

**Toxicology Review of BLA/STN 125324 Pneumococcal 13-Valent Conjugate
(Prevnar plus Diphtheria CRM197saccharide conjugates for Types 1, 3, 5, 6A, 7F and
19A) Vaccine, Alum Adsorbed**

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From: Claudia Wrzesinski

Through: Martin Green

To: Julienne Vaillancourt, Chair, Review Committee

File: BLA 125324

Product: Prevnar 13™ [Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein)]

Subject: Review of toxicology data

Reviewer: Claudia Wrzesinski

Reference: BLA sections reviewed:
4.2.3.2 Repeat-dose Toxicity
4.2.3.5 Juvenile toxicity study
4.2.3.6 Local tolerance

Sponsor: Wyeth Pharmaceuticals

Product: 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC)

Proposed use: 13vPnC is indicated for active immunization against invasive disease and otitis media caused by *Streptococcus pneumoniae* Serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F in infants and children. The routine schedule is 2, 4, 6, and 12-15 months of age.

EXECUTIVE SUMMARY

Five repeat-dose toxicology studies were performed in rats, rabbits and ----(b)(4)---- monkeys to evaluate the preclinical safety of 13vPnC. These were two (2) 13-week studies in rats, one (1) 13-week study in ----(b)(4)---- monkeys, one (1) 5-cycle study in rabbits, one (1) 8 week study in juvenile rats. In addition, the local tolerance of 13vPnC was evaluated in rabbits. Single-dose toxicity was evaluated using data collected after the first dose in repeat-dose studies. All studies were conducted under Good Laboratory Practice (GLP) regulations.

Eight Week SC Toxicity Study In Juvenile Rats (study #900742, RPT 59901)

13vPnC (30.8 µg polysaccharide, 29 µg CRM 197, and 0.125 mg AlPO₄) was administered subcutaneously (SC) on postnatal days (PNDs) 7, 21, 35, 49, and 63 to SD juvenile rats, initial age 7 days, 40/gender, (0.15 ml/injection on PND 7 and 0.5 ml/injection on subsequent occasions). Control groups received 0.15 ml 0.9% sodium chloride for injection. Note that the route of administration (SC) differed from the clinical route (IM). On PNDs 23 (2 days after the 2nd dose) and PND 65 (2 days after the 5th dose) pathology, serology, hematology and clinical chemistry was performed. Injection-site reactions consisting of macroscopic nodules correlating with microscopic subcutaneous inflammation with degeneration and necrosis. Symptoms did not completely resolve by end of the short recovery phase (2 days). Overall, there was no systemic toxicity noted in this study. Most animals had a positive serum IgG antibody response to each of the 13 serotypes of 13vPnC after 5 doses.

Thirteen Week SC Toxicity Study In Monkeys (study #501297, RPT-59900)

13vPnC (30.8 µg polysaccharide, 29 µg CRM 197, and 0.125 mg AlPO₄) was administered SC on days 1, 15, 29, 43, 57, 71, and 85 to ----(b)(4)----monkeys, age 2.5-3.5 years, (6/gender) at a dose of 0.5 ml/injection. Control groups received 0.5 ml 0.9% sodium chloride for injection. Note that the route of administration (SC) differed from the clinical route (IM). On day 87 (2 days after the last dose) 3 animals/gender/group were sacrificed and another 3 animals/gender/group were sacrificed on day 115 (4 weeks after the last dose). Effects observed upon macroscopic and microscopic evaluations were injection-site related and consisted of nodules starting with 2nd dose administration that correlated with minimal to moderate microscopic inflammation. Overall, there was no systemic toxicity noted in this study as evidenced by lack of treatment-related mortality and lack of changes in body weight, relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight. 13vPnC induced specific serum antibodies to each of the 13 pneumococcal serotypes contained in the vaccine.

5-Cycle IM Toxicity Study In Rabbits (study #6115A1, RPT-72055)

13vPnC (31.3 µg polysaccharide, 36.4 µg CRM 197, and 0.125 mg AlPO₄) was administered IM on days 1, 22, 43, 64, and 85 to (b)(4) rabbits, 5 months of age, (10/gender) at a dose of 0.5 ml/injection into the right thigh muscle. Control groups (10/gender) received 0.5 ml 0.9% sodium chloride for injection. The AlPO₄ group (10/gender) received 0.125 mg ALPO₄ as adjuvant. Five (5) animals/gender/group underwent a 4-week dose free recovery period after the last injection. No treatment-related mortality nor any other toxicologically relevant change in relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight was observed supporting lack of systemic toxicity. Injection site reactions were observed and consistent of microscopic degeneration/necrosis at the injection site which completely reversed at the end of the observation period. Chronic inflammation at the injection site were slight to mild in the vaccine treated group and slight in the ALPO₄ treated group and partially reversed at the end of the observation period. 13vPnC induced specific serum antibodies to each of the 13 pneumococcal serotypes of 13vPnC.

Single Dose IM Irritation Study In Rabbits (study # 501549, RPT-62420)

13vPnC (30.8 µg polysaccharide, 29 µg CRM 197, and 0.125 mg AlPO₄) with and without ALPO₄ adjuvant were administered IM as a single injection into the left thigh muscle at a dose

of 0.5 ml/injection to 3 male rabbits/group, 5 months of age. The control group (3 males) received 0.5 ml 0.9% sodium chloride for injection. There was no evidence of treatment-related mortality, changes in body weight, relative food consumption, or treatment related macroscopic or microscopic lesions. Local toxicity manifested itself as microscopic hemorrhage, was detected in all three study groups and correlated with microscopic degeneration/necrosis and inflammation in the saline control and the 13vPnC without adjuvant group. Notably, in the repeated dose toxicity studies, local toxicity was also not observed after the first injection and only appeared after repeated vaccine administration.

Thirteen Week SC Toxicity Study In Rats (study #501296, RPT-59899)

13vPnC (30.8 µg polysaccharide, 29 µg CRM 197, and 0.125 mg AlP₀₄) was administered SC on days 1, 15, 29, 43, 57, 71, and 85 to groups of S-D rats, age 56 days, (20/gender) at a dose of 0.5 ml/injection. Control groups received 0.5 ml 0.9% sodium chloride for injection (20/gender). Ten (10) animals/gender/group were euthanized on day 87 (2 days after the last dose) and 10 animals/gender/group were euthanized on day 115 (4 weeks after the last dose). Note that the route of administration (SC) differed from the clinical route (IM). There were no treatment-related mortality or toxicologically relevant changes in clinical signs, