcium Phosphorus Sodium Potassium Chloride
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION:	Aspartate aminotransferase (AST) SD29 M G2 \uparrow 1.7	Alanine aminotransferase (ALT) Glutamate dehydrogenase (ND) Sorbitol dehydrogenase (ND) Total bile acids (ND) Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids (ND) Total bilirubin
ACUTE PHASE REACTANTS	C-reactive protein (see tables 12 and 13)	Fibrinogen (see table 14)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5, i.e. \uparrow 1.6 or \downarrow 1.6)</i>	<i>NOT OF NOTE</i>
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Creatine kinase (CK) SD29 M G4 \uparrow 1.8 Fasting Triglycerides (TRIG) SD87 F G2 \uparrow 1.6 T4, Total SD87 F G2 \uparrow 1.7	Total protein Albumin (A) Globulin (G) A/G Ratio Total Cholesterol Cholinesterase (ND) Lactate Dehydrogenase (LDH) Amylase Lipase

Table 11: Clinical chemistry results – ND = not determined; fold changes above between treated animals and saline control; statistical significance determined between treated groups and adjuvant control

C-reactive protein:

<i>Study Day</i>	<i>N</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
-8	10	8.2 \pm 22.46	0.9 \pm 0.34	1.5 \pm 1.16	1.3 \pm 1.17	1.1 \pm 1.12
3	10	0.8 \pm 0.61	0.6 \pm 0.35	21.9 \pm 25.08**	15.3 \pm 23.64**	44.5 \pm 64.66**
15	10	1.6 \pm 1.71	1.0 \pm 0.63	1.6 \pm 1.52	1.2 \pm 1.01	0.6 \pm 0.29
29	10	2.0 \pm 2.18	1.4 \pm 1.01	2.0 \pm 1.9	1.9 \pm 1.42	1.8 \pm 2.26
57	10	2.1 \pm 2.54	1.9 \pm 1.62	4.1 \pm 3.94	2.1 \pm 1.94	5.7 \pm 7.00
59	5	2.2 \pm 2.17	1.7 \pm 0.31	37.5 \pm 12.90**	13.9 \pm 8.32**	22.0 \pm 10.64**
87	5	2.6 \pm 1.46	2.1 \pm 0.96	4.2 \pm 4.79	2.7 \pm 2.20	4.0 \pm 2.81

Table 12: Male C-reactive protein results – data presented in mean $\mu\text{g/ml} \pm$ standard deviation;
**p<0.01

<i>Study Day</i>	<i>N</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
-8	10	7.4 \pm 14.57	6.5 \pm 8.07	4.2 \pm 2.21	3.5 \pm 1.48	3.7 \pm 2.64
3	10	2.5 \pm 2.30	5.0 \pm 7.05	20.4 \pm 15.25**	8.5 \pm 7.22*	11.1 \pm 7.26**
15	10	4.6 \pm 8.52	1.9 \pm 0.87	2.0 \pm 0.83	2.3 \pm 1.66	2.0 \pm 0.86
29	10	5.0 \pm 7.67	4.5 \pm 6.09	2.8 \pm 1.50	3.0 \pm 2.41	3.5 \pm 1.82
57	10	4.4 \pm 5.54	2.6 \pm 0.92	2.9 \pm 2.07	3.5 \pm 2.13	2.1 \pm 1.26
59	5	3.4 \pm 2.65	2.4 \pm 1.23	22.6 \pm 26.19**	5.7 \pm 2.83*	10.4 \pm 8.94*
87	5	4.2 \pm 1.17	4.1 \pm 0.70	3.4 \pm 2.39	4.3 \pm 2.83	3.0 \pm 1.92

Table 13: Female C-reactive protein results – data presented in mean $\mu\text{g/ml} \pm$ standard deviation;
*p<0.05; **p<0.01

Hematology and coagulation:

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5², i.e. ↑ 1.6 or ↓ 1.6)</i>	<i>NOT OF NOTE</i>
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) Red Cell Distribution Width (RDW) Reticulocytes Burr cells Acanthocytes Stomatocytes
WHITE BLOOD CELLS	Monocyte count SD3 M G2 ↑ 1.6 Large unstained cells (LUC) SD3 M G2 ↑ 1.8 SD15 M G5 ↑ 2.0 SD29 M G3 ↑ 2.0 SD57 M G5 ↑ 2.0 SD3 F G2 ↑ 2.5 SD3 F G5 ↑ 2.0 SD29 F G2 ↑ 3.0 SD29 F G5 ↑ 2.0 SD57 F G2 ↑ 2.0 SD57 F G3 ↑ 2.0 SD57 F G4 ↑ 2.0 SD87 F G2 ↑ 2.0 SD87 F G3 ↑ 2.0	Total leukocytes (WBC) Neutrophil count Lymphocyte count Eosinophil count Basophil count
CLOTTING POTENTIAL		Activated partial-thromboplastin clotting time (APTT) Prothrombin time (PT) Platelet count Mean platelet volume (ND) Fibrinogen
OTHER		Bone marrow cytology (ND)

Table 14: Hematology results – ND = not determined; fold changes above between treated animals and saline control; statistical significance determined between treated groups and adjuvant control

Systemic toxicity: No treatment-related mortality or any toxicologically relevant changes in clinical signs, relative food consumption, ophthalmologic parameters, and body temperature were found. There was an increased incidence of “masses” at the injection sites of treated animals, but considering how these were rapidly self-resolving, they were inflammatory in

² 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.

nature. These did not cause any other clinical abnormalities nor were there any reports of the animals displaying any overt evidence of pain.

While treated animals of both sexes gained weight over the course of the study, a non-statistically significant but dose-dependent decrease in body weight gain was observed in treated animals of both sexes. For males this ranged between 7-20% and for females this ranged between 8-27%. Body weight gains appeared to normalize during the time points observed during the recovery period, thus making this finding recoverable. The lack of body weight data for all female animals on study day 86 was recorded as a study deviation. There was not a direct correlate to any decreases in food consumption, though there was a lack of food consumption at a few critical time points, such as the days following the 1st and final vaccinations.

Among the clinical pathology data, the only treatment-related, toxicologically-relevant changes observed were increases in LUC, hyperfibrinogenemia and elevations in CRP on study days 3 and 59 (the only time points within 2 days post-administrations). The increases in fibrinogen were observed in both sexes and ranged from approximately 12-49% for animals receiving 20vPnC. CRP increases for animals receiving 20vPnC demonstrated large variability, but the mean values ranged from approximately 2 to 60-fold over saline control animals. All three of these parameters were comparable between treated animals and controls by the next time point (study day 15 and 86) and were thus considered recoverable.

Organ weights:

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
Number of Animals	5/5	5/5	5/5	5/5	5/5
Terminal body weight	3462.52/ 3725.40	3532.26/ 3583.22	3548.15/ 3467.32	3476.56/ 3692.58	3527.94/ 3588.82
Brain, absolute weight (g)	9.6/ 10.1	10.0/ 10.0	9.6/ 9.5	9.0/ 9.9	9.7/ 9.3
Brain, body weight ratio (%)	0.28/ 0.27	0.28/ 0.28	0.27/ 0.27	0.26/ 0.27	0.28/ 0.26
Adrenals, absolute weight (g)	0.566/ 0.684	0.521/ 0.548	0.501/ 0.618	0.619/ 0.672	0.566/ 0.538
Adrenals to body weight ratio (%)	0.016/ 0.018	0.015/ 0.015	0.014/ 0.018	0.018/ 0.018	0.016/ 0.015
Adrenals to brain weight ratio (%)	0.059/ 0.067	0.052/ 0.055	0.053/ 0.065	0.069/ 0.068	0.058/ 0.059
Heart, absolute weight (g)	7.2/ 9.6	8.9/ 9.2	8.7/ 8.1	10.3/ 10.0	7.7/ 8.8
Heart to body weight ratio (%)	0.21/ 0.25	0.25/ 0.25	0.25/ 0.24	0.30/ 0.27	0.22/ 0.25
Heart to brain weight ratio (%)	0.76/ 0.94	0.89/ 0.91	0.91/ 0.86	1.14/ 1.02	0.80/ 0.95
Kidney, absolute weight (g)	17.4/ 20.2	17.7/ 17.8	17.9/ 19.4	18.2/ 19.8	18.7/ 19.4
Kidney to body weight ratio (%)	0.50/ 0.54	0.50/ 0.50	0.50/ 0.56	0.52/ 0.54	0.53/ 0.54
Kidney to brain weight ratio (%)	1.82/ 1.98	1.77/ 1.77	1.86/ 2.05	2.02/ 2.01*	1.94/ 2.10*
Liver, absolute weight (g)	83.0/ 99.0	91.1/ 98.0	95.7/ 95.4	92.6/ 92.2	91.8/ 94.4

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
Liver to body weight ratio (%)	2.383/ 2.658	2.570/ 2.713	2.707/ 2.734	2.641/ 2.483	2.589/ 2.634
Liver to brain weight ratio (%)	8.658/ 9.748	9.065/ 9.703	9.961/ 10.080	10.273/ 9.412	9.396/ 10.104
Pituitary, absolute weight (g)	0.50/ 0.027	0.043/ 0.056	0.29/ 0.33	0.032/ 0.031	0.066/ 0.030
Pituitary to body weight ratio (%)	0.001/ 0.001	0.001/ 0.002	0.001/ 0.001	0.001/ 0.001	0.002/ 0.001
Pituitary to brain weight ratio (%)	0.005/ 0.003	0.004/ 0.005	0.003/ 0.004	0.004/ 0.003	0.007/ 0.003
Prostate, absolute weight (g)	3.885/ 2.483	3.057/ 2.580	3.203/ 2.803	2.994/ 2.760	3.752/ 2.606
Prostate to body weight ratio (%)	0.117/ 0.066	0.087/ 0.072	0.091/ 0.082	0.088/ 0.075	0.107/ 0.074
Prostate to brain weight ratio (%)	0.420/ 0.243	0.300/ 0.259	0.335/ 0.297	0.336/ 0.285	0.388/ 0.284
Spleen, absolute weight (g)	1.17/ 1.55	1.20/ 1.30	1.28/ 1.41	1.14/ 1.40	1.38/ 1.17
Spleen to body weight ratio (%)	0.03/ 0.04	0.03/ 0.04	0.04/ 0.04	0.03/ 0.04	0.04/ 0.03
Spleen to brain weight ratio (%)	0.12/ 0.15	0.12/ 0.13	0.13/ 0.15	0.13/ 0.14	0.14/ 0.12
Testes, absolute weight (g)	6.88/ 7.20	6.84/ 6.61	6.08/ 6.78	6.44/ 6.45	5.79/ 7.33
Testes to body weight ratio (%)	0.20/ 0.19	0.19/ 0.19	0.17/ 0.20	0.18/ 0.18	0.16/ 0.20
Testes to brain weight ratio (%)	0.72/ 0.71	0.68/ 0.66	0.63/ 0.72	0.71/ 0.6	0.59/ 0.79*
Thymus, absolute weight (g)	3.980/ 3.947	3.161/ 3.883	3.331/ 3.741	3.957/ 4.087	3.945/ 4.923
Thymus to body weight ratio (%)	0.117/ 0.106	0.091/ 0.108	0.096/ 0.108	0.115/ 0.110	0.113/ 0.137
Thymus to brain weight ratio (%)	0.423/ 0.389	0.314/ 0.387	0.355/ 0.394	0.443/ 0.419	0.413/ 0.534
Thyroid, absolute weight (g)	0.310/ 0.414	0.381/ 0.427	0.319/ 0.463	0.382/ 0.473	0.330/ 0.418
Thyroid to body weight ratio (%)	0.009/ 0.011	0.011/ 0.012	0.009/ 0.013	0.011/ 0.013	0.009/ 0.012
Thyroid to brain weight ratio (%)	0.033/ 0.040	0.038/ 0.042	0.034/ 0.049	0.044/ 0.048	0.034/ 0.045

Table 15: Male organ weights – absolute weights are expressed as mean grams; treatment and recovery phase animals separated by a ‘/’; *p<0.05

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
Number of Animals	5/5	5/5	5/5	5/5	5/5
Terminal body weight	3706.92/ 3977.20	3608.18/ 3903.52	3798.46/ 3884.28	3524.52/ 4011.44	3470.34/ 3881.82
Brain, absolute weight (g)	10.0/ 9.5	9.7/ 9.7	9.7/ 9.6	9.7/ 9.7	9.7/ 9.3
Brain, body weight ratio (%)	0.27/ 0.24	0.27/ 0.25	0.26/ 0.25	0.28/ 0.24	0.29/ 0.24
Adrenals, absolute weight (g)	0.570/ 0.483	0.592/ 0.576	0.639/ 0.645	0.551/ 0.520	0.534/ 0.563

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
Adrenals to body weight ratio (%)	0.015/ 0.012	0.016/ 0.015	0.017/ 0.017	0.016/ 0.013	0.016/ 0.015
Adrenals to brain weight ratio (%)	0.057/ 0.051	0.061/ 0.059	0.065/ 0.067	0.057/ 0.054	0.055/ 0.060
Heart, absolute weight (g)	9.7/ 9.4	8.2/ 8.7	8.7/ 8.9	7.6/ 9.3	8.9/ 9.8
Heart to body weight ratio (%)	0.26/ 0.24	0.23/ 0.23	0.23/ 0.23	0.22/ 0.23	0.26/ 0.25
Heart to brain weight ratio (%)	0.96/ 0.99	0.84/ 0.90	0.90/ 0.93	0.79/ 0.96	0.91/ 1.05
Kidney, absolute weight (g)	17.6/ 16.9	16.0/ 17.3	17.7/ 18.2	16.5/ 18.2	16.1/ 17.8
Kidney to body weight ratio (%)	0.47/ 0.42	0.44/ 0.44	0.46/ 0.47	0.47/ 0.45	0.47/ 0.46
Kidney to brain weight ratio (%)	1.75/ 1.78	1.64/ 1.78	1.83/ 1.91	1.69/ 1.89	1.67/ 1.92
Liver, absolute weight (g)	83.7/ 79.7	77.3/ 85.7	89.1/ 79.0	73.0/ 85.7	76.7/ 84.4
Liver to body weight ratio (%)	2.252/ 1.996	2.138/ 2.196	2.326/ 2.043	2.063/ 2.130	2.195/ 2.169
Liver to brain weight ratio (%)	8.275/ 8.341	7.942/ 8.806	9.258/ 8.324	7.505/ 8.857	7.917/ 9.147
Ovaries, absolute weight (g)	0.430/ 0.558	0.495/ 0.582	0.519/ 0.519	0.633/ 0.453	0.469/ 0.460
Ovaries to body weight ratio (%)	0.012/ 0.014	0.014/ 0.015	0.014/ 0.014	0.018/ 0.011	0.014/ 0.012
Ovaries to brain weight ratio (%)	0.043/ 0.058	0.051/ 0.060	0.053/ 0.054	0.066/ 0.047	0.049/ 0.050
Pituitary, absolute weight (g)	0.050/ 0.043	0.029/ 0.034	0.050/ 0.028	0.059/ 0.023	0.037/ 0.032
Pituitary to body weight ratio (%)	0.001/ 0.001	0.001/ 0.001	0.001/ 0.001	0.002/ 0.001	0.001/ 0.001
Pituitary to brain weight ratio (%)	0.005/ 0.004	0.003/ 0.004	0.005/ 0.003	0.006/ 0.002	0.004/ 0.003
Spleen, absolute weight (g)	1.91/ 2.01	1.41/ 1.79	1.83/ 1.85	1.83/ 2.13	1.90/ 1.69
Spleen to body weight ratio (%)	0.05/ 0.05	0.01/ 0.05	0.05/ 0.05	0.05/ 0.05	0.05*/ 0.04
Spleen to brain weight ratio (%)	0.19/ 0.21	0.14/ 0.18	0.19/ 0.19	0.19/ 0.22	0.20/ 0.18
Thymus, absolute weight (g)	4.214/ 6.133	4.458/ 6.138	5.751/ 5.575	3.260/ 6.184	4.353/ 6.898
Thymus to body weight ratio (%)	0.114/ 0.154	0.124/ 0.157	0.152/ 0.144	0.095/ 0.155	0.124/ 0.174
Thymus to brain weight ratio (%)	0.421/ 0.645	0.459/ 0.631	0.600/ 0.597	0.338/ 0.641	0.460/ 0.745
Thyroid, absolute weight (g)	0.327/ 0.425	0.428/ 0.462	0.427/ 0.419	0.385/ 0.410	0.409/ 0.425
Thyroid to body weight ratio (%)	0.009/ 0.011	0.012/ 0.012	0.011/ 0.011	0.011/ 0.010	0.012/ 0.011
Thyroid to brain weight ratio (%)	0.032/ 0.045	0.044/ 0.048	0.044/ 0.044	0.039/ 0.043	0.043/ 0.045

Table 16: Female organ weights – absolute weights are expressed as mean grams; treatment and recovery phase animals separated by a ‘/’; *p<0.05

There were no treatment-related effects on organ weights or weight ratios for animals of either sex receiving the vaccine of either valency or dosage. The above differences are considered incidental to the study because either they did not correlate with any gross or histopathological findings, had differing absolute weights but comparable weight ratio, lacked a correlation between sexes or is considered within the acceptable bounds of biologic variation.

Gross Pathology:

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>
Skin and adnexa, hair loss	0	0	0	0	0	1	0	0	0	0
Injection site, abnormal color	0	0	0	0	0	0	1	0	0	0
<i>Recovery Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>
Skin and adnexa, hair loss	0	0	0	0	0	1	0	0	0	0
Skin and adnexa, wound/scar/crust	0	1	0	0	0	0	0	0	0	1

Table 17: Macroscopic pathology findings – a reading of '-' should be read as zero

Histopathology:

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>
Adrenal, sinusoidal dilation	0	1	0	1	1	0	0	0	0	0
Adrenal, nodular cortical hyperplasia	0	0	0	1	1	1	0	0	0	0
Eye (left), RPE detachment/hypertrophy/pigmentation	0	2	0	1	1	1	0	1	2	1
Eye (right), RPE detachment/hypertrophy/pigmentation	0	1	0	1	1	1	0	0	2	0
Eye (right), retinal dysplasia	0	0	0	0	0	1	0	0	0	0
Heart, aorta adventitia thickening	0	0	0	0	0	0	1	0	0	0
Heart, inflammation with degeneration/necrosis	0	0	0	1	0	0	0	0	0	1
Heart, valvulopathy	0	0	0	0	0	0	0	0	0	1
Heart, chronic endocardial inflammation	1	0	0	0	0	0	0	0	0	0
Heart, mixed cell infiltration	0	0	0	0	0	1	0	0	0	0
Heart, mononuclear cell infiltration	1	1	1	0	0	1	1	1	0	1
Heart, interstitial fibrosis	0	0	0	1	0	0	0	0	1	1
Injection site, hemorrhage	0	0	1	1	1	1	0	0	0	1
Injection site, myofiber degeneration/necrosis	0	0	1	1	1	0	1	2	2	1
Injection site, chronic active inflammation	2	5	5	5	5	2	5	5	5	5
Injection site, deposition material (skin)	0	0	1	0	0	0	0	0	0	0
Joint (stifle), perivascular inflammation	0	0	0	0	0	1	0	0	0	1
Joint (stifle), mononuclear cell inflammation	0	0	0	0	0	0	1	0	0	0
Kidney, tubular basophilia	4	2	2	2	1	3	3	3	2	3
Kidney, deposition material	0	1	0	0	0	1	1	0	0	0
Liver, cytoplasmic rarefaction	1	1	2	1	1	0	0	0	0	0
Liver, mononuclear cell infiltration	0	0	0	0	0	0	0	0	1	1
Liver, acute inflammation	0	0	0	0	0	0	0	1	0	0
Lung, hemorrhage	0	0	0	1	0	0	0	0	1	0
Lung, perivascular and alveolar inflammation	3	2	3	4	3	3	2	2	5	3
Lung, thrombus	0	0	0	1	0	0	0	0	0	0

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>
Lung, interstitial osseous metaplasia	0	0	0	0	1	0	0	0	0	0
Lymph node (iliac), increased germinal centers	0	3	4	3	5	0	1	4	4	5
Lymph node (iliac), increased lymphoid cellularity	0	0	0	0	0	1	0	0	0	0
Lymph node (inguinal), increased germinal centers	0	1	5	1	2	0	3	3	1	2
Pancreas, acinar exocrine necrosis	2	1	1	2	1	3	3	1	3	2
Pancreas, inflammation	0	0	0	0	0	1	0	0	0	0
Prostate, squamous metaplasia	0	0	1	1	0	NA	NA	NA	NA	NA
Salivary gland, chronic salivary duct inflammation	0	0	0	0	1	0	0	0	0	0
Skeletal muscle, mononuclear cell inflammation	0	0	0	0	0	0	0	0	0	1
Spleen, increased germinal centers	0	4	3	5	5	0	4	3	4	4
Testis, hypospermatogenesis	3	1	2	1	4	NA	NA	NA	NA	NA
Testis, seminiferous tubule hypoplasia	0	1	0	0	2	NA	NA	NA	NA	NA
Thymus, ectopic tissue	0	1	0	0	0	0	0	0	0	0
Thyroid, cyst	0	0	1	0	0	0	1	0	0	4
Thyroid, increased colloid	0	0	1	0	0	0	0	0	0	0
Urinary bladder, deposition material	0	0	1	0	0	0	0	0	0	0

Table 18: Treatment phase animal microscopic pathology findings – values represent total occurrence; NA = not applicable

<i>Recovery Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>
Adrenal, deposition pigment	0	2	0	1	2	0	0	0	0	0
Adrenal, sinusoidal dilation	1	3	1	1	1	0	0	0	0	0
Adrenal, nodular cortical hyperplasia	0	0	0	0	0	1	0	0	0	1
Brain, ventricular dilation	0	0	0	0	0	0	0	0	1	0
Cecum, inflammation	0	0	0	0	0	0	0	1	0	0
Duodenum, inflammation	0	0	0	0	0	0	0	0	1	0
Eye (left), RPE detachment/hypertrophy/pigmentation	1	2	2	2	0	0	0	0	1	0
Eye (left), retinal dysplasia	0	0	1	0	0	0	0	0	0	0
Eye (left), choroid hemosiderophages	0	0	0	0	1	0	0	0	0	0
Eye (right), RPE detachment/hypertrophy/pigmentation	1	4	3	2	0	0	1	0	1	0
Eye (right), retinal dysplasia	0	0	1	0	0	0	0	0	0	0
Gall bladder, mononuclear cell infiltration	0	0	0	0	1	0	0	0	0	1
Heart, mononuclear cell infiltration	0	2	0	0	3	2	1	2	1	3
Heart, interstitial fibrosis	0	0	0	0	0	0	0	0	1	0
Heart, degeneration/fibrosis	0	1	0	0	0	0	0	0	0	0
Injection site, chronic inflammation	0	2	5	3	4	0	4	5	3	5
Injection site, myofiber degeneration/necrosis	0	0	2	1	1	0	0	0	0	0
Injection site, dermal and subcutaneous fibrosis	0	0	0	0	0	1	0	0	0	0
Kidney, tubular basophilia	2	4	4	2	2	4	3	4	5	4
Kidney, pelvic inflammation	1	0	0	0	0	0	0	0	0	1
Kidney, mononuclear cell infiltration	1	0	0	0	0	0	0	0	0	1
Kidney, glomerulopathy	0	0	1	0	0	0	0	0	0	1

<i>Recovery Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>
Liver, centrilobular hepatocyte vacuolation	0	1	0	0	0	0	0	0	0	0
Liver, periportal hepatocyte vacuolation	0	0	0	0	0	0	1	0	0	0
Liver, cytoplasmic rarefaction	0	0	1	1	0	0	0	0	0	0
Liver, mononuclear cell infiltration	1	0	0	0	1	1	0	0	2	2
Liver, periportal fibrosis	0	0	0	0	0	0	2	0	0	0
Lung, alveolar hemorrhage	0	0	0	0	0	1	1	1	0	0
Lung, perivascular and alveolar inflammation	4	3	1	2	3	3	1	4	3	2
Lymph node (iliac), increased germinal centers	0	1	4	1	4	0	0	3	3	3
Lymph node (inguinal), increased germinal centers	0	0	4	0	0	0	0	1	2	4
Lymph node (mesenteric), medullary foamy macrophages	0	0	0	0	1	0	0	0	0	0
Pancreas, ectopic spleen	0	1	1	2	0	0	2	0	0	0
Pancreas, acinar exocrine necrosis	0	0	0	0	0	0	0	0	0	1
Peripheral nerve, infiltrate	0	0	0	0	0	1	0	0	0	0
Prostate, squamous metaplasia	0	1	0	1	2	NA	NA	NA	NA	NA
Seminal vesicle, infiltrate	1	0	0	0	0	NA	NA	NA	NA	NA
Skeletal muscle, mononuclear cell infiltration	0	0	0	0	0	1	0	0	0	0
Skin and adnexa, dermal inflammation	0	1	0	0	0	0	0	0	0	0
Spleen, hemosiderin deposition	2	0	0	0	1	2	0	0	0	1
Spleen, increased germinal centers	0	1	3	5	3	0	0	3	3	3
Testis, hypospermatogenesis	0	3	1	3	2	NA	NA	NA	NA	NA
Thoracic cavity, granulomatous inflammation	0	0	0	0	0	0	0	1	0	0
Thymus, mediastinal fibrosis	0	0	0	0	1	0	0	0	0	0
Thyroid, cyst	1	3	2	2	1	1	3	1	0	1
Thyroid, follicular dilation	0	1	0	0	0	0	0	0	0	0
Trachea, mucosal and submucosal eosinophilic infiltration	1	0	0	0	1	1	1	0	1	0
Urinary bladder, submucosal inflammation	1	0	1	0	1	0	0	0	0	0

Table 19: Treatment phase animal microscopic pathology findings – values represent total occurrence; NA = not applicable

An extensive number of tissues were examined for histology. Treatment-related increases in germinal center formation, characterized by immature B lymphocytes, were observed in all treatment groups of both sexes both in the spleen and in the draining lymph nodes (iliac and inguinal). This finding was unaffected by valency or dose but was observed in higher incidence in vaccinated animals compared to those receiving just the adjuvant. These germinal centers were still present at the end of the recovery phase, though in lower frequency.

In the animals receiving 20vPnC, there were microscopic findings observed in the heart which were considered test article-related and adverse by the study pathologist. These consisted of mild to moderate multifocal inflammation with degeneration/necrosis of cardiac myocytes and minimal fibrosis in the left ventricle of female animal 95 (group 5) and male animal 34 (group 4). Animal 95 also had minimal valvulopathy. These lesions were in

the left ventricle (specifically the papillary muscle) and were characterized by accumulations of “primarily mononucleated cells (macrophages), fewer enlarged stromal cells with occasional mitoses, rare heterophils, and increased extracellular basophilic matrix around hypereosinophilic homogenous or fragmented cardiac myocytes. Masson’s Trichrome stain demonstrated minimal increased interstitial fibrosis. Frequently, the inflammation extended to the endocardium adjacent to affected areas. Minimal valvulopathy in female Animal 95 was characterized by enlarged stromal cells with occasional mitoses and increased extracellular basophilic matrix.” These findings were not observed in any saline control, adjuvant control or (b) (4) vaccinated rabbits.

These two animals were tested and found negative for rabbit coronavirus, encephalomyocarditis virus and encephalitozoon cuniculi, to rule out these pathogens as possible causes of the cardiac findings.

Otherwise, no increased incidence of histological findings indicative of potential adverse events was observed in treated animals relative to controls. The remainder of the findings listed in table 16 are considered incidental to the study either due to even distribution between treated and control animals or they are considered recognized and established background pathology for the species.

Local toxicity: Draize scoring of the injection site revealed the following as presented in the tables below³:

Erythema						
<i>Dose</i>	<i>Score</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
1	1	1	5	2	2	4
1	2	0	0	0	0	2
1	3	0	0	0	0	0
1	4	0	0	0	0	0
2	1	2	1	2	0	1
2	2	0	0	0	0	0
2	3	0	0	0	0	0
2	4	0	0	0	0	0
3	1	1	1	3	2	4
3	2	0	0	0	0	0
3	3	0	0	0	0	0
3	4	0	0	0	0	0
4	1	0	4	9	7	8
4	2	1	0	0	0	0
4	3	0	0	0	0	0
4	4	0	0	0	0	0
5	1	2	2	6	3	2
5	2	0	0	0	0	0

³ Draize, Dermal Toxicity, In: Association of Food and Drug Officials US Appraisal of the Safety of Chemicals and Food, Drugs and Cosmetics, pp 46-59, Texas State Dept of Health, Austin, 1959.

<i>Dose</i>	<i>Score</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
5	3	0	0	0	0	0
5	4	0	0	0	0	0

Table 20: Draize scoring for erythema at injection sites – values represent total incidence within 3 days following administrations; values from males and females combined

Edema						
<i>Dose</i>	<i>Score</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>
1	1	1	2	1	2	4
1	2	0	0	0	1	0
1	3	0	0	0	0	0
1	4	0	0	0	0	0
2	1	1	2	2	2	6
2	2	0	0	1	0	0
2	3	0	0	0	0	0
2	4	0	0	0	0	0
3	1	2	1	1	5	5
3	2	0	0	2	0	0
3	3	0	0	0	0	1
3	4	0	0	0	0	0
4	1	0	2	4	9	5
4	2	0	0	2	0	1
4	3	0	0	0	0	0
4	4	0	0	0	0	0
5	1	1	1	5	2	1
5	2	0	0	0	2	2
5	3	0	0	0	0	0
5	4	0	0	0	0	0

Table 21: Draize scoring for edema at injection sites – values represent total incidence within 3 days following administrations; values from males and females combined

Draize scoring of the injection sites revealed a slight increase in frequency and severity of irritation in the treated animals compared to control animals.

Histologically, the injection sites of treated animals at the end of the treatment phase showed an increased incidence of minimal to moderate chronic-active inflammation with myofiber degeneration/necrosis. The inflammation was characterized by accumulations of large macrophages containing basophilic material (suspected to be aluminum by the study pathologist) and variable numbers of heterophils and lymphocytes. These cells were observed within and/or along the muscle bundles of the injection tract and/or accumulated in the skin. While these changes were not dose-dependent and unaffected by valency, the study pathologist notes there was a correlation between the injection site pathology and increases in fibrinogen and CRP. These findings were still present at the end of the recovery period, though improved in severity.

1 Serology:

<i>Study Day</i>	<i>-8</i>	<i>-8</i>	<i>-8</i>	<i>-8</i>	<i>-8</i>	<i>15</i>	<i>15</i>	<i>15</i>	<i>15</i>	<i>15</i>	<i>87</i>	<i>87</i>	<i>87</i>	<i>87</i>	<i>87</i>
<i>Serotype</i>	<i>Grp. 1</i>	<i>Grp. 2</i>	<i>Grp. 3</i>	<i>Grp. 4</i>	<i>Grp. 5</i>	<i>Grp. 1</i>	<i>Grp. 2</i>	<i>Grp. 3</i>	<i>Grp. 4</i>	<i>Grp. 5</i>	<i>Grp. 1</i>	<i>Grp. 2</i>	<i>Grp. 3</i>	<i>Grp. 4</i>	<i>Grp. 5</i>
1	1.49	1.16	1.64	1.49	1.49	1.49	1.49	119.13	75.86	59.99	1.49	1.49	166.09	132.53	166.51
3	1.12	1.35	1.27	1.32	1.26	1.16	1.31	71.48	48.65	56.60	1.12	1.27	1021.39	938.66	984.04
4	1.50	1.50	1.50	1.50	1.50	1.50	1.50	151.26	90.10	150.70	1.50	1.50	759.42	570.29	518.50
5	0.68	0.68	0.68	0.68	0.68	0.68	0.68	191.37	114.55	154.26	0.68	0.68	503.54	453.01	389.10
6A	0.86	0.86	0.86	0.86	0.86	0.86	0.86	116.59	244.51	184.39	0.86	0.86	234.02	377.02	292.87
6B	0.87	0.87	0.87	0.87	0.87	0.87	0.87	42.96	69.19	42.78	0.87	0.97	436.45	550.95	367.36
7F	1.17	1.27	1.31	1.24	1.11	1.21	1.24	1701.60	1478.74	1694.94	1.29	1.37	556.74	570.48	495.66
9V	5.68	5.93	6.36	5.83	8.59	5.92	6.41	161.72	129.94	203.41	7.40	8.00	329.73	332.80	266.27
14	2.00	3.06	6.33	2.28	1.73	1.54	4.96	364.35	172.53	284.00	1.38	4.71	1166.81	646.61	656.47
18C	1.85	1.85	1.85	1.97	1.85	1.85	1.85	375.55	655.74	499.92	1.85	1.85	1033.03	1489.32	1003.45
19A	0.77	0.77	0.81	0.77	0.77	0.77	0.77	244.63	291.61	182.60	0.77	0.77	1335.93	1398.24	1112.02
19F	0.61	0.61	0.61	0.61	0.61	0.61	0.61	572.43	620.37	715.63	0.61	0.61	1566.19	1844.41	1558.09
23F	24.43	24.43	24.43	24.43	24.43	24.43	24.43	44.88	44.51	43.82	24.43	24.43	1381.01	1406.21	1335.33
8	29.25	29.25	32.48	29.25	30.74	29.25	29.25	NT	4808.16	4565.12	29.25	29.25	NT	2315.63	2460.55
10A	68.40	74.60	68.40	68.40	68.40	68.40	75.04	NT	831.00	1415.66	68.40	82.67	NT	2210.54	1698.23
11A	43.87	43.87	43.87	43.87	46.05	43.87	43.87	NT	5831.26	5334.10	43.87	43.87	NT	5945.97	2992.60
12F	6.30	6.30	6.30	6.30	6.30	6.30	6.30	NT	685.39	905.73	6.30	6.30	NT	992.94	918.77
15B	14.39	14.39	16.50	14.39	14.39	14.39	16.28	2420.72	1626.70	1648.85	14.39	16.44	2579.26	2207.38	1619.87
22F	57.38	57.38	60.66	57.38	57.38	57.38	57.38	4647.88	3729.95	4365.15	57.38	57.38	2638.71	3204.06	2000.38
33F	9.07	8.72	8.34	8.94	9.40	8.99	9.33	844.46	879.73	851.32	10.49	9.97	447.87	678.86	485.54

2 Table 22: Serology results – data presented in geometric mean titers with both sexes combined; Grp. = group; NT = not tested

Analysis of serum samples for IgG against all 20 serotypes was carried out via a validated (b) (4) assay at the Pfizer VRU laboratory in Pearl River, NY. Serum samples were collected pretest and on study days 15 and 87. Results are presented as Log₁₀ transformed titers. Results were not calculated for serotypes 8, 10A, 11A and 12F for group 3 after the pretest sample because they were not part of the (b) (4) formulation.

Results of the (b) (4) assay revealed a robust antibody response to all of the tested serotypes for animals of both sexes in groups 3, 4 and 5 when compared to both the adjuvant and saline control groups from study day 15 onward. All animals were seronegative at the pretest analysis and the animals receiving saline or the adjuvant alone did not seroconvert at any point in the study. See table 19 below for results:

Assessment: There was no treatment-related mortality or any toxicologically-relevant effects on clinical signs, relative food consumption, ophthalmologic parameters, and body temperature. Treatment-related effects were limited to changes in local reactogenicity, clinical pathology and histopathology.

At the injection sites of treated animals, there were mild increases in the incidence of very slight to well defined erythema and edema which all resolved within a few days. Additionally, increased incidence of minimal to moderate chronic-active inflammation with myofiber degeneration/necrosis was observed on histology of injection sites. This was only partially recoverable but is considered an anticipated effect associated with the immunological response to vaccine administration rather than as a sign of frank toxicity.

Among the clinical pathology parameters, treatment-related changes were limited to mild to moderate increases in LUC and the acute phase reactants fibrinogen and CRP. The latter with the severity of the chronic-active inflammation at injection sites, per the study pathologist. Further evidence of a systemic immune response to the vaccine was observed through increased lymphocytic germinal center formation in injection site draining lymph nodes and the spleens of treated animals. These were all fully or partially-recoverable, considered anticipated effects associated with an immunological response and did not occur at a degree which should create any cause for concern with proceeding with the planned clinical protocol with the exception of CRP. The elevation in CRP observed after the 5th dose was to a degree where there can be some anticipated risk of human subjects experiencing malaise, fatigue and nausea.

Of most notable concern, however, were the changes observed in hearts on histology. Though at low incidence, the administration of 20vPnC was associated with up to moderate inflammation with degeneration/necrosis of cardiac myocytes and interstitial fibrosis in the papillary muscle of the left ventricle, with a valvulopathy observed amongst the high dose group. This finding was deemed to be test-article related and adverse by the study pathologist. While there were no outward clinical effects on the animals in this study, this should be considered test-article related.

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

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

4.2.3.5.1 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

A COMBINED FERTILITY AND DEVELOPMENTAL STUDY (INCLUDING TERATOGENICITY AND POSTNATAL INVESTIGATIONS) OF PF-06482077 VACCINE BY INTRAMUSCULAR ADMINISTRATION IN THE RABBIT

Study number: 18GR287

Performing laboratory: (b) (4)

Study initiation date: February 19th, 2019

Final report date: February 13th, 2020

Test article batch/lot: CSM13195_18-002661_X29567-2, expiry date not provided

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

Breeder/supplier: (b) (4)

Number of animals per group and sex: 44 females per group; males in this study were stock studs not kept on study

Age: 17-18 weeks at time of first administration (stud males were at least 53 weeks at time of pairing with females)

Body weight range: 3561 to 4442g at the time of mating

Means of administration: Intramuscular injection, needle and syringe

Site of administration: Alternating quadriceps musculature

Volume of injection: 0.5 mL

Frequency of administration: 2 pre-mating doses, 2 gestational doses

Dose: 46.2 µg (4.4 µg/mL per serotype with the exception of 6B at 8.8 µg/mL)

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use, pre-filled syringes (one syringe per dose). Stability studies were performed by Pfizer

(b) (4) Specification Review Team on the same batches of vaccine and adjuvant control as used in this study.

Report status: Final

Experimental design: Animals were acclimated for at least 7 days, randomized then assigned to 1 of 2 groups according to the table below. Administration of saline control and 20vPnC occurred 4 and 14 days prior to mating and gestation days (GD) 10 and 24. Does were assigned to have terminal caesareans on GD 29 or to be humanely terminated on postnatal day (PND) 35.

<i>Group</i>	<i>Treatment</i>	<i>Caesarean Subset</i>	<i>Littering Subset</i>
1	Saline Control	22	22
2	20vPnC	22	22

Table 23: Group assignments – value represent number of female rabbits assigned to group subsets

Methods for blood collection: Blood from unfasted does was drawn from ear arteries without anesthetic or sedative assistance. Blood samples from fetuses and kits were collected

via intracardiac puncture and pooled per litter following euthanasia (fetuses) or anesthesia (pups)

Randomization procedure: Yes, based on body weight classes

Statistical analysis plan: Yes, with assistance from the (b) (4) data acquisition system

The following parameters were evaluated:

<i>Parameter</i>	<i>Frequency of Testing</i>
Cageside observations ⁴	Twice daily
Clinical examinations ⁵	Weekly
Injection site observations	Twice post-dose on days of administrations
Body weight	PMD: Weekly, days of administration GD: 0, 6, 10, 13, 16, 20, 24, 27, and 29 PND: 4, 7, 11, 14, 17, 21, 28, and 35
Food consumption	PMD: 1-7, 7-14, 14-17 and 1-17 GD: 0-6, 6-10, 10-13, 13-16, 16-20, 20-24, 24-27, 27-29 and 0-29 PND: 0-4, 4-7, 7-11, 11-14, 14-17, 17-21, 21-28, 28-35 and 0-35
Body temperature:	Not collected
Littering data:	
No. kits alive	PND 0, 4, 7, 11, 14, 17, 21, 28 and 35
Live kit weight	PND 4, 7, 11, 14, 17, 21, 28 and 35
Surface righting reflex	Not evaluated
Pupil reflex	PND 35
Auditory reflex	PND 35
Postmortem examinations	Caesarean subset: GD 29 Littering subset: PND 35
Immunological response*	Does: PMD -17, 1; GD 29, PND 35 Fetuses: GD 29 Kits: PND 35

Table 24: Experimental design – PMD = pre-mating day; GD = gestation day; PND = postnatal day; *blood collected from ear arteries from does and intracardiac puncture from fetuses and kits

Postmortem procedures: Does were humanely euthanized on their scheduled termination days (GD 29 and PND 29 for caesarean and littering subsets, respectively) via an intravenous injection of sodium pentobarbitone followed by exsanguination. The carcasses were then weighed and underwent a macroscopic necropsy examination. For caesarean subset females, the following were recorded: pregnancy status, number and distribution of corpora lutea, gravid uterine weight, pre- and post-implantation loss, number and distribution of implantations to correlate with live fetuses, dead fetuses, early resorptions and late resorptions, individual fetal weights and fetal sexing.

Fetuses from the caesarean subset were humanely euthanized by oral administration of sodium pentobarbitone then then sexed and examined viscally. Approximately half of the fetuses had their heads removed and fixed for subsequent examination. Eviscerated fetal

⁴ Cageside observations include mortality, morbidity, general health and signs of toxicity.

⁵ Clinical examination details not provided.

carcasses were fixed and processed for skeletal examinations via maceration in potassium hydroxide, staining with Alizarin red and passage into glycerol.

Kits from the littering subsets were humanely euthanized in the same fashion (except they received an intramuscular injection of ketamine and xylazine prior to euthanasia) and underwent a macroscopic necropsy examination in the same fashion as the does.

RESULTS

Morbidity and mortality: A group 2 doe was euthanized on PND 2 following decreased food consumption and total litter death. Another group 2 doe was euthanized for ethical reasons on PND 17 due to poor motor coordination, irregular breathing and reduced feces. There were also “red traces” observed in the cage. Neither of these incidents were considered to be related to treatment as the total litter loss was within the bounds of the historical control data and the latter incident was considered to be related to housing trauma.

Clinical observations: There were no treatment-related clinical signs observed in this study. Those abnormal signs observed in this study were considered incidental to the study either because of sporadic incidence comparable between treated and control animals or the signs are considered recognized background findings in laboratory rabbits.

Body weight change:

<i>Study Days</i>	<i>Group 1</i>	<i>Group 2</i>
PMD -8 to -3	97.3	79.0
PMD -7 to -1	113.1	106.3
PMD -3 to -1	52.9	16.6
PMD 1 to 7	123.1	141.4
PMD 7 to 14	173.7	170.7
PMD 1 to 14	296.8	312.1
GD 0 to 6	168.3	164.0
GD 6 to 10	68.2	72.8
GD 10 to 13	71.0	69.9
GD 13 to 16	90.8	87.0
GD 16 to 20	-0.5	-34.0
GD 20 to 24	-4.9	-5.8
GD 24 to 27	16.0	24.6
GD 27 to 29	40.0	15.1
GD 0 to 29	448.9	393.4
PND 4 to 7	31.8	54.9
PND 7 to 11	49.4	52.1
PND 11 to 14	6.5	46.5*
PND 14 to 17	24.1	17.3
PND 17 to 21	-29.6	-50.8
PND 21 to 28	-14.3	-13.0
PND 28 to 35	-17.1	-57.2
PND 4 to 35	38.3	59.5

Table 25: Body weight change – values presented as change in body weight in mean grams (g) between study days; PMD = prenatting day; GD = gestation day; PND = postnatal day; *p<0.05

There were no treatment-related, toxicologically-relevant differences in body weight gain in does during this study. The differences observed are considered incidental because they are minimal in nature and within the normal bounds of biologic variation or did not correlate with any other evidence of illness.

Food consumption:

<i>Study Days</i>	<i>Group 1</i>	<i>Group 2</i>
PMD 1 to 7	182.90	185.77
PMD 7 to 14	186.69	191.02
PMD 14 to 17	188.73	191.53
PMD 1 to 17	185.65	189.15
GD 0 to 6	186.53	188.11
GD 6 to 10	186.26	193.24
GD 10 to 13	179.39	181.41
GD 13 to 16	153.74	151.23
GD 16 to 20	159.11	155.37
GD 20 to 24	127.38	119.69
GD 24 to 27	97.41	85.57
GD 27 to 29	113.09	100.98
GD 0 to 29	156.14	153.74
PND 0 to 4	229.80	214.34
PND 4 to 7	315.44	306.30
PND 7 to 11	324.09	324.46
PND 11 to 14	334.18	346.82
PND 14 to 17	350.77	358.88
PND 17 to 21	359.93	362.96
PND 21 to 28	526.11	513.28
PND 28 to 35	795.00	819.92
PND 0 to 35	455.55	456.44

Table 26: Food consumption – values presented as mean grams/animal/day between study days; (C) = caesarean subset; (L) = littering subset; PMD = pre mating day; GD = gestation day; PND = postnatal day

There were no treatment-related, toxicologically-relevant differences in food consumption in does during this study. The differences observed are considered incidental because they are minimal in nature and within the normal bounds of biologic variation, or the treated does consumed more food than control does.

Mating performance:

<i>Parameter (Unit)</i>	<i>Group 1</i>	<i>Group 2</i>
Does paired (N)	44	44
Does that failed to mate	2C	1C
Does inseminated	42 (20C+22L)	43 (21C+22L)
Does pregnant	38 (19C+19L)	42 (20C+22L)
Does not pregnant	4 (1C+3L)	1C
Copulation index (%)	95	98

<i>Parameter (Unit)</i>	<i>Group 1</i>	<i>Group 2</i>
Pregnancy rate (%)	86	96
Fertility index (%)	90	98
Caesarean subset does with viable fetuses	19	19
Lactation subset does pregnant that littered	19	22
Lactation subset does that died post-partum	0	1
Lactation subset does with total litter death post-partum	0	1
Lactation subset does that reared kits to weaning	19	22
Gestation index (%)	100	100

Table 27: Mating performance and fertility assessment

The indices in table 27 were calculated as follows:

- Copulation index = (no. of inseminated females/no. of paired females) x 100
- Pregnancy rate = (no. of pregnant females/no. of paired animals) x 100
- Fertility index = (no. of pregnant females/no. of inseminated females) x 100
- Gestation index = (no of females with live kits/no. of pregnant females) x 100

There were no treatment-related effects observed on female fertility and mating performance in this study. The single isolated incident of total litter loss is within the bounds of the historical control data of the testing facility.

Caesarean weights:

<i>Parameter (Unit)</i>	<i>Group 1</i>	<i>Group 2</i>
Gravid uterus weight (mean grams)	592.71	562.16
Body weight at necropsy (mean grams)	4539.7	4515.0
Body weight adjusted for uterine weight (mean grams)	3947.03	3952.84
Net body weight change from day 0 (mean grams)	385.32	330.79
Day 0 net weight change – uterine weight (mean grams)	-207.39	-231.37
Mean fetal weight (mean grams)	39.79	32
No. live fetuses (mean N)/litter	10.2	9.5

Table 28: Gravid uterine weight and maternal body weight change

Caesarean parameters:

<i>Parameter (Unit)</i>	<i>Group 1</i>	<i>Group 2</i>
Females pregnant (N)	19	19
Does with viable fetuses (N)	19	19
No. of corpora lutea (mean N)	11.1	10.9
No. of implantations (mean N)	10.6	10.0
Pre-implantation loss (calculated mean)	0.5	0.9
Pre-implantation loss (calculated %)	4.92	8.66*
No. of early resorptions (mean N)	0.2	0.3
Early resorptions (mean %)	1.90	2.65
No. of late resorptions (mean N)	0.2	0.3
Late resorptions (mean %)	1.77	2.75

<i>Parameter (Unit)</i>	<i>Group 1</i>	<i>Group 2</i>
No. of dead fetuses (mean N)	0.0	0.0
Post-implantation loss (calculated N)	0.4	0.5
Post-implantation loss (calculated %)	3.67	5.39
No. of live fetuses/litter (mean N)	10.2	9.5
No. of male fetuses/litter (mean N)	4.6	4.6
No. of female fetuses/litter (mean N)	5.6	4.9
Male fetuses (mean %)	46.41	46.58
Total litter weight (mean grams)	393.24	365.07
Mean fetal weight, both sexes (mean grams)	39.79	39.32
Mean fetal weight, males (mean grams)	39.99	39.54
Mean fetal weight, females (mean grams)	38.66	38.64

Table 29: Caesarean examination results – *p<0.05

There were no treatment-related effects observed on any of the pregnancy parameters assessed in this study. This included female fertility and maternal performance, gravid uterine weight, fetal viability, fetal weights, number of corpora lutea, number of implantations, implantation loss and resorptions. Those differences observed in this study were considered incidental either because they were minimal and within the bounds of biologic variation or were within the bounds of the provided historical control data for the facility.

Fetal examinations:

<i>Fetal Examination Finding</i>	<i>Group 1</i>	<i>Group 2</i>
Limb/paw/digit, paw hyperflexion (M)	0/0	1/1
Gall bladder, absent (M)	1/1	0/0
Gall bladder, small (A)	2/2	2/2
Heart, misshapen (M)	0/0	1/1
Heart, three-chambered (M)	1/1	0/0
Heart, ventricular chamber large (M)	0/0	1/1
Heart, ventricular septum defect (M)	1/1	0/0
Heart, ventricular wall thick (M)	1/1	0/0
Kidney, dilated renal pelvis (A)	1/1	0/0
Kidney, malpositioned (A)	1/1	0/0
Kidney, misshapen (A)	1/1	0/0
Liver, lobe cyst (A)	0/0	1/1
Liver, discolored (A)	0/0	1/1
Liver, misshapen (A)	1/1	0/0
Lung, absent lobe (A)	1/1	4/3
Lung, small lobe (A)	1/1	1/1
Lung, abnormal location (M)	0/0	1/1
Lung, small (A)	1/1	0/0
Major blood vessel, dilated aortic arch (M)	1/1	0/0
Major blood vessel, absent common carotid trunk (V)	37/13	33/11
Major blood vessel, narrowed pulmonary trunk (M)	1/1	0/0
Major blood vessel, retroesophageal subclavian artery (A)	1/1	0/0
Ovary, cyst (A)	0/0	2/2

<i>Fetal Examination Finding</i>	<i>Group 1</i>	<i>Group 2</i>
Spleen, discolored (A)	1/1	0/0
Ureter, retrocaudal (A)	4/4	7/5
Skull, cranium sutural bone (A)	1/1	2/1
Skull, hyoid incomplete ossification (A)	3/3	1/1
Skull, small interparietal (A)	1/1	0/0
Skull, interparietal unossified line (A)	1/1	0/0
Skull, nasal unossified line (A)	0/0	1/1
Skull, parietal unossified area (A)	1/1	1/1
Skull, supraoccipital misshapen (A)	1/1	0/0
Forepaw, metacarpal, incomplete ossification of 2 nd to 5 th digit (A)	1/1	0/0
Forepaw, metacarpal, unossified 1 st digit (V)	7/4	6/3
Forepaw, phalanx, incomplete proximal ossification (A)	1/1	0/0
Forepaw, phalanx, middle unossified (V)	0/0	6/4
Hindpaw, unossified tarsal bone (A)	1/1	2/1
Pelvic girdle, malpositioned (A)	8/6	10/5
Pubis, incomplete ossification (A)	4/4	6/3
Ribs, detached (A)	6/3	6/5
Ribs, number of full ribs = 12/12 (V)	127/19	111/18
Ribs, number of full ribs = 12/13 (V)	25/14	28/14
Ribs, short (A)	14/11	18/9
Ribs, supernumerary lumbar (A)	17/11	24/13
Sternebra, extra ossification site (A)	1/1	1/1
Sternebra, fused (M)	3/3	1/1
Sternebra, minor fusion (A)	1/1	4/2
Sternebra, misshapen (A)	1/1	0/0
Sternebra, multiple abnormalities (M)	0/0	1/1
Sternebra, 5 th unossified (V)	41/15	35/13
Sternebra, 6 th unossified (V)	7/4	10/5
Sternebra, 2 nd and 4 th incomplete ossification (V)	2/2	0/0
Sternebra, 6 th incomplete ossification (V)	27/10	18/8
Vertebra, caudal, number <14 (A)	1/1	1/1
Vertebra, cervical, extra ossification (A)	0/0	1/1
Vertebra, lumbar, number = 6 (V)	32/15	38/15
Vertebra, lumbar, number = 8 (V)	1/1	6/4
Vertebra, thoracic, 1 st to 9 th incomplete ossification (A)	1/1	0/0
Vertebra, thoracic, multiple abnormalities (M)	0/0	1/1
Vertebra, thoracic, number = 12 (V)	127/19	111/18

Table 30: Fetal caesarean examination findings – values representing individual incidence and litter incidence separated by a '/'; (A) = anomaly; (V) = variation; (M) = malformation

There were no treatment-related findings among the fetal caesarean examinations observed that have a clear concern for human recipients of 20vPnC. With the exception of two findings, all of the above findings are considered incidental either because they were in comparable incidence between treated and control animals or were within the historical reference range for the test facility. The two exceptions include absent lung lobes and

unossified forepaw middle phalanxes. These were near the upper bounds of the historical reference range in terms of percentage incidence at 2.2% (historical incidence ~1.0 – 1.5%) and 3.3% (historical incidence 2.2 – 2.9%).

Delivery and litter data:

<i>Parameter (Unit)</i>	<i>Group 1</i>	<i>Group 2</i>
Females completing delivery (N)	19	21
Females completing delivery with liveborn pups (N)	19	21
Females completing delivery with stillborn pups (N)	3	6
Females completing delivery with all stillborn pups (N)	0	0
Females completing delivery with all dead on PND 35 (N)	0	1
Gestation length (mean days)	31.2	30.7*
Number of implantation sites (mean N)	8.9	9.7
Pre-birth loss (mean %)	3.02	4.80
Pups delivered/litter (mean N)	8.3	9.1
Live kits on PND 0 (mean N)	8.1	8.6
Live kits on PND 4 (mean N)	8.0	8.8
Live kits on PND 7 (mean N)	7.6	8.3
Live kits on PND 11 (mean N)	7.5	8.2
Live kits on PND 14 (mean N)	7.4	8.2
Live kits on PND 17 (mean N)	7.3	8.2
Live kits on PND 21 (mean N)	7.3	8.2
Live kits on PND 28 (mean N)	7.3	8.1
Live kits on PND 35 (mean N)	7.3	8.1
Kits dead, missing or cannibalized on PND 0 (N)	4	11
Kits dead, missing or cannibalized over PND 1-7 (N)	9	15
Kits dead, missing or cannibalized over PND 8-14 (N)	4	3
Kits dead, missing or cannibalized over PND 15-21 (N)	3	0
Kits dead, missing or cannibalized over PND 22-28 (N)	NVR	1
Kits dead, missing or cannibalized over PND 29-35 (N)	0	0
Kits dead, missing or cannibalized over PND 1-35 (N)	16	19
Live birth index (%)	97.5	94.3
Viability index (PND 0-4) (%)	98.7	97.2
Lactation index (PND 4-35) (%)	90.8	92.0
Sex ratio PND 35- (mean % males)	53.2	46.8

Table 31: Delivery and litter data – PND = postnatal day; NVR = no value reported; *p<0.05

The indices in table 31 were calculated as follows:

- Pre-birth loss = (no. kits born/no. implantation sites) x 100
- Live birth index = (no kits born alive/no. pups born) x 100
- Viability index = (no. kits alive on PND 4/no. kits live at birth) x 100
- Lactation index = (no. kits alive on PND 35/no. kits alive on PND 4) x 100

There were no treatment-related effects observed on gestation length, implantation sites, parturition and kit viability postpartum for the littering subsets in this study. Those differences observed above were considered incidental to the study and within the normal

bounds of biologic variation or within the background historical incidence for the test facility.

Clinical observations (F1 generation): There were no clinical findings observed in the F1 generation kits during the postnatal period that could be attributed to the does being treated with 20vPnC. Those abnormal clinical signs which were observed were considered incidental to the study either due to being sporadic (observed in a single kit), in comparable incidence to kits from treated and control does or are recognized background findings in laboratory rabbits.

Body weights (F1 generation):

<i>Postnatal Day</i>	<i>Group 1</i>	<i>Group 2</i>
4	89.46	82.55
7	126.27	113.94
11	170.35	158.81
14	204.90	191.87
17	242.92	222.93
21	295.39	265.88
28	517.19	467.50
35	805.38	750.65

Table 32: F1 generation body weights – values presented as mean pup absolute body weight in grams (g)

There was a minimally (<10%) decreased mean body weight observed in pups from vaccinated does, but this could be attributed to normal biologic variation and was within the bounds of the historical reference range for the test facility. Therefore, this difference should be considered incidental to the study.

Functional development (F1 generation): All live kits from both vaccinated and control does were found to have normal pupillary and auditory reflexes on postnatal day 35.

Postmortem examinations:

<i>Necropsy Finding</i>	<i>Group 1</i>	<i>Group 2</i>
Oviduct (right), several cysts	0	2
Oviduct (right), single cyst	2	2
Oviduct (left), several cysts	2	1
Oviduct (left), single cyst	0	2
Oviduct (bilateral), several cysts	1	0
Oviduct (bilateral), single cyst	2	0
Ears (bilateral), several sores/crusts	0	1
Skin/subcutis, left hindlimb many sores/crusts	3	0

Table 33: Macroscopic examination findings, F0 generation, caesarean subset

<i>Necropsy Finding</i>	<i>Group 1</i>	<i>Group 2</i>
Oviduct (right), several cysts	1	0
Oviduct (right), single cyst	1	0

<i>Necropsy Finding</i>	<i>Group 1</i>	<i>Group 2</i>
Oviduct (left), several cysts	1	0
Oviduct (bilateral), several cysts	1	1
Oviduct (bilateral), single cyst	0	1

Table 34: Macroscopic examination findings, F0 generation, littering subset

There were no findings on postmortem necropsy examinations considered to be related to treatment in either the does or the F1 generation of kits from the subsets allowed to litter. Those findings in does in the tables above were considered incidental to the study since they were either sporadic or in comparable incidence between treated and control animals. For doe 25 which was found moribund shortly after parturition, no abnormal findings were observed on necropsy and the cause of the rabbit's condition was not determined. Doe 42 which was euthanized for humane reasons on PND 17 was found to have a single dark focus in two lung lobes but no other findings on necropsy. The cause of death of this animal was believed to be due to trauma from housing.

Necropsy examination findings for the F1 generation kits was not presented in tabulated fashion, but review of the individual data did not demonstrate any evidence of an increased incidence of findings between kits from vaccinated does compared to those from control does. There was an increased incidence of thoracic autolysis, but this was isolated to the litter from doe 25 which had complete litter loss and is considered within the historical reference range for the facility.

Serology: Analysis of serum samples for anti-pneumococcal IgG antibodies was performed for all 20 strains in 20vPnC using qualified (b) (4). This portion of the study was not performed under GLP conditions and was conducted by the sponsor. Serum samples for immunogenic response were collected from dams once during the pretest, once right before pairing, at the end of the gestation period and at the end of the postnatal phase. F1 generation kits had serum collected (and pooled per litter) from fetuses at caesarean and kits during necropsy at the end of the study.

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
1	1	Maternal	PMD -17	0.59
1	1	Maternal	PMD 1	0.59
1	1	Maternal	GD 29	0.59
1	1	Maternal	PND 35	0.59
1	1	Fetuses	GD 29	0.59
1	1	Kits	PND 35	0.59
1	2	Maternal	PMD -17	0.59
1	2	Maternal	PMD 1	197.92
1	2	Maternal	GD 29	147.95
1	2	Maternal	PND 35	29.02
1	2	Fetuses	GD 29	221.28
1	2	Kits	PND 35	5.09
3	1	Maternal	PMD -17	0.68
3	1	Maternal	PMD 1	0.65

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
3	1	Maternal	GD 29	0.59
3	1	Maternal	PND 35	0.69
3	1	Fetuses	GD 29	0.59
3	1	Kits	PND 35	0.31
3	2	Maternal	PMD -17	0.32
3	2	Maternal	PMD 1	874.61
3	2	Maternal	GD 29	586.02
3	2	Maternal	PND 35	91.01
3	2	Fetuses	GD 29	747.07
3	2	Kits	PND 35	20.20
4	1	Maternal	PMD -17	2.12
4	1	Maternal	PMD 1	2.12
4	1	Maternal	GD 29	2.12
4	1	Maternal	PND 35	2.12
4	1	Fetuses	GD 29	2.12
4	1	Kits	PND 35	2.12
4	2	Maternal	PMD -17	2.12
4	2	Maternal	PMD 1	928.93
4	2	Maternal	GD 29	849.73
4	2	Maternal	PND 35	236.80
4	2	Fetuses	GD 29	884.50
4	2	Kits	PND 35	33.03
5	1	Maternal	PMD -17	0.65
5	1	Maternal	PMD 1	0.65
5	1	Maternal	GD 29	0.65
5	1	Maternal	PND 35	0.65
5	1	Fetuses	GD 29	0.65
5	1	Kits	PND 35	0.65
5	2	Maternal	PMD -17	0.65
5	2	Maternal	PMD 1	518.52
5	2	Maternal	GD 29	341.05
5	2	Maternal	PND 35	102.94
5	2	Fetuses	GD 29	379.15
5	2	Kits	PND 35	19.49
6A	1	Maternal	PMD -17	1.14
6A	1	Maternal	PMD 1	1.14
6A	1	Maternal	GD 29	1.14
6A	1	Maternal	PND 35	1.25
6A	1	Fetuses	GD 29	1.14
6A	1	Kits	PND 35	1.14
6A	2	Maternal	PMD -17	1.20
6A	2	Maternal	PMD 1	385.01
6A	2	Maternal	GD 29	231.47
6A	2	Maternal	PND 35	39.02

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
6A	2	Fetuses	GD 29	319.80
6A	2	Kits	PND 35	9.72
6B	1	Maternal	PMD -17	0.56
6B	1	Maternal	PMD 1	0.56
6B	1	Maternal	GD 29	0.56
6B	1	Maternal	PND 35	0.56
6B	1	Fetuses	GD 29	0.56
6B	1	Kits	PND 35	0.56
6B	2	Maternal	PMD -17	0.56
6B	2	Maternal	PMD 1	186.75
6B	2	Maternal	GD 29	542.74
6B	2	Maternal	PND 35	53.74
6B	2	Fetuses	GD 29	53.57
6B	2	Kits	PND 35	638.55
7F	1	Maternal	PMD -17	0.76
7F	1	Maternal	PMD 1	0.76
7F	1	Maternal	GD 29	0.69
7F	1	Maternal	PND 35	0.70
7F	1	Fetuses	GD 29	0.72
7F	1	Kits	PND 35	0.64
7F	2	Maternal	PMD -17	0.73
7F	2	Maternal	PMD 1	841.31
7F	2	Maternal	GD 29	283.90
7F	2	Maternal	PND 35	79.74
7F	2	Fetuses	GD 29	378.88
7F	2	Kits	PND 35	19.81
8	1	Maternal	PMD -17	3.34
8	1	Maternal	PMD 1	3.35
8	1	Maternal	GD 29	3.28
8	1	Maternal	PND 35	3.28
8	1	Fetuses	GD 29	3.28
8	1	Kits	PND 35	3.28
8	2	Maternal	PMD -17	3.39
8	2	Maternal	PMD 1	2710.06
8	2	Maternal	GD 29	630.78
8	2	Maternal	PND 35	105.29
8	2	Fetuses	GD 29	1086.45
8	2	Kits	PND 35	30.56
9V	1	Maternal	PMD -17	1.59
9V	1	Maternal	PMD 1	1.79
9V	1	Maternal	GD 29	1.77
9V	1	Maternal	PND 35	1.46
9V	1	Fetuses	GD 29	2.31
9V	1	Kits	PND 35	1.46

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
9V	2	Maternal	PMD -17	1.60
9V	2	Maternal	PMD 1	447.89
9V	2	Maternal	GD 29	316.36
9V	2	Maternal	PND 35	64.90
9V	2	Fetuses	GD 29	335.88
9V	2	Kits	PND 35	12.25
10A	1	Maternal	PMD -17	1.31
10A	1	Maternal	PMD 1	1.31
10A	1	Maternal	GD 29	1.44
10A	1	Maternal	PND 35	1.31
10A	1	Fetuses	GD 29	1.75
10A	1	Kits	PND 35	1.31
10A	2	Maternal	PMD -17	1.69
10A	2	Maternal	PMD 1	650.66
10A	2	Maternal	G29	1208.60
10A	2	Maternal	PND 35	395.99
10A	2	Fetuses	GD 29	1398.26
10A	2	Kits	PND 35	53.27
11A	1	Maternal	PMD -17	0.72
11A	1	Maternal	PMD 1	0.72
11A	1	Maternal	G29	0.72
11A	1	Maternal	PND 35	0.72
11A	1	Fetuses	GD 29	0.72
11A	1	Kits	PND 35	0.72
11A	2	Maternal	PMD -17	0.74
11A	2	Maternal	PMD 1	2429.11
11A	2	Maternal	GD 29	2236.51
11A	2	Maternal	PND 35	1771.67
11A	2	Fetuses	GD 29	1699.31
11A	2	Kits	PND 35	135.71
12F	1	Maternal	PMD -17	1.64
12F	1	Maternal	PMD 1	1.60
12F	1	Maternal	GD 29	1.31
12F	1	Maternal	PND 35	1.54
12F	1	Fetuses	GD 29	1.45
12F	1	Kits	PND 35	1.31
12F	2	Maternal	PMD -17	1.59
12F	2	Maternal	PMD 1	590.04
12F	2	Maternal	GD 29	540.84
12F	2	Maternal	PND 35	82.06
12F	2	Fetuses	GD 29	641.60
12F	2	Kits	PND 35	20.57
14	1	Maternal	PMD -17	0.70
14	1	Maternal	PMD 1	0.70

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
14	1	Maternal	GD 29	0.70
14	1	Maternal	PND 35	0.70
14	1	Fetuses	GD 29	0.70
14	1	Kits	PND 35	0.70
14	2	Maternal	PMD -17	0.70
14	2	Maternal	PMD 1	186.26
14	2	Maternal	GD 29	240.99
14	2	Maternal	PND 35	107.22
14	2	Fetuses	GD 29	368.41
14	2	Kits	PND 35	18.17
15B	1	Maternal	PMD -17	0.79
15B	1	Maternal	PMD 1	0.86
15B	1	Maternal	GD 29	0.86
15B	1	Maternal	PND 35	1.75
15B	1	Fetuses	GD 29	1.04
15B	1	Kits	PND 35	0.63
15B	2	Maternal	PMD -17	0.96
15B	2	Maternal	PMD 1	1648.40
15B	2	Maternal	GD 29	1302.01
15B	2	Maternal	PND 35	505.25
15B	2	Fetuses	GD 29	1430.72
15B	2	Kits	PND 35	54.52
18C	1	Maternal	PMD -17	0.67
18C	1	Maternal	PMD 1	0.67
18C	1	Maternal	GD 29	0.67
18C	1	Maternal	PND 35	0.67
18C	1	Fetuses	GD 29	0.67
18C	1	Kits	PND 35	0.67
18C	2	Maternal	PMD -17	0.67
18C	2	Maternal	PMD 1	1113.58
18C	2	Maternal	GD 29	1216.57
18C	2	Maternal	PND 35	251.84
18C	2	Fetuses	GD 29	1454.19
18C	2	Kits	PND 35	46.69
19A	1	Maternal	PMD -17	0.58
19A	1	Maternal	PMD 1	0.58
19A	1	Maternal	GD 29	0.58
19A	1	Maternal	PND 35	0.58
19A	1	Fetuses	GD 29	0.58
19A	1	Kits	PND 35	0.58
19A	2	Maternal	PMD -17	0.58
19A	2	Maternal	PMD 1	1374.20
19A	2	Maternal	GD 29	949.20
19A	2	Maternal	PND 35	366.78

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
19A	2	Fetuses	GD 29	1209.12
19A	2	Kits	PND 35	46.87
19F	1	Maternal	PMD -17	0.74
19F	1	Maternal	PMD 1	0.74
19F	1	Maternal	GD 29	0.74
19F	1	Maternal	PND 35	0.74
19F	1	Fetuses	GD 29	0.74
19F	1	Kits	PND 35	0.74
19F	2	Maternal	PMD -17	0.74
19F	2	Maternal	PMD 1	1869.33
19F	2	Maternal	GD 29	1066.27
19F	2	Maternal	PND 35	374.67
19F	2	Fetuses	GD 29	1389.55
19F	2	Kits	PND 35	44.55
22F	1	Maternal	PMD -17	3.67
22F	1	Maternal	PMD 1	3.38
22F	1	Maternal	GD 29	3.28
22F	1	Maternal	PND 35	3.28
22F	1	Fetuses	GD 29	3.28
22F	1	Kits	PND 35	3.28
22F	2	Maternal	PMD -17	3.28
22F	2	Maternal	PMD 1	3490.44
22F	2	Maternal	GD 29	1777.80
22F	2	Maternal	PND 35	452.78
22F	2	Fetuses	GD 29	2466.33
22F	2	Kits	PND 35	86.80
23F	1	Maternal	PMD -17	0.60
23F	1	Maternal	PMD 1	0.60
23F	1	Maternal	GD 29	0.60
23F	1	Maternal	PND 35	0.60
23F	1	Fetuses	GD 29	0.60
23F	1	Kits	PND 35	0.60
23F	2	Maternal	PMD -17	0.60
23F	2	Maternal	PMD 1	259.49
23F	2	Maternal	GD 29	980.26
23F	2	Maternal	PND 35	154.79
23F	2	Fetuses	GD 29	1046.00
23F	2	Kits	PND 35	35.16
33F	1	Maternal	PMD -17	3.40
33F	1	Maternal	PMD 1	3.65
33F	1	Maternal	GD 29	3.89
33F	1	Maternal	PND 35	3.59
33F	1	Fetuses	GD 29	4.05
33F	1	Kits	PND 35	3.28

<i>Serotype</i>	<i>Group</i>	<i>Animal</i>	<i>Study Day</i>	<i>GMT (U/mL)</i>
33F	2	Maternal	PMD -17	3.34
33F	2	Maternal	PMD 1	426.34
33F	2	Maternal	GD 29	271.06
33F	2	Maternal	PND 35	40.94
33F	2	Fetuses	GD 29	325.90
33F	2	Kits	PND 35	10.57

Table 35: Serology – results presented as geometric mean titers (GMT) in U/mL; PMD = prematuring day; GD = gestation day; PND = postnatal day

Serologic analysis of serum titers demonstrated a robust immunogenic response to all 20 pneumococcal serotypes in the vaccinated does in this study by the time of conception. There was no evidence of detectable antibodies in any control does at any point in the study nor in any animal pretest. There was demonstrable evidence of passive antibody transfer to the F1 generation in both subsets, though there was significant attenuation by the end of the end of the study in those kits from the vaccinated littering does.

Assessment: There was no treatment-related mortality in does, fetuses or kits observed in this study, nor was there any evidence of treatment-related clinical signs on the does which would imply a risk of maternal toxicity that may have affected the F1 generation. For the two does who were prematurely terminated, there was no clear relation to treatment. One was likely related to complications with parturition while the other appears to be related to housing trauma. Overall, 20vPnC was well tolerated by the study does and there was no treatment-related effect on body weight change, food consumption, female fertility, maternal performance, gestational length, parturition and lactation. There were also no treatment-related effects observed in the does during postmortem necropsy examinations.

Additionally, administration of does with 20vPnC did not result in any clear effect on the F1 generation from either subset. There were no clear treatment-related malformations, variations, or effects on number of corpora lutea, implantations, implantation loss, resorptions, litter size, litter weight or sex distribution. Similarly, there was no effect on the littering subset as the kits from vaccinated does showed no difference compared to those from control does in regard to litter size, viability, growth and developmental milestones. The two variations observed in higher frequency in fetuses from vaccinated does, absent lung lobes and unossified forepaw middle phalanges, should be considered incidental even though they were slightly above the historical reference range because of the lack of a correlating observation on kit development and postmortem necropsy examinations.

Immunology testing confirmed administration of the vaccine to does as all of those given 20vPnC demonstrated a sufficient immune response to all 20 serotypes while the none of the control does demonstrated detectable antibodies to any of the serotypes. Additionally, passive transfer of antibodies was demonstrated in both the fetuses of the caesarean subset and kits from the littering subset of does which received 20vPnC.

4.2.3.6 LOCAL TOLERANCE

SINGLE DOSE SUBCUTANEOUS LOCAL TOLERANCE STUDY OF PF-06482077 IN FEMALE
(b) (4) RABBITS

Study number: 19GR379

Performing laboratory: Pfizer (b) (4)

Study initiation date: January 27th, 2020Final Report date: May 22nd, 2020

Test article batch/lot: CL3156

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

Breeder/supplier: (b) (4)

Number of animals per group and sex: 6 female rabbits in a single group

Age: 7 months

Body weight range: 3.5 – 3.6 kg

Means of administration: *Subcutaneous* injection

Site of administration: Left and right craniodorsal thoracic regions

Volume of injection: 0.5 mL (x2)

Frequency of administration and study duration: A single administration followed by either a 4- or 15-day observation period

Dose: 46.2 µg (4.4 µg/mL per serotype with the exception of 6B at 8.8 µg/mL)

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use syringes (one syringe per dose). Stability studies were performed by Pfizer (b) (4) on the same lot of vaccine as used in this study.

Report status: Final

Experimental design: Animals were acclimated for a minimum of 14 days and all assigned to a single group. These rabbits, all female, received a single injection of 20vPnC on their left side and a single injection of saline control on their right side on study day 1. Half of the study animals were humanely euthanized on study day 4 while the other half humanely euthanized on study day 15.

The following parameters were evaluated:

<i>Parameters</i>	<i>Frequency of Testing</i>
Cageside observation ⁶	Twice daily and 4 hours post-dosing
Detailed clinical observations ⁷	SD 1 (pre-dose), 4, 8 and 15
Body weight	SD 1 (pre-dose), 4, 8 and 15
Food consumption	Daily
Body temperature	Not collected
Ophthalmologic exam	Not performed
Clinical chemistry	Not collected
Hematology	Not collected
Coagulation	Not collected

⁶ Cageside observations include mortality, morbidity, general health and signs of toxicity.

⁷ Clinical observations details not provided.

<i>Parameters</i>	<i>Frequency of Testing</i>
Immunological response	Not collected
Evaluation of site of inoculation (dermal Draize scoring method)	Pre-dose, 4- and 24-hours post-dose
Necropsy	SD 4 and 15
Organ weights	Not performed
Tissues for histopathology	SD 4 and 15

Table 36: Experimental design – SD = study day

Postmortem procedures: Animals were humanely euthanized at scheduled terminations via intravenous barbiturate administration followed by exsanguination. All carcasses underwent a necropsy examination to include the external surface and both thoracic and abdominal cavities as well as their contents. The following tissues were collected and processed for slide preparation and histologic examination: left and right injection site axillary lymph nodes (draining the injection sites), macroscopic necropsy findings, and both left and right injection sites.

RESULTS

Morbidity and mortality: There were no unscheduled deaths in this study. All animals survived to their scheduled terminations.

Clinical signs: One animal had a transient injection site mass associated with 20vPnC administration. This mass resolved by termination on study day 15. The only other observation was bruising at injection sites that occurred more frequently at control sites which indicates it was related to the injection procedure.

Body weight: All of the animals in this study had comparable body weight changes during the antemortem phase of the study.

Food consumption: The amount of food consumed post-administration was comparable to the amount consumed pre-test in all of the study animals.

Local toxicity: Dermal Draize scoring of the injection sites found no abnormalities at the saline injection sites while all 6 animals had grade 1 edema and 2 animals had grade 1 erythema at the vaccinated sites. This resolved after 24 hours.

Necropsy observations: For the saline treated side, a single animal was found to have an abnormal color to the injection site while another was found to have an abnormal color to the injection site draining lymph node. For the vaccinated side, a single animal was found to have abnormal color to the injection site which correlated to microscopic hemorrhage.

Microscopic observations: There were no test article-related findings observed at either study day 4 or 15 terminations among the limited selection of tissues collected for histology in this study. Minimal mixed cell inflammation and hemorrhage were observed subcutaneously at injection sites, but this was considered related to the injection procedure as it was observed on both sides of the animals.

Assessment: There was no treatment-related mortality or any effects on body weight, food consumption or post-mortem findings. Overall, the vaccine was well tolerated under the conditions of this study and the only treatment-related finding when administered subcutaneously was minimal, transient injection site erythema and edema and a single incidence of a transient mass.

This study provided some insight into potential differences in safety risk in case 20vPnC is unintentionally administered SC rather than IM. This study demonstrates a comparable amount of risk of local reactogenicity when administered SC, but it should be noted how this study was limited in scope in regard to endpoints selected and study design. This route of administration was not tested on both sexes, and there was not a dedicated group which received only saline. Additionally, there was no assessment of clinical pathology or body temperature as well. Despite that, this study provided additional insight into the overall safety and risk assessment of 20vPnC.

4.2.3.7 OTHER TOXICITY STUDIES

59-DAY INTRAMUSCULAR STUDY OF PF-06482077 IN RABBITS

Study number: 13GR165

Performing laboratory: Pfizer (b) (4)

Study initiation date: May 29th, 2013

Final Report date: March 4th, 2014

Test article batch/lot:

- *PF-06482077* (20v): test article lot PROT-16293, expiration date April 9th, 2014
- *0.9% Sodium Chloride injection*: control article lot (b) (4), expiration date April 1st, 2014

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

Breeder/supplier: (b) (4)

Number of animals per group and sex: 36

Age: Males 8-9 months, females 7-8 months

Body weight range: Males 3.0 – 4.0 kg, females 2.9 – 4.7 kg

Means of administration: Intramuscular injection via needle and syringe

Site of administration: Left and right quadriceps muscles

Volume of injection: 1.0 mL (0.5 mL x2)

Frequency of administration and study duration: A total of 5 biweekly administrations and a total study duration of 59 days

Dose: 4.4 µg/serotype except for 6B which was dosed at 8.8 µg

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor on the same batches of vaccine and adjuvant control as used in this study.

Report status: Final

Experimental design: Animals were acclimated for a minimum of 32 days, randomized and assigned to one of two groups according to table 20 below. Administrations of test and

control articles were administered on study days 1, 15, 29, 43 and 57. Animals were humanely euthanized and necropsied two days after the final injection, on study day 59.

<i>Group</i>	<i>Treatment</i>	<i>Dose (μg)*</i>	<i>Dose Volume**</i>	<i>Treatment Phase Animals (No.)</i>	<i>Recovery Phase Animals (No.)</i>
1	Saline	0	1.0	5	5
2	PF-06842077	4.4 (8.8)	1.0	5	5

Table 37: Group assignments – *dose presented for all serotypes except for 6B which is presented in parentheses

Methods for blood collection: Unfasted blood was drawn from an ear vein with saphenous veins used as an alternative

Randomization procedure: Yes, computer-assisted based on body weight

Statistical analysis plan: Yes, SAS programs, one-way ANOVA and two-sided t-test

The following parameters were evaluated:

<i>Parameter</i>	<i>Frequency of Testing</i>
Clinical signs	Once daily pre-test and on days of scheduled terminations; twice daily on non-dosing days; pre-dose, approximately 4-hours post the last animal dosed and at the end of the work day.
Sham injection site evaluation*	Animals were removed from their cages and handled for sham injection site evaluations prior to dosing on Days 1, 15, 29, 43, and 57, and at approximately 4- and 24-hours after dosing. However, since this was a sham procedure, no observations were made.
Body weight	Animals were weighed three times during the pretreatment phase (prior to Day 1). During the dosing phase, animals were weighed at approximately the same time of day on Days 1, 4, 8, 15, 18, 22, 29, 32, 36, 43, 46, 50, and 57. A fasted weight was obtained just prior to scheduled necropsy.
Sham body temperature evaluation*	Animals were removed from their cages and handled for sham body temperature evaluations prior to dosing and at approximately 4- and 24-hours post-dose on each day of dosing. However, since this was a sham procedure, body temperature was not measured.
Food consumption	Qualitative food consumption was assessed daily beginning approximately 2 weeks pretreatment prior to Day 1.
Sham ophthalmology*	Animals were removed from their cages and handled for sham ophthalmologic examinations pretreatment and Week 8 (Day 52). However, since this was a sham procedure, no ophthalmology observations were made.

Table 38: Experimental design – adapted from a figure provided by the sponsor; *conducted as sham procedures to mimic procedures used in study 12GR385

Blood for clinical pathology parameters was drawn pretest and on study days -4, 3, 15, 29, 57 and 59. Blood for serologic response was drawn pretest and on study days 15, 29, 57 and 59.

Postmortem procedures: Animals were humanely euthanized via intravenous barbiturate administration on study day 59. Only the hearts of animals and any macroscopic gross lesions were examined microscopically. These were examined in all animals of both sexes in both groups. Brains, hearts, kidneys and livers were weighed.

Representative samples of collected organs were fixed in 10% neutral buffered formalin. After standard histology sections were obtained, sections were frozen in Optimal Cutting Temperature medium for potential analysis. For the heart, a section of the left ventricle was obtained. Hearts and gross lesions were sectioned and stained with hematoxylin and eosin. Formalin-fixed paraffin-embedded blocked heart, liver and kidney tissue from animals 73 and 116 were shipped to (b) (4) for immunohistochemistry analysis.

Microscopic lesions were graded on a scale of 1 to 5 as minimal, mild, moderate, marked or severe with lesions not graded being listed as present. Masson's trichrome stain for collagen was performed on animals 73 and 116.

RESULTS

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

Clinical observations: Similar to what was observed in study 12GR385, small, firm masses were observed in approximately 7 of 72 treated animals with none occurring in control animals. These were transient in nature with only 2 still present at necropsy. Aside from these masses, there were no treatment-related clinical observations through the course of the study. Those observed were considered incidental and commonly observed in laboratory rabbits.

Ophthalmologic observations: not conducted as part of the study design

Body temperature: not conducted as part of the study design

Body weight:

<i>Study Day</i>	<i>Group 1M</i>	<i>Group 2M</i>	<i>Group 1F</i>	<i>Group 2F</i>
1	3522.91	3517.69	3790.31	3790.76
4	3509.86	3520.60	3770.80	3780.37
8	3548.54	3542.76	3842.90	3839.39
15	3485.39	3477.65	3798.78	3790.30
18	3573.92	3570.55	3889.79	3903.90
22	3604.69	3599.22	3924.55	3937.84
29	3533.17	3536.90	3916.06	3913.17
32	3646.70	3652.92	3977.80	3993.46
36	3674.63	3672.36	4021.43	4027.88
43	3739.73	3746.89	4053.10	4089.16
46	3739.45	3735.73	4073.36	4090.78
50	3763.92	3768.76	4113.62	4139.80
57	3720.25	3742.26	4056.68	4081.40

Table 39: Body weight – data presented in mean grams; data was statistically analyzed but no statistically-significant differences observed

Food consumption: There were no statistically-significant or toxicologically-relevant differences in food consumption between treated and control animals of either sex.

Clinical chemistry:

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5, i.e. \uparrow 1.6 or \downarrow 1.6)</i>	<i>NOT OF NOTE</i>
ELECTROLYTE BALANCE		Calcium Phosphorus Sodium Potassium Chloride
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Glutamate dehydrogenase (ND) Sorbitol dehydrogenase (ND) Total bile acids (ND)
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids (ND) Total bilirubin
ACUTE PHASE REACTANTS	C-reactive protein (see table 42 below) Fibrinogen (see table 43)	
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Total protein Albumin (A) Globulin (G) A/G Ratio Total Cholesterol Cholinesterase (ND) Fasting Triglycerides Creatine kinase (CK) Lactate dehydrogenase (LDH) Amylase/lipase T4

Table 40: Clinical Chemistry Results – ND = not determined

C-reactive protein:

<i>Study Day</i>	<i>Group 1M</i>	<i>Group 2M</i>	<i>Group 1F</i>	<i>Group 2F</i>
-4	1.48	1.64	10.88	9.75
3	0.70	22.27**	6.63	58.89**
15	1.08	1.08	2.64	3.89
29	3.17	2.75	4.80	3.71
57	4.19	5.361	5.24	5.32
59	6.55	63.31**	5.04	17.72**

Table 41: C-reactive protein – results presented in mean µg/mL; **p≤0.01

Hematology and coagulation:

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5⁸, i.e. ↑ 1.6 or ↓ 1.6)</i>	<i>NOT OF NOTE</i>
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) Red Cell Distribution Width (RDW) Reticulocytes
WHITE BLOOD CELLS	Monocyte count SD29 M G2 ↑ 1.7* SD59 M G2 ↑ 1.7*	Total leukocytes (WBC) Neutrophil count Lymphocyte count Eosinophil count Basophil count Large unstained cells (LUC)
CLOTTING POTENTIAL	Fibrinogen SD3 M G2 ↑ 1.6**	Activated partial-thromboplastin clotting time (APTT) Prothrombin time (PT) Platelet count Mean platelet volume (ND)
OTHER		Bone marrow cytology (ND)

Table 42: Hematology and coagulation results – ND = not determined; * p≤0.05; ** p≤0.01

Systemic toxicity: No treatment-related mortality or any toxicologically relevant changes in clinical signs, body weight change, relative food consumption and hematology and coagulation parameters were found. Similar to study 12GR385, transient lumps were observed at injection sites and most resolved by the time of necropsy.

Among the hematology parameters, the only toxicologically relevant changes observed were statistically-significant increases in fibrinogen (37-55%) and CRP (4- to 32-fold) in treated animals of both sexes on study days 3 and 59, the only time-points where blood was drawn within 48 hours after an administration. The increases in CRP correlated with the severity of chronic-active inflammation noted on microscopic examination of injection sites. The changes were considered reversible based on the return to baseline after the changes on study day 3 (i.e. by study day 15). However, due to the lack of a recovery period incorporated into the study design, the increases observed on study day 59 could not be assessed for reversibility.

⁸ 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.

Organ weight:

<i>Parameter</i>	<i>Group 1M</i>	<i>Group 2M</i>	<i>Group 1F</i>	<i>Group 2F</i>
Number of animals (N)	36	36*	36	36
Body weight (mean g)	3740.106	3753.142	4081.817	4105.531
Brain, absolute weight (mean g)	9.27	9.27	9.33	9.25
Brain to body weight ratio (%)	0.002	0.002	0.002	0.002
Heart, absolute weight (mean g)	8.48	7.97/7.75*	7.50	7.33
Heart to body weight ratio (%)	0.002	0.002	0.002	0.002
Heart to brain weight ratio (%)	0.918	0.865	0.809	0.794
Kidneys, absolute weight (mean g)	17.95	17.95	16.32	16.44
Kidneys to body weight ratio (%)	0.005	0.005	0.004	0.004
Kidneys to brain weight ratio (%)	1.948	1.940	1.761	7.783
Liver, absolute weight (mean g)	90.04	91.06	76.62	80.99
Liver to body weight ratio (%)	0.0241	0.0241	0.0187	0.0197
Liver to brain weight ratio (%)	9.770	9.8127	8.2631	8.7742

Table 43: Organ weights – data statistically analyzed, but no statistically-significant differences observed; *see discussion below regarding data discrepancy

There were no treatment-related effects on brain, heart, kidney or liver weights or weight ratios receiving 20vPnC. In the summary tables for male organ weights on page 230 of the study report, the N for group 2 heart weights was listed as 35 instead of 36 with a mean reported as 7.97 grams. When looking at the individual data table on pages 582-583 of the study report, all 36 animals have heart weight data and the mean is listed as 7.75 grams. It is not clear why there is this data discrepancy occurred nor is there any explanation. It is also not apparent which animal was omitted for the summary table. The difference is minimal and should not affect the overall risk assessment of 20vPnC.

Gross Pathology:

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>1F</i>	<i>2F</i>
Injection site, abnormal color	8	16	9	18
Injection site, abnormal surface	0	2	0	0
Skin and adnexa, abnormal surface	0	0	1	0
Skin and adnexa, wound/scar/crust	0	1	0	0

Table 44: Macroscopic pathology findings

Histopathology:

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>1F</i>	<i>2F</i>
Heart, inflammation with degeneration/necrosis (grade 1)	0	0	0	2
Heart, inflammation with degeneration/necrosis (grade 2)	0	0	0	1
Heart, deposition of mineral: tunica media aorta (grade 2)	1	0	0	1
Heart, mixed cell infiltration (grade 1)	3	0	1	1
Heart, epicardial mesothelium inflammation/hypertrophy (grade 1)	3	4	0	2
Heart, mononuclear cell infiltration (grade 1)	6	6	6	7
Injection site, hemorrhage (grade 1)	2	6	4	12
Injection site, hemorrhage (grade 2)	4	9	2	5
Injection site, hemorrhage (grade 3)	0	1	0	0
Injection site, chronic active inflammation (grade 1)	4	0	2	0
Injection site, chronic active inflammation (grade 2)	4	5	1	11
Injection site, chronic active inflammation (grade 3)	0	13	0	7

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>1F</i>	<i>2F</i>
Injection site, chronic inflammation (grade 1)	0	0	5	0
Injection site, myofiber degeneration/necrosis (grade 1)	4	1	4	9
Injection site, myofiber degeneration/necrosis (grade 2)	2	17	0	8

Table 45: Microscopic pathology findings

A limited number of tissues were examined for histology because the purpose of this study was to investigate reproducibility of cardiac myocyte pathology observed in study 12GR385. Adverse test article-related cardiac myocyte inflammation with degeneration/necrosis was observed in female animals 116 and 123. For animal 116, the lesion was mild, multi-focal and present mostly in the interventricular septum, though also in the papillary muscle of the left ventricle. For animal 123, the lesion was minimal, focal and present in the papillary muscle of the left ventricle.

These were characterized by accumulations of mononucleated cells (macrophages), heterophils, hypereosinophilic homogenous or fragmented cardiac myocytes and increased extracellular basophilic matrix. There were also observable Anichov (caterpillar) cells which have been observed in myocarditis and in both naturally occurring and experimentally induced myocardial necrosis⁹. The study pathologist considered these lesions adverse, test article-related and morphologically comparable to those observed in study 12GR385.

Local toxicity: Draize scoring of the injection sites was not conducted as part of the study design.

Histologically, the injection site in treated animals at the end of the in-life portion or terminal kill showed an increased incidence of minimal to moderate hemorrhage and chronic-active inflammation as well as minimal to mild myofiber degeneration/necrosis. The chronic-active inflammation was characterized by accumulations of large macrophages containing basophilic cytoplasmic material and variable numbers of heterophils and lymphocytes. Per the study pathologist, the increases in CRP and fibrinogen correlated with the chronic-active inflammation at injection sites.

For those saline control animals which demonstrated chronic-active inflammation at the injection sites, it was of lower severity than those animals receiving 20vPnC and there were no macrophages containing basophilic cytoplasmic material.

Serology: A serology study was conducted using (b) (4) assays (b) (4) to not only measure anti-20vPnC IgG, but also to determine whether these antibodies had the capacity to bind non-dosed rabbit heart antigen. Data was measured in (b) (4). See figure 1 below which details which animals were tested for serology:

⁹ Maxie and Robinson, Cardiovascular system. Pathology of Domestic Animals. 2007. 35.

<i>Animal ID</i>	<i>Dose</i>	<i>Heart lesion</i>
73	saline	NO
74	saline	NO
91	saline	NO
92	saline	NO
115	PF-06482077 (20v)	NO
117	PF-06482077 (20v)	NO
116	PF-06482077 (20v)	YES
123	PF-06482077 (20v)	YES

Figure 1: Animal selection for serologic testing – figure provided by the sponsor

All four of the group 2 animals seroconverted to 20vPnC whereas none of the group 1 control animals seroconverted. No antibodies against non-dosed rabbit heart homogenate antigens were detected in the serum samples of any animal in this serology testing.

This serology report does demonstrate that 4 animals did seroconvert (including the two with heart lesions in question), but unlike the serology testing in study 12GR385, the report does not demonstrate that every group 2 animal received the active 20vPnC vaccine. It should also be noted that the anti-20vPnC individual data was not provided, only the (b) (4) ratios discussed above.

Assessment: There was no treatment-related mortality or any clear treatment-related effects on general clinical presentation, food consumption, body weight and most clinical pathology parameters. The exploratory nature of this study reduced the number of endpoints assessed, though the animals were handled equally between 12GR385 and this study. This was the purpose for all of the “sham” treatments.

Treatment-related changes included transient lumps at the injection sites of approximately 10% of treated animals, elevated CRP and fibrinogen, injection site chronic-active inflammation and degeneration/necrosis and two additional instances of cardiac inflammation with degeneration necrosis.


The injection site lumps, elevations in acute-phase reactants and injection site pathology are comparable to those observed in study 12GR385 in both incidence and severity. Unlike study 12GR385 where all of the injection site lumps resolved prior to necropsy, two of the lumps were still present at port-mortem examinations and were characterized by chronic-active inflammation. The acute-phase reactant elevations observed on study day 3 recovered by study day 15, but due to the lack of a recovery period in this study, the elevations observed on study day 59 could not be assessed for recoverability.

Similar to study 12GR385, two animals receiving 20vPnC were found to have minimal to mild inflammation of cardiac myocytes with degeneration/necrosis characterized by large macrophages and hypereosinophilic fragmentation of said myocytes. This was also found in the same location (interventricular septum and left papillary muscle) as in study 12GR385. The study pathologist again believes that the finding is not background in nature and should be considered both test article-related and adverse.

Immunology performed in this study verified that an active dose was administered to a select few animals, including the two animals with cardiac pathology, and that no cross-reactivity with rabbit heart homogenate antigen occurred with 20vPnC antibodies. However, the study only assessed serology in 4 out of 72 animals treated with 20vPnC and individual IgG data was not provided.

GLP study deviations or amendments: This study report states the following: “Minor deviations from the protocol and/or current standard operating procedures occurred and did not affect the quality, integrity or interpretations of the data or the conclusions of the study. The deviations are documented in the study file.”

(b) (4)



59-DAY INTRAMUSCULAR INJECTION TOXICITY STUDY WITH PF-06482077, (b) (4)
IN RABBITS

Study number: 13GR370

Performing laboratory: (b) (4)

Study initiation date: January 17th, 2014

Final Report date: January 23rd, 2015

Test article batch/lot:

- (b) (4) test article lot no. (b) (4), expiration date December 2nd, 2014
- (b) (4) test article lot no. (b) (4), expiration date December 2nd, 2014
- (b) (4) test article lot no. (b) (4), expiration date December 4th, 2014
- (b) (4) test article lot no. (b) (4), expiration date December 4th, 2014
- PF-06482077 (20vPnC): positive-control lot no. PROT-16293, expiration date October 9th, 2014 (Note this is not the same lot as was used in study 12GR385)
- 0.9% Sodium Chloride: saline control lot no. (b) (4), expiration date October 1st, 2015 and lot no. (b) (4), expiration date November 1st, 2015

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

Breeder/supplier: (b) (4)

Number of animals per group and sex: Group 1 – 10; Group 2 – 40

Age: 5 months

Body weight range: Males 2.5-3.5 kg, females 2.5-3.6 kg

Means of administration: Intramuscular injection, needle and syringe

Site of administration: Left and right quadriceps at each administration

Volume of injection: 1.0 mL (0.5 mL x2)

Frequency of administration and study duration: 5 biweekly administrations with a study duration of 59 days

Dose: 4.4 µg/serotype except for 6B which was dosed at 8.8 µg

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study.

Report status: Final

Experimental design: Animals were acclimated, randomized and assigned to one of six groups according to table 34 below. Administrations of test article and control articles on study days 1, 15, 29, 43 and 57. All animals were humanely euthanized on study day 59 without a recovery period incorporated into the study design.

<i>Group</i>	<i>Treatment</i>	<i>Treatment Phase Animals (No.)</i>	<i>Recovery Phase Animals (No.)</i>
1	Saline Control	10	0
2	20vPnC (positive control)	40	0
3	(b) (4)	40	0
4	(b) (4)	40	0
5	(b) (4)	40	0
6	(b) (4)	40	0

Table 54: Group assignments

Methods for blood collection: Fasted blood samples were collected via a jugular vein or medial auricular ear artery. Details on analyzers not provided.

Randomization procedure: Yes, computer-assisted based on body weight

Statistical analysis plan: Yes, ANOVA, Levene's test

The following parameters were evaluated:

<i>Parameters</i>	<i>Frequency of Testing</i>
Cageside observation ¹¹	Twice daily
Clinical observations ¹²	Weekly
Body weight	SD
Qualitative food consumption	1, 4, 8, 15, 22, 29, 32, 36, 43, 46, 50 and 57
Body temperature (SHAM)	Pre-dose, 4- and 24-hours post-dose
Ophthalmologic exam (SHAM)	Pretest, week 8
Clinical chemistry*	Pretest, SD 59
Hematology*	Pretest, SD 59
Coagulation*	Pretest, SD 59
Immunological response	Pretest, SD 59
Evaluation of site of inoculation (SHAM)	Pre-dose, 4- and 24-hours post-dose
Necropsy	SD 59
Organ weights	SD 59
Tissues for histopathology	SD 59

Table 55: Experimental design – *blood collected from a jugular vein or medial auricular artery; SD = study day; SHAM = animals were handled in same manner as if actual procedure was performed but no data were collected for these procedures

Postmortem procedures: On study day 59, fasted animals were humanely euthanized with sodium pentobarbital followed by exsanguination. Terminal body weights were recorded then full macroscopic post-mortem examinations were conducted.

Similar to the other exploratory studies following 12GR385, only the hearts of animals and any gross lesions elsewhere were examined microscopically. These tissues were embedded in paraffin, sectioned and stained hematoxylin and eosin. For selected animals, additional slides of heart tissue were prepared and stained with Masson Trichrome stain in order to highlight

¹¹ Cageside observations include mortality, morbidity and signs of pain or distress.

¹² Details regarding clinical examinations not provided.

fibrous connective tissue. No further procedural details on the evaluation of heart tissues were provided.

RESULTS

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

Clinical observations: Similar to the other studies in this submission, the only test-article related observation was transient swelling at injection sites. This was observed in animals receiving 20vPNC and all four groups receiving (b) (4), with none being observed in those animals receiving saline. These swellings all resolved within 2 days. The rest of the observations during the study were considered incidental because of sporadic frequency, transiency, or were observed at comparable incidences between treated and control animals.

Ophthalmic observations: Not included as part of the study design

Body temperature: Not included as part of the study design.

<i>Study Day</i>	<i>Group 1M</i>	<i>Group 2M</i>	<i>Group 3M</i>	<i>Group 4M</i>	<i>Group 5M</i>	<i>Group 6M</i>
1	3038	3040	3042	3055	3080	3073
4	3105	3103	3094	3140	3127	3132
8	3174	3173	3159	3158	3210	3169
15	3134	3144	3148	3143	3176	3190
22	3297	3308	3352	3321	3340	3317
29	3302	3284	3304	3291	3324	3282
32	3403	3405	3373	3417	3427	3426
36	3445	3430	3440	3433	3471	3413
43	3473	3475	3481	3486	3525	3459
46	3525	3511	3501	3515	3548	3531
50	3560	3533	3551	3556	3595	3547
57	3468	3463	3491	3477	3514	3476

Table 56: Male body weight – data presented as mean weights in grams; data was statistically analyzed but none of the results were deemed statistically significant

<i>Study Day</i>	<i>Group 1F</i>	<i>Group 2F</i>	<i>Group 3F</i>	<i>Group 4F</i>	<i>Group 5F</i>	<i>Group 6F</i>
1	3063	3083	3079	3076	3119	3119
4	3125	3133	3165	3139	3207	3180
8	3221	3241	3203	3237	3248	3238
15	3224	3221	3227	3217	3266	3264
22	3453	3470	3422	3442	3444	3414
29	3385	3390	3412	3392	3438	3388
32	3513	3533	3554	3522	3549	3507
36	3554	3583	3560	3580	3587	3536
43	3566	3602	3621	3634	3665	3578
46	3616	3599	3667	3649	3668	3636
50	3668	3669	3651	3670	3670	3635
57	3614	3600	3637	3611	3640	3621

Table 57: Female body weight – data presented as mean weights in grams; data was statistically analyzed but none of the results were deemed statistically significant

Food consumption: Only qualitative measurement of food consumption was recorded with the following results below per the clinical observations data from the study:

<i>Sex</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>	<i>Group 6</i>
Male	3	24	14	14	13	14
Female	4	13	7	13	13	20

Table 58: Food consumption – data represents number of animals within a group which demonstrated qualitatively low food consumption at some point during the study

Quantitative food consumption was not measured in this study, nor were there any summary tables showing on which study days the above qualitative decreases occurred. The numbers above demonstrate how many animals had decreased food consumption for at least one day, though the large majority of animals exhibited this on more than one study day. However, a review of the individual data showed that a large majority of the incidents of decreased food consumption did not occur within 3 days after vaccine administrations. They were sporadic in nature and did not appear to demonstrate any particular pattern.

Clinical chemistry:

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5, i.e. ↑ 1.6 or ↓ 1.6)</i>	<i>NOT OF NOTE</i>
ELECTROLYTE BALANCE		Calcium Phosphorus Sodium Potassium Chloride
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Glutamate dehydrogenase (ND) Sorbitol dehydrogenase (ND) Total bile acids (ND)
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids (ND) Total bilirubin
ACUTE PHASE REACTANTS	C-reactive protein (see table 61 below)	Fibrinogen (ND)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Total protein Albumin (A) Globulin (G) A/G Ratio Total Cholesterol Cholinesterase (ND) Creatine kinase (CK) Fasting Triglycerides

Table 59: Clinical chemistry results – ND = not determined

C-reactive protein:

<i>Sex</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>	<i>Group 6</i>
Male	5.0	8.0	8.0	8.0	8.0	9.0
Female	3.0	6.0	7.0	6.0	6.0	5.0

Table 60: C-reactive protein results – data presented in mean µg/mL on study day 59**Hematology and coagulation:**

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5¹³, i.e. ↑ 1.6 or ↓ 1.6)</i>	<i>NOT OF NOTE</i>
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) Red cell distribution width (RDW) Reticulocytes
WHITE BLOOD CELLS		Total leukocytes (WBC) Neutrophil count Monocyte count Lymphocyte count Eosinophil count Basophil count Large unstained cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin clotting time (APTT) Prothrombin time (PT) Platelet count Mean platelet volume (MPV) Fibrinogen (ND)
OTHER		Bone marrow cytology (ND)

Table 61: Hematology and coagulation results – ND = not determined

Systemic toxicity: No treatment-related mortality or any clinically relevant changes in clinical signs, body weight gain, relative food consumption or clinical pathology parameters were found. Similar to the other toxicology studies in this submission, small transient swellings were observed at the injection sites which did not cause the animals to demonstrate any outward sign of pain.

Unlike the other studies in this submission, food consumption was not measured quantitatively. Though there was an apparent treatment-related correlation to overall number of animals experiencing decreased qualitative food consumption, there did not appear to be any correlation to treatment dates or any consistent trends on when the decreases occurred. Additionally, there was not an apparent increase in C-reactive protein as was observed in the

¹³ 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.

other studies in this submission. Considering this lack of consistency plus the difference in reporting (i.e. in mg/dL with low precision) and the different laboratory used for this study (b) (4), there were likely differences in the assay used to measure CRP in this study compared the other studies. It should also be pointed out how the other commonly assessed acute phase reactant fibrinogen was not measured in this study.

Organ Weight: Not included as part of the study design.

Gross Pathology:

<i>Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>6M</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5M</i>	<i>6M</i>
Heart, large	0	0	0	0	0	0	0	1	0	0	0	0
Liver, rough surface	0	0	0	0	0	0	0	1	0	0	0	0
Lung, discolored	0	0	0	1	0	0	0	0	0	0	0	0
Nicitating membrane, discolored	0	0	0	1	0	0	0	0	0	0	0	0
Skin/subcutis, scab	0	0	0	1	0	0	0	0	0	0	0	0
Stomach, discolored	0	0	1	0	0	0	0	0	0	0	0	0
Thymus, discolored	0	0	0	0	0	0	0	1	0	0	0	0

Table 62: Macroscopic pathology results

Histopathology:

<i>Male Treatment Group Finding</i>	<i>1M</i>	<i>2M</i>	<i>3M</i>	<i>4M</i>	<i>5M</i>	<i>6M</i>
Aorta, mononuclear cell infiltrate (grade 1)	0	1	1	0	0	0
Aorta, media mineralization (grade 1)	1	2	2	2	0	1
Aorta, media mineralization (grade 2)	0	1	0	1	0	0
Heart myocardiocyte degeneration (grade 1)	1	4	3	2	0	2
Heart, fibrosis (confirmed by Masson Trichrome; grade 1)	0	1	0	0	0	0
Heart, hemorrhage (grade 1)	1	1	0	0	0	0
Heart, mixed cell infiltrate (grade 1)	3	9	5	6	4	4
Heart, mixed cell infiltrate (grade 2)	0	0	0	1	0	0
Heart, mononuclear cell infiltrate (grade 1)	2	15	24	14	19	17
Heart, mononuclear cell infiltrate (grade 2)	0	0	1	0	0	0
Heart, myocyte inflammation with degeneration/necrosis (grade 1)	0	1	0	0	0	0
Heart, examined with Masson Trichrome	6	1	1	0	0	2
Lung, hemorrhage (grade 3)	0	0	0	1	0	0
Nicitating membrane, mixed cell infiltrate (grade 1)	0	0	0	1	0	0
Skin/subcutis, erosion/ulcer (grade 2)	0	0	0	1	0	0
Skin/subcutis, inflammation (grade 2)	0	0	1	0	0	0

Table 63: Microscopic pathology findings in male animals

<i>Female Treatment Group Finding</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>	<i>6F</i>
Aorta, mixed cell infiltrate (grade 1)	0	0	1	0	0	0
Aorta, media mineralization (grade 1)	0	0	3	2	3	0
Heart myocardiocyte degeneration (grade 1)	0	3	3	5	4	6
Heart, fibrosis (confirmed by Masson Trichrome; grade 1)	0	0	0	1	0	2
Heart, mixed cell infiltrate (grade 1)	4	6	6	4	3	9
Heart, mixed cell infiltrate (grade 2)	0	0	0	1	0	0
Heart, mononuclear cell infiltrate (grade 1)	5	18	26	25	22	21
Heart, mononuclear cell infiltrate (grade 2)	0	0	1	0	1	0
Heart, myocyte inflammation with degeneration/necrosis (grade 1)	0	0	0	0	0	1

<i>Female Treatment Group Finding</i>	<i>1F</i>	<i>2F</i>	<i>3F</i>	<i>4F</i>	<i>5F</i>	<i>6F</i>
Heart, myocyte inflammation with degeneration/necrosis (grade 2)	0	0	0	0	0	1
Heart, examined with Masson Trichrome	5	3	2	1	0	9
Liver, capsule fibrosis (grade 1)	0	1	0	0	0	0

Table 64: Microscopic pathology findings in female animals

Similar to studies 12GR165 and (b) (4), only the hearts and gross lesions were examined histologically due to the exploratory nature of this study. Also similar to the previous toxicology studies in this submission, adverse test article-related microscopic changes were observed in the hearts of three treated animals while none was observed in animals that received saline.

- Animal F32949 (b) (4) focal, minimal inflammation with degeneration/necrosis of the left ventricular free wall
- Animal F32945 (b) (4): multifocal, slight inflammation with degeneration/necrosis of the left ventricular free wall; minimal multifocal fibrosis
- Animal F32577 (20vPnC): multifocal minimal inflammation with degeneration/necrosis of the papillary muscle of the left ventricle; minimal, multifocal fibrosis

The inflammation was characterized by a mixture of mononuclear cells (macrophages and heterophils) with the degeneration/necrosis being characterized by hypereosinophilic and/or fragmented cardiac myocytes with increased pale basophilic or lacy material separating the cells. In addition, the multifocal, minimal fibrosis in the two animals above was collocated with the finding of inflammation with degeneration/necrosis.

It should be noted that the remainder of the microscopic findings were considered incidental to the study pathologist, including the minimal myocardiocyte degeneration and fibrosis that were not associated with inflammation with degeneration/necrosis.

Local toxicity: Assessment of local reactogenicity, either through Draize scoring or histologic examination, was not included as part of the study design.

Serology: Similar to the other studies in this submission, analysis of IgG responses to the Pneumococcal serotypes in this study was analyzed via a (b) (4) assay.

<i>Serotype</i>	<i>Study Day</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>	<i>Group 6</i>
1	Pre-dose	1.49	1.49	1.49	1.51	1.49	1.49
1	57	1.49	141.62	203.96	175.56	193.40	141.78
3	Pre-dose	1.22	1.23	1.26	1.20	1.18	1.29
3	57	1.33	1532.25	1275.76	1044.89	1121.92	1079.81
4	Pre-dose	1.50	1.50	1.50	1.52	1.50	1.50
4	57	1.50	681.16	677.64	575.68	577.03	501.24
5	Pre-dose	0.68	0.68	0.68	0.69	0.68	0.68
5	57	0.68	329.63	423.12	341.97	319.27	277.49
6A	Pre-dose	0.86	0.87	0.86	0.86	0.86	0.86
6A	57	0.86	230.46	277.00	205.37	276.45	203.69

<i>Serotype</i>	<i>Study Day</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>	<i>Group 4</i>	<i>Group 5</i>	<i>Group 6</i>
6B	Pre-dose	0.87	0.90	0.87	0.88	087	0.89
6B	57	0.98	460.78	619.48	505.47	600.26	468.55
7F	Pre-dose	1.37	1.44	1.35	1.48	1.28	1.46
7F	57	1.86	339.88	444.24	376.55	465.49	367.59
8	Pre-dose	29.25	29.85	30.40	30.18	30.81	29.94
8	57	29.25	2247.56	2372.68	2042.90	2386.05	41.85
9V	Pre-dose	5.36	5.09	5.21	5.07	5.68	5.10
9V	57	6.68	341.87	329.98	258.15	261.80	238.71
10A	Pre-dose	68.40	68.40	68.40	68.40	68.40	68.40
10A	57	68.40	1818.66	2459.21	2051.83	69.06	2141.82
11A	Pre-dose	43.87	45.11	43.87	43.87	43.87	43.87
11A	57	43.87	5407.05	5931.71	45.35	6113.73	4728.17
12F	Pre-dose	6.62	6.30	6.30	6.30	6.30	6.30
12F	57	6.30	970.63	6.45	1032.87	1133.16	1119.30
14	Pre-dose	2.40	2.59	2.32	2.30	2.80	1.97
14	57	3.31	774.19	964.94	1018.92	915.78	932.68
15B	Pre-dose	14.39	14.78	14.58	14.39	14.39	14.68
15B	57	14.39	2802.31	2990.36	2876.36	3066.55	2787.04
18C	Pre-dose	1.95	1.89	1.87	1.85	1.85	1.96
18C	57	2.12	1020.98	1103.10	898.40	1052.43	828.72
19A	Pre-dose	0.77	0.77	0.77	0.77	0.78	0.79
19A	57	0.77	1363.18	1246.38	1127.47	1270.80	969.23
19F	Pre-dose	0.61	0.72	0.67	0.64	0.62	0.62
19F	57	0.61	1229.18	1467.64	1183.36	1423.04	1131.37
22F	Pre-dose	57.38	58.12	57.99	58.24	59.08	57.90
22F	57	57.38	2019.55	2388.92	2011.40	2161.10	2038.56
23F	Pre-dose	24.43	24.43	24.43	24.43	24.43	24.43
23F	57	24.43	1096.82	1268.95	1190.64	1533.09	1129.57
33F	Pre-dose	8.34	8.97	8.82	8.50	9.26	9.35
33F	57	8.75	799.57	843.54	689.31	762.45	722.40

Table 65: Serology results – data presented in mean (b) (4) units; data from sexes combined

The results of the (b) (4) assay showed a strong immune response to the various serotypes contained in each formulation on study day 57 with 100% seroconversion across the sexes. No animals were considered seroconverted pretest and none of the group 1 animals seroconverted by study day 57.

Assessment: There was no treatment-related mortality or any clinically relevant effects on any of the in-life endpoints in this study, though they were limited in scope due to the exploratory nature of this study. Similar to the other toxicology studies in this submission, transient swellings were observed in treated animals. Unlike the other studies, there was not as notable an increase in CRP with the most likely reason being the use of a different assay for measuring rabbit CRP. This study differed from the others thus far in this submission in performing laboratory (and pathologists), different formulations (the (b) (4) formulations),

different 20vPnC lot than in study 12GR385, and differing group sizes (N=10 for saline treated animals, N=40 for vaccinated animals).

Histologically, a similar profile of cardiac myocyte changes was observed in this study in regard to nature, location and distribution. Again, a low incidence of minimal to slight inflammation with hypereosinophilic degeneration/necrosis and irreversible fibrosis of cardiac myocytes of the left ventricle and papillary muscle was observed in animals receiving 20vPnC and (b) (4). This change was confirmed as test-article related by (b) (4) pathologists who are different than those involved in the previous studies. Additionally, these changes were not observed in animals receiving saline, though it should be reiterated that the saline group was smaller in number.

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

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

(b) (4)

(b) (4)

**59-DAY INTRAMUSCULAR TOXICITY STUDY OF PROCEDURES IN FEMALE (b) (4)
RABBITS**

Study number: 15GR382

Performing laboratory: Pfizer (b) (4)

Study initiation date: October 8th, 2015

Final Report date: June 14th, 2016

Test article batch/lot: (b) (4) – 0.9% sterile saline for injection, manufactured by (b) (4)
(b) (4), expiration date January 31st, 2017

Animal species and strain: (b) (4) rabbit (outbred)

Breeder/supplier: (b) (4)

Number of animals per group and sex: 70, *female animals only*

Age: 6-8 months

Body weight range: 2770.4 – 3998.3 g

Means of administration: Intramuscular injection

Site of administration: Left and right quadriceps at each dosing

Volume of injection: 1.0 mL (0.5 mL x 2)

Frequency of administration and study duration: Biweekly administrations, study duration of 59 days

Dose: Not applicable

Stability: Analysis of stability, homogeneity and concentration of the saline under test conditions was not performed as part of the study.

Report status: Final

Experimental design: Animals were acclimated for a minimum of 26 days, randomized and allocated to one of two groups according to table 20 below. Administrations of saline were administered on study days 1, 15, 29, 43 and 57. One half of the animals had an increased number of procedures compared to the other half in order to simulate increased cause of stress. See the experimental design tables below for details. Animals were humanely euthanized on study day 59. There was no recovery phase in this study.

<i>Group</i>	<i>Treatment</i>	<i>Treatment Phase Animals (No.)</i>	<i>Recovery Phase Animals (No.)</i>
1	Increased procedures	70	0
2	Minimal procedures	70	0

Table 83: Group assignments – all animals received two injections of 0.5 mL sterile saline at each administration point, one in each quadriceps group

Methods for blood collection: Fasted blood samples were collected from the medial ear artery with a saphenous vein used as an alternate site in group 1 animals when needed for humane reasons. No sedation or anesthesia was used.

Randomization procedure: Yes, stratified randomization based on body weight

Statistical analysis plan: Yes, hematology, coagulation and hematology only

<i>Parameter</i>	<i>Frequency of Testing</i>
Cageside observations	Group 1: Pre-dose, twice post-dose Group 2: Pre-dose, once post-dose
Clinical observations	Group 1: Pre-test, SD 1, 8, 15, 22, 29, 36, 43, 50 and 57 Group 2: Pre-test
Body weight	Group 1: Pre-test, SD 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, 50, 53, 57 and 59 Group 2: Pre-test, SD 1 and 59
Injection site and body temperature evaluation (SHAM)	Group 1: Pre-dose, 4- and 24-hours post-dose Group 2: Not performed
Ophthalmology exam	Group 1: Pre-test and during week 8 Group 2: Not performed
Blood collection*	Group 1: Pre-test, SD 1, 15, 29, 43, 57 and 59 Group 2: Pre-test, SD 59

Table 84: Experimental design – SD = study day; *see clinical pathology measurements table below; SHAM = animals were removed from cages and handled in the same manner as when the procedure is performed, but no data collected

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>
Cageside observations	15	10
Clinical observations	10	1
Body weight	19	3
Injection site and body temperature evaluation	15	0
Ophthalmology exam	2	0
Blood collection*	21	2

Table 85: Frequency of observations – values represent total number of observations for each group

<i>Parameter</i>	<i>Frequency of Testing</i>
Hematology	Group 1: Pre-test only Group 2: Pre-test only
Coagulation	Group 1: Pre-test only Group 2: Pre-test only
Clinical chemistry (core chemistry)	Group 1: Pre-test and SD 59 Group 2: Pre-test and SD 59
Clinical chemistry (other biomarkers)/serum*	Group 1: Pre-test, SD 1, 15, 29, 43, 57 and 59 Group 2: Pre-test and SD 59
Urine chemistry (other biomarkers)	Group 1: SD 59 Group 2: SD 59

Table 86: Clinical pathology measurements – SD = study day; *blood was collected pre-dose and 4-, 24-, and 48-hours post-dose on SD 1, 15, 29 and 43 and blood was collected pre-dose and 4, 24-, and 48-hours post-dose on SD 57

On day 59, the amount of blood originally required from all animals for all analyses was not possible to obtain from the saphenous vein. Therefore, the hematology and coagulation sample collection were eliminated for Day 59 and there were no results to interpret.

Postmortem procedures: Only the heart was examined on post-mortem evaluation in this study. Tissues were first examined macroscopically for gross findings, then sectioned and stained with hematoxylin and eosin. Special stains including Gram, Periodic acid Schiff, Crocott's methenamine silver, Geimsa, Masson's trichrome, and/or Phosphotungstic acid hematoxylin were performed on heart sections from select animals.

Because the purpose of this study was to establish if cardiac inflammation with degeneration/necrosis is a background finding in (b) (4) rabbits, the following methodology from the study should be mentioned here:

“The heart was examined microscopically from all animals. Microscopic findings were graded on a scale of 1 to 5 as minimal, mild, moderate, marked or severe; findings not graded were listed as present. Evaluation of the heart was performed and recorded separately for the left ventricle, right ventricle, interventricular septum, right atrium and left atrium. In addition, a final morphologic finding was also recorded under the heart as an integrated summation of findings present in the sub regions listed above. Lesions within the specific sub regions of the heart were recorded separately according to their fundamental components: 1) infiltration [either mononuclear (primarily macrophages with rare lymphocytes) or mixed (included heterophils)]; 2) cardiac myocyte necrosis (characterized as hypereosinophilic or lytic cardiac myocytes); and 3) fibrosis, interstitial. These lesions were frequently but not always co-localized. The finding in the organ ‘Heart’ reflected an integrated interpretation of the findings from the specific heart sub regions. Findings that were interpreted to be typical of spontaneous background lesions were termed ‘Typical spontaneous findings’ (minimal and focal/multifocal infiltration of mononuclear/mixed cells and/or necrosis of cardiac myocytes) whereas findings that were out of the range of these typical spontaneous findings were termed ‘Inflammation with degeneration/necrosis; cardiac myocyte’ and given a severity score (minimal to moderate and focal/multifocal infiltration of mononuclear /mixed cells and necrosis of cardiac myocytes). Under heart, a morphologic finding of ‘Not present’ was used when the animal did not have ‘Typical spontaneous

findings’ or ‘Inflammation with degeneration/necrosis; cardiac myocyte’. Other microscopic findings were also entered under sub regions of the heart, but they were not included in the morphologic finding of ‘Typical spontaneous findings.’”

RESULTS

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

Clinical observations: There were no treatment-related changes in clinical signs during the course of the study. Those observed are considered incidental or commonly observed signs in laboratory rabbits.

Body temperature: Not included in the design of this study.

<i>Study Day</i>	<i>Group 1</i>	<i>Group 2</i>
1	3411.91	3418.50
4	3451.20	Not performed
8	3505.89	Not performed
11	3540.90	Not performed
15	3545.72	Not performed
18	3569.29	Not performed
22	3626.38	Not performed
25	3638.39	Not performed
29	3628.05	Not performed
32	3662.78	Not performed
36	3715.49	Not performed
39	3726.22	Not performed
43	3744.13	Not performed
46	3783.29	Not performed
50	3837.95	Not performed
53	3860.93	Not performed
57	3854.26	Not performed
59	3775.41	3743.78

Table 87: Body weight – absolute body weights reported in mean grams; weights only recorded for group 2 animals on study days 1 and 59 due to the nature of the study design

Food consumption: Not included in the design of this study.

Clinical chemistry:

<i>MEASUREMENT RELATED TO</i>	<i>END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE (if greater or less than 1.5, i.e. \uparrow 1.6 or \downarrow 1.6)</i>	<i>NOT OF NOTE</i>
ELECTROLYTE BALANCE		Calcium Phosphorus Sodium Potassium Chloride
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Glutamate dehydrogenase (ND) Sorbitol dehydrogenase (ND) Total bile acids (ND)
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids (ND) Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein Fibrinogen
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Cardiac Troponin I (CTNI) Cortisol Norepinephrine See discussion below	Total protein Albumin (A) Globulin (G) A/G Ratio Total Cholesterol Cholinesterase (ND) Creatine kinase Triglycerides Corticosterone Epinephrine

Table 88: Clinical chemistry results – ND = not determined

All animals had baseline CTN I values of ≤ 0.03 ng/mL with > 0.06 ng/mL being considered above the normal reference range. In group 1 animals, CTN I values ranged between 0.07 and 2.04 ng/mL or 2.33 to 68-fold higher than baseline for that particular animal, at 4 and/or 24 hours post-dose.

In group 1 animals, mean norepinephrine was increased at 4, 24 and/or 48 hours post-dose up to 2.67-fold over baseline which was determined to be ≤ 2.07 ng/mL. Of those animals, 87% had elevations above this baseline at 1 to 11 time points during the study, though there was large variability.

In group 1 animals, mean cortisol was increased up to 1.70-fold over baseline which was determined to be ≤ 2.2 μ g/mL. Of those animals, 33% had increases above this baseline

value at 24- or 48-hours post-dose at 1 to 4 time points during the study, though there was large variability.

See the figures below for a graphical data presentation of norepinephrine and cortisol.

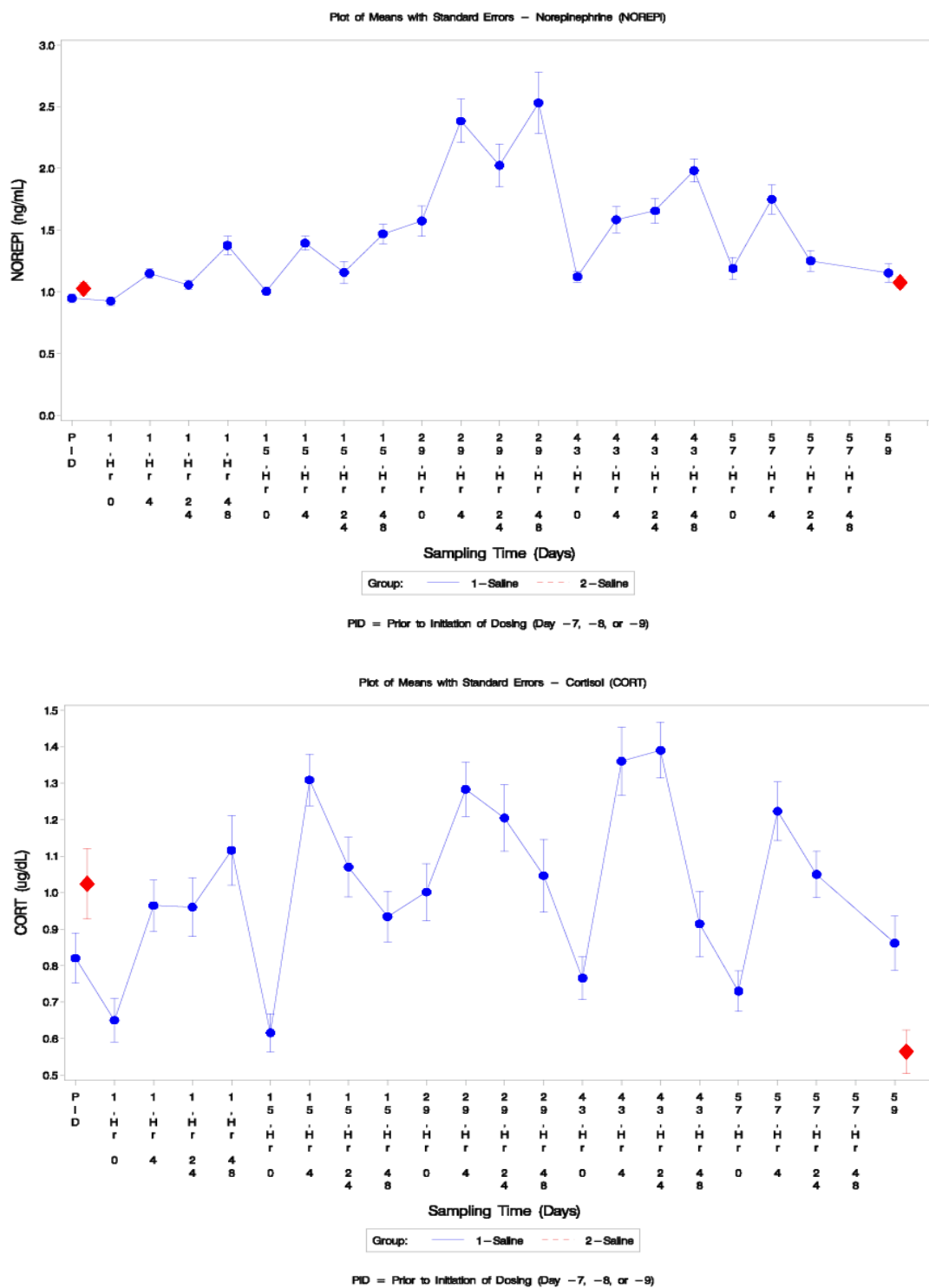


Figure 3: Norepinephrine and cortisol results – figures provided by the sponsor

Hematology and coagulation: Due to the study deviation described above, insufficient blood was drawn to measure these values at the end of the study, so these parameters were only measured pretest. Therefore, they have not been reviewed or reported here since pretest values alone carry no weight in regard to the conclusions drawn from this study.

Systemic toxicity: No treatment (or procedural)-related mortality or any toxicologically relevant changes in clinical signs were observed in this study. In general, the nature of the study design does not allow for effective evaluation of most parameters assessed for systemic toxicity. Since the purpose of this study was to assess the effect of stress from increased handling and blood draws, there was only a comparison against a separate control group pre-test and at the end of the study. In between that time, group 1 animals were only compared against their baseline pre-test values. For example, body weight was measured throughout the study, but the only control-based comparison was made at the end of the study.

Among the clinical pathology parameters measured in this study, those with results of note were the CTN I, norepinephrine and cortisol. The latter two biomarkers were generally elevated above the baseline value for individual animals, though CTN I was elevated in one-third of group 1 animals at any time point in the study. The study draws a correlation and conclusion from the results from group 1 animals that there is a correlation with increased handling and elevations in these biomarkers. However, because of the study design, there was no determination of how these values compared to group 2 animals that were not handled in between study days 1 and 57. These results did demonstrate high variability both between and within time points.

Organ Weight: Not included in the design of this study.

Gross Pathology: The only abnormality noted on gross post-mortem examinations was abnormal coloration of the heart noted in 8/70 group 1 animals and 13/70 group 2 animals.

Histopathology:

<i>Treatment Group Finding</i>	<i>1F</i>	<i>2F</i>
Heart, typical spontaneous findings	55	55
Heart, not present	6	14
Heart, inflammation with degeneration/necrosis; grade 1	5	1
Heart, inflammation with degeneration/necrosis; grade 2	2	0
Heart, inflammation with degeneration/necrosis; grade 3	2	0
Heart, interstitial fibrosis; grade 1	0	1
Ventricle (left), mononuclear cell infiltration; grade 1	46	35
Ventricle (left), interstitial fibrosis; grade 1	0	1
Ventricle (left), mixed cell infiltration; grade 1	28	7
Ventricle (left), mixed cell infiltration; grade 2	1	0
Ventricle (left), mixed cell infiltration; grade 3	1	0
Ventricle (left), focal fibrosis; grade 1	1	0
Ventricle (left), ascending aorta deposition mineral; grade 1	4	7
Ventricle (left), ascending aorta deposition mineral; grade 2	0	1
Ventricle (left), ascending aorta deposition mineral; grade 3	0	1
Ventricle (left), ascending aorta hyperplasia adventitia; grade 2	1	0
Ventricle (left), cardiac myocyte necrosis; grade 1	31	15
Ventricle (left), cardiac myocyte necrosis; grade 2	2	0

<i>Treatment Group Finding</i>	<i>1F</i>	<i>2F</i>
Ventricle (right), mononuclear cell infiltration; grade 1	11	8
Ventricle (right), mixed cell infiltration; grade 1	9	9
Ventricle (right), cardiac myocyte necrosis; grade 1	10	4
Ventricle (right) pulmonary artery deposition material	0	1
Interventricular septum, mixed cell infiltration; grade 1	9	9
Interventricular septum, mixed cell infiltration; grade 2	1	0
Interventricular septum, mixed cell infiltration; grade 3	2	0
Interventricular septum, cardiac myocyte necrosis; grade 1	16	7
Interventricular septum, cardiac myocyte necrosis; grade 2	2	0
Interventricular septum, mononuclear cell infiltration; grade 1	29	11
Atrium (left), hypertrophy; grade 1	0	5
Atrium (left), mixed cell infiltration; grade 1	8	8
Atrium (left), mononuclear cell infiltration; grade 1	7	1
Atrium (left), cardiac myocyte necrosis; grade 1	3	2
Atrium (right), hemorrhage; grade 1	1	0
Atrium (right), mononuclear cell infiltration; grade 1	6	5
Atrium (right), mixed cell infiltration; grade 1	9	6
Atrium (right), mixed cell infiltration; grade 2	1	0
Atrium (right), cardiac myocyte necrosis; grade 1	4	2

Table 89: Microscopic pathology findings

As per the study design, only the hearts were examined for microscopic changes post-mortem. An increased incidence of inflammation with degeneration/necrosis was observed in group 1 animals compared to group 2 (7 to 1). These ranged in severity from minimal to moderate with the two animals with moderate inflammation having elevations in CTN I of 56 and 68-fold over their respective baselines. The inflammation and degeneration/necrosis were primarily localized within the left ventricle and interventricular septum and not associated with fibrosis. One group 2 animal had interstitial fibrosis of the left ventricular papillary muscle that was interpreted to be a chronic, resolving focus of inflammation with degeneration/necrosis.

Local toxicity: No report of any gross abnormalities or clinical signs was noted at the injection sites for any animal in this study. The study design did not call for histologic examination of injection sites.

Serology: Since the animals only received injectable saline in this study, serology was not included in the design nor was it possible.

Assessment: There was no treatment/procedural-related mortality or clinical signs observed in this study. The design of this study limited the endpoints in this study to body weights, clinical chemistry (with select biomarkers) and histology of the heart. No toxicologically-relevant changes were observed in body weights or clinical chemistries at the end of the study between group 1 and 2 animals.

The study as a whole demonstrates some level of correlation between the stress from handling and changes in CTN I, norepinephrine and cortisol. There is also an established correlation between elevations in CTN I and histologic changes in cardiac myocytes as evidenced by those animals with inflammation and degeneration/necrosis having notable elevations above baseline levels. However, it should be made clear that there was anticipated

variability in all of the biomarkers evaluated in this study and with only the baseline level used as a control as opposed a separate control. This is mostly due to the nature of the study design. Since the “treatment” in this study was the procedural elements themselves, there was no feasible way to have a control group for comparison at every point during the study.

Additionally, this study used a fundamentally different methodology for histological preparation and analysis than what was used in any of the preceding toxicology studies. In the prior studies, the heart was opened at necropsy and affixed to cardboard then fixed for 24 hours and sectioned. In this current, saline-only study, the heart was flushed with formalin but not opened, then after 48 hours of fixation, two longitudinal sections were taken using external landmarks to promote consistency of sampling between animals. In the prior studies, the grading of findings was a composite of pathological systems (i.e. inflammation in the context of necrosis), whereas in this study, there was classification by pathological system (e.g. inflammation, necrosis). Additionally, the overall score for the heart was based on the worst findings. Previously, the heart findings were subsumed under the heading “heart”, but in this study, they assigned an overall score to the entire heart based on the most severe finding regardless of whether or not it was an irreversible finding. For example, a finding of grade 3 white blood cell infiltrates would give rise to an overall “heart” score of 3 in this study despite how there may have been grade 2 irreversible findings like fibrosis reportedly separately in the previous reporting system.

In addition, despite how the scientific literature (prior to publication of this study¹⁹) demonstrates how typical background lesions for the hearts of rabbits have yet to be definitively understood, this study arbitrarily assigned what the pathologist felt was considered “typical background findings” to include “minimal and focal/multifocal infiltration of mononuclear/mixed cells and/or necrosis of cardiac myocytes.” This is in contrast to a prior citation used in the published version of this study which states that the recognized background findings in rabbits are “...inert cellular infiltrates with no accompanying myocardial necrosis or fibrosis associated with the lesion.”²⁰

Considering how different the histological analysis is in this study, it is of this reviewer’s opinion that this study does not absolve the adverse finding from study 12GR385 from being treatment-related. When you take into account the 110 animals which were lumped as having “typical spontaneous findings” which may have been similar to something previously observed, the different means of lumping and scoring lesions, plus the fact that no animals in this study received 20vPnC, there is no true, verifiable means to assume the histological findings from this study would compare to those vaccinated animals in past toxicology studies. This could have been resolved by including a third group of animals in this study which received increased procedures and 20vPnC.

GLP study deviations or amendments: As previously mentioned, there was insufficient blood to perform hematology and coagulation testing on animals at the end of the study period. Therefore, no useful data was produced for those parameters. No significant

¹⁹ Sellers RS, Pardo I, Hu G, et al. Inflammatory cell findings in the female rabbit heart and stress-associated exacerbation with handling and procedures used in nonclinical studies. *Toxicol Pathol* 2017;45(3):416-26.

²⁰ (b) (4)

deviations or amendments were recorded that influenced the quality, integrity or interpretation of the results.

**PATHOLOGY WORKING GROUP REPORT FOR HEART FINDINGS IN RABBIT
TOXICOLOGY STUDIES FROM THE EXPANDED VALENCY PNEUMOCOCCAL CONJUGATE
(PNC) VACCINE PROJECT**

Methods: In order to get an outside opinion on the cardiac histopathology findings, the sponsor convened a pathology working group (PWG) consisting of three board-certified veterinary pathologists in April 2016 to evaluate heart tissue slides from studies 12GR385, 13GR165, (b) (4), 13GR370, (b) (4) and 15GR382. All cardiac slides from those animals which had abnormal findings were included as well as three animals with cardiac findings (one each of those receiving non-adjuvant control, vehicle or test-article) from archived historical samples from unrelated rabbit vaccine toxicity studies, for a total number of 74 slides evaluated. Animals were randomized and the pathologists were blinded regarding animal number group designation and study origin in order to reduce bias. Consensus diagnoses were made for each rabbit following discussion from the participants.

Findings were classified and defined as:

- **Infiltrate, monocyte:** “infiltrates of cells in the myocardium that were morphologically consistent with monocytes/histiocytes and lymphocytes”
- **Infiltrate, mixed:** “infiltrates of cells in the myocardium that included a significant proportion of heterophils, in addition to a monocytic component”
- **Degeneration/necrosis, cardiomyocyte:** “individual or multiple myofibers that were shrunken and eosinophilic, fragmented, vacuolated, and/or infiltrated by monocyte cells”
- **Fibrosis:** “expansion of the interstitium by mature collagen, characterized by being homogenous, eosinophil material on H&E-stained slides, or as intensely blue on trichrome-stained slides, when trichrome stains were available.”

These findings were then graded for severity from a scale of 0-5 as absent, minimal, mild, moderate, marked and severe, respectively. An overall severity grade was given for monocytic infiltrate and mixed infiltrate combined. The PWG were also requested to opine on whether the microscopic changes were consistent with background changes in the hearts of rabbits based on their experience. Following the examinations, the slides were unmasked for comparison across groups and studies.

Results: The consensus diagnoses from the PWG were presented in tabular form in an appendix to the report. They agreed that inflammation was the primary lesion in most cases and while degeneration/necrosis and fibrosis were not always present, they were “considered to derive from a similar process and variations reflected different stages of lesion chronicity (findings are along a continuum of change).” The lesions were found across all locations in the heart but were more prevalent in the left ventricle and interventricular septum.

Unlike in the toxicology studies, the PWG during their masked evaluations did not classify any of the lesions higher than a severity grade of 2 (mild). Additionally, they believed that the appearance of the individual lesions was indistinguishable across groups and there was

“...no difference in the pattern or occurrence of lesions across groups based on specific diagnoses, distribution of lesions, or severity of lesions.” Additionally, they agreed with the conclusions of the saline-only study 15GR382 in that more cardiac changes occurred in the group that received increased handling and procedures.

Of note, the PWG suggested a cross-study comparison of the incidences of the various findings in assessing the toxicology studies. Additionally, they state that, while the participants considered many of the lesions to be “...consistent with background findings and could therefore be spontaneous changes that may occur in rabbit hearts in toxicology studies”, they agreed some were *not*. Lastly, following unmasking and seeing how there were lesions graded mild in control groups, they admitted that they have not observed mild lesions of this sort in the control groups of routine rabbit toxicology studies which they would consider spontaneous in their combined experience.

WHITE PAPER ON ASSESSMENT OF MICROSCOPIC CHANGES IN HEART OF (b) (4) RABBITS IN TOXICITY STUDIES

This document was written and by provided by the sponsor and details in length how they arrived at their position that the cardiac changes observed in 12GR385 are not related to the vaccine or its components, nor does it necessarily translate to human risk. Included is a detailed overview and summary of the findings from all of the studies included here as well as the results of the PWG assessment. The sponsor provided the following summarized rationale with citations within the submission:

- The findings were not due to direct cardiac effects, as they were sporadic and rare (<1% incidence in animals administered antigenic components).
- Rabbits administered vehicle containing AlPO₄ had heart findings indistinguishable in nature, distribution, incidence, and severity from those seen in rabbits administered vaccine PnC_s (b) (4) or all 20 of the conjugates in 20vPnC).
- The findings were not immune-mediated based on lack of antibodies cross-reactive with rabbit heart muscle homogenates.
- Analysis of the vaccine vehicle excipients and their source materials (all of which are in marketed vaccines and other biotherapeutic products) revealed no safety concerns.
- Myocardial inflammation with cardiac myocyte necrosis up to moderate in severity may occur in rabbits unrelated to test article.
- The incidence and severity (up to moderate) of myocardial inflammation with cardiac myocyte necrosis in rabbits administered saline IM may be increased by study procedures.
- No heart findings were identified in rabbits, rats, or (b) (4) monkeys administered 13vPnC, or in rats administered the 7 new serotypes in 20vPnC.

- There were no increases in the cardiac damage biomarker cTnI in the 20vPnC [First-in-Human] clinical trial (B7471001).
- Approximately 4500 participants receiving 1 or more doses of 20vPnC have been followed for safety through at least 6 months (younger and older adults). Safety review has been ongoing and to date there have been no cardiac safety signals.

As mentioned in the sponsor's summary, reference is made to certain documents and toxicology studies which were not included in the original BLA submission. These include:

- Reference to toxicity studies in rabbits, rats and (b) (4) monkeys in 13vPnC (Prevnam 13) which has the same non-antigenic components. These changes were not observed in those studies.
- Discussion of a root cause analysis of vehicle constituents which included "...identification of all matrix components and sourcing, review of manufacturing process changes associated with purchased components, review of manufacturing process changes associated with aluminum phosphate suspension and review of the supporting supplier management quality system." This analysis did not identify any specific root cause which could attribute to the cardiac changes.
- A review of the sponsor's historical database to include 18 other injectable vaccine toxicology studies including 1254 rabbits from 2000 to 2012. Mild to moderate cardiac myocyte inflammation comparable to those observed in study 12GR385 were reported in 2 control rabbits and 1 treated rabbit, with 2 of these instances of inflammation associated with degeneration/necrosis. Minimal inflammation degeneration/necrosis was observed in 7/1254 rabbits.
- Discussion of a repeat-dose toxicity study performed in (b) (4) rats using "c7vPnC" a vaccine formulation which includes the 7 polysaccharides in 20vPnC which were not in Prevnam 13. No cardiac myocyte inflammation was observed in these rats.

The sponsor responded to an information request for these items and were submitted in an amendment to the BLA. The included information in that amendment confirms these claims in these portions of the white paper. Regarding the historical database, it is not clear if histologic preparation, examination and reporting of cardiac changes was identical across studies.

Assessment: This document is a summary of all the nonclinical toxicology data plus a shortened review of the PWG document included in this submission as well as some mention of clinical data from this submission plus nonclinical data not included in this submission. There is no new data to review. A discussion of these points is included in the conclusions section of this review below.

CONCLUSIONS

<i>Test article related effects</i>	<i>Effects considered incidental</i>
<ul style="list-style-type: none"> • Mild to moderate, recoverable elevations in fibrinogen, CRP and LUC • Minimal to moderate, recoverable injection site chronic-active inflammation with myofiber degeneration/necrosis • Increased lymphoid germinal centers in the spleen and draining lymph nodes • Minimal to moderate, low-incidence chronic inflammation with degeneration/necrosis and fibrosis of cardiac myocytes 	<ul style="list-style-type: none"> • Minimally increased incidence of absent lung lobes and unossified middle forepaw phalanges in fetal kits (but not newborn kits) of vaccinated rabbit does

Table 90: Summary of observations

Overall assessment: A total of six repeat-dose toxicity studies, one single-dose toxicity study (using an alternate route of administration) and one DART study were included in this submission to support the safety and risk assessment of 20vPnC in (b) (4) rabbits. Across all of these studies, the vaccine was well tolerated by the study rabbits and most of the test article-related findings were considered anticipated sequelae of the intended local (injection site) and systemic immune response to vaccination rather than as signs of frank toxicity. Serology assessments in these studies confirmed the administration of the test articles as well as passive transfer of antibodies to fetuses in the DART study.

In the repeat-dose toxicity studies, the rabbits received 5 intramuscular doses of the intended clinical dose of 20vPnC (or higher) or other formulations containing (b) (4) 20 polysaccharides. Test article-related findings during the antemortem phase of the studies included elevations in inflammatory biomarkers on clinical pathology assessments and a sporadic incidence of lumps at the injection sites. These were all considered reversible and did not result in any clinical manifestations in the vaccinated rabbits. They are also comparable to what was observed with Prevnar 13. During the postmortem phase of the studies, outside of the cardiac changes which are discussed below, treatment-related observations were limited to minimal to moderate injection site inflammation with myofiber degeneration/necrosis and increased lymphoid germinal centers at injection site-draining lymph nodes and the spleen. These findings were also considered fully or partially reversible by the end of the recovery phase and are related to the antemortem evidence of the anticipated inflammatory response observed during the antemortem phase of the study.

IM administration of 20vPnC at a dose of 46.2 µg to pregnant rabbits did not result in any evidence of maternal toxicity, nor was there any clear evidence of toxicity or teratologic effect on their kit litters observed either *in utero* or postpartum. There were no clear treatment-related malformations, variations, or effects on number of corpora lutea, implantations, implantation loss, resorptions, litter size, litter weight or sex distribution. Similarly, there was no effect on the littering subset as the kits from vaccinated does showed no difference compared to those from control does in regard to litter size, viability, growth and developmental milestones. The two variations observed in higher frequency in fetuses from vaccinated does, absent lung lobes and unossified forepaw middle phalanges, should be considered incidental even though they were slightly above the historical reference range because of the lack of a correlating observation on kit development and postmortem

necropsy examinations. Immunologic testing demonstrated that there was evidence of passive transfer of antibodies observed in both in utero and postpartum.

The single-dose toxicity study included in this submission demonstrated that 20vPnC was well tolerated when administered subcutaneously. While this study was limited in design and scope, it provided additional insight into the overall safety and risk assessment should 20vPnC be inadvertently administered subcutaneously.

Cardiac findings assessment: While the sponsor provides detailed rationale in the included white paper, based on the results of the studies reviewed here, the etiology and mechanism for the cardiac findings is unknown and the relevance of the cardiac findings to human subjects should be considered inconclusive. Despite the citations supplied by the sponsor, there is a paucity of literature on susceptibility to rabbits to non-specific myocardial fiber degeneration, necrosis, inflammation and necrosis, unlike the (b) (4) rat where this finding is well established²¹. The cardiomyocyte inflammation with degeneration/necrosis was deemed adverse and test article-related by the original study pathologist plus one from a contract research organization, and the finding was repeatable (study 13GR165).

See table 92 below for a summary of the cardiac findings and the differences in design between the 7 submitted toxicology studies. The studies included in this submission are inconsistent in regard to number of animals per group, treatment received and number of phases. For example, the study investigating the first (b) (4) formulations of (b) (4) had a saline control group, but the second study investigating the other (b) (4) formulations had a vehicle control group. Additionally, only the initial study, 12GR385 had a recovery phase. These studies tested various formulations containing (b) (4) or 20 serotypes to try to identify whether or not a particular serotype was the culprit for these cardiac changes, though none of these studies investigated a (b) (4) formulation: i.e. Prevnar 13 plus (b) (4) of the serotypes in 20vPnC. The sponsor also investigated a vaccine which included CRM-conjugated serotypes for the 7 that separate Prevnar 13 and 20vPnC which is referenced in their white paper in this submission. While there was no evidence of treatment-related cardiac changes in the toxicology study conducted to provide a nonclinical risk assessment, the sponsor elected to use rats which have an established background rate of spontaneous cardiomyopathy.

<i>Study Number</i>	<i>Article Administered</i>	<i>No. Animals Receiving Article*</i>	<i>No. Animals with ID/N</i>	<i>No. Animals with Fibrosis</i>
12GR385	Saline	20	0	0
12GR385	Vehicle-only	20	0	0
12GR385	(b) (4)	20	0	0
12GR385	20vPnC	40	2	4
13GR165	Saline	72	0	0
13GR165	Vehicle-only	0	NA	NA

(b) (4)

<i>Study Number</i>	<i>Article Administered</i>	<i>No. Animals Receiving Article*</i>	<i>No. Animals with ID/N</i>	<i>No. Animals with Fibrosis</i>
13GR165	20vPnC	72	2	0
(b) (4)				
13GR370	Saline	20	0	0
13GR370	Vehicle-only	0	NA	NA
13GR370	(b) (4)	320	3	2
13GR370	20vPnC	80	1	1
(b) (4)				
14GR064	Saline	0	NA	NA
14GR064	Vehicle-only	72	7	0
14GR064	20vPnC	0	NA	NA
15GR382	Saline**	140	10	1
15GR382	Vehicle-only	0	NA	NA
15GR382	20vPnC	0	NA	NA
TOTAL	Saline	132	0	1
TOTAL	Saline**	140	10	1
TOTAL	Vehicle-only	212	9	3
TOTAL	(b) (4)	134	2	1
TOTAL	(b) (4)	860	5	6
TOTAL	20vPnC	192	5	5

Table 91: Summary of adverse histologic cardiac changes – *number of animals include both sexes combined; **histologic preparation and examination of hearts notably different than in other studies, discussed below; ID/N = inflammation with degeneration/necrosis; NA = not applicable

While the saline-only study 12GR382 adequately demonstrates a correlation between increased procedures and cardiac inflammation and degeneration, no conclusions or correlation should be drawn from this study in the context of 20vPnC. The catecholamine correlation that the sponsor proposes carries scientific validity based on the results of that study, but because of the intentional exclusion of 20vPnC from 12GR382 and the stark differences in histological preparation and analysis in 12GR382 (specifically the introduction of an entirely new classification system and the arbitrary assignment of a new category of “typical spontaneous findings”), no clear distinction and comparison should be made between the histologic findings from this study and those seen in any previous toxicology study involving 20vPnC.

The sponsor correctly points out in their white paper rationale how these cardiac changes were also observed in some rabbits which received only the vehicle. The components of the vehicle, including the adjuvant aluminum phosphate, have been used extensively in both humans and animals for decades without any historical evidence of cardiac toxicity. Therefore, unless rabbits truly have spontaneous cardiomyopathy similar to rats, there is

either a very low-grade, low incidence change which these ingredients may be causing or there is some interaction with the serotypes and/or CRM in this product resulting in these cardiac changes. Further investigation is recommended through truly investigational studies where a possible mechanism or biodistribution is sought rather than further toxicology studies. An ideally-designed repeat-dose toxicology study with rabbits could be considered where there are 4 arms to the study: saline, vehicle, Prevnar 13 and 20vPnC.

Looking back at the toxicology study pathology data submitted for Prevnar13, 2/40 saline-treated rabbits had grade 1 focal cardiac mononuclear inflammation, 2/20 vehicle-treated rabbits had grade 1 multi-focal cardiac mononuclear cell inflammation, 3/20 vaccinated rabbits had grade 1 focal cardiac mononuclear cell inflammation and 1/10 vehicle-treated had grade 1 focal mixed cell inflammation, though no further details were provided. The sponsor did provide the historical reference data from 2000 and 2012 used to draw their conclusion in the submitted white paper and there was a comparable incidence of cardiac findings between vaccinated and control rabbits, but it should be pointed out how it is not clear if the findings from the historical data are morphologically similar because of how they are presented (e.g. inflammation and myocardial degeneration are listed separately). Additionally, the control data is for saline and vehicle-only rabbits lumped together.

This is the first time in this reviewer's years of professional experience reviewing investigational vaccines observing a pathologist not only report cardiac inflammation paired with degeneration/necrosis but to also consider a postmortem cardiac finding adverse. A retrospective review of nonclinical repeat-dose toxicology studies for investigational vaccines using rabbits submitted to CBER was conducted for comparison to the sponsor's historical control data. Among 568 rabbits that received saline only, a total of 27 instances of cardiomyocyte inflammation (of varying classifications), 15 instances of mononuclear or mixed cell infiltrates and 3 instances of degeneration/necrosis. The latter was always an isolated finding and not listed along with inflammation.

As was included in this review and mentioned in the supplementary documentation provided in this BLA, CBER and the sponsor had multiple PreIND communications regarding the cardiac findings. It should be noted that none of the studies included in this submission were conducted at the request of CBER, nor were they designed based on advice from CBER. Rather than conducting a study with design recommendations from CBER, the sponsor convened two different working groups with outside pathologists for consultation, their PWG and an Expert Working Group (EWG). These working groups generally support the notion that these cardiac findings are unlikely to be of translational risk to humans, but there were some conclusions and statements of note from these pathologists (in no particular order):

- The EWG considered that the primary change in both the spontaneous heart findings and the heart lesions of concern are likely degeneration and necrosis of cardiac myocytes with subsequent infiltration of inflammatory cells in response to the cell damage, suggesting the lesions seen with the vehicle or expanded valency PnC vaccines are on a continuum and on one end of the same spectrum.
- While the EWG considered the cardiac lesions in the rabbits to be of no translational risk to humans, their confidence was limited by the paucity of literature on

susceptibility to rabbits to non-specific myocardial fiber degeneration, necrosis inflammation and fibrosis. They even go so far as to admit that the literature on the susceptibility of rabbits to heart lesions is sparse at best and the translational importance of these lesions is uncertain.

- The EWG admitted and agreed that there was a clear difference in the microscopic findings in the heart between the saline, matrix and PnC conjugate groups with only heart findings of low severity being identified in saline treated animals.
- The PWG concluded that saline treated rabbits had only what were considered spontaneous heart findings with no true cardiac lesions, unlike matrix- and PnC-treated groups which had both spontaneous findings and cardiac lesions.
- The EWG thought that the incidence and severity of the cardiac lesions between the matrix-only and PnC vaccine treatment groups were not different; indicating that the lesions were not due to the polysaccharide conjugates but rather was associated with the matrix component of the vaccines.
- The EWG felt that the heart lesions were of generally greater severity and distribution than spontaneous lesions, although they were otherwise similar to each other.

In conclusion, the finding of cardiac myocyte inflammation with degeneration/necrosis and fibrosis, though sporadic, was deemed test-article related and adverse by the original study pathologist. The sponsors white paper provides some valid rationale for why they believe this lesion does not translate to human subjects, and study 15GR382 does establish a baseline understanding that rabbits may develop stress-related cardiomyopathy, but the efforts to better understand and investigate these lesions have not resulted in any definitive information and the mechanism and etiology are poorly understood at this time. This reviewer considers the relevance and translation to human subjects to be inconclusive at this time and should be based on the clinical data available, though it will be difficult to assess for subclinical changes in humans beyond what was done in the Phase 1 trial. Post-marketing surveillance of any increased risk of cardiac disease may be considered as well. Extra consideration should be given when reviewing clinical data for any evidence of cardiac disease.

Conclusions: This submission is acceptable with regards to nonclinical toxicology and adequate data has been presented demonstrating safety and tolerability of 20vPnC when administered with the adjuvant aluminum phosphate IM. There was no clinically-relevant evidence of maternal toxicity (to include female fertility) or teratology when administered to pregnant rabbit does. Evidence of systemic inflammation and positive titers on serology adequately demonstrate the immunomodulatory effect of the vaccine. There are no toxicologic issues identified which would preclude approval of the BLA in the intended human population, but post-marketing surveillance for cardiac symptoms and further exploration of the cardiac changes in rabbits can be considered.

Concurrence: Martin D. Green, PhD, Supervisory Toxicologist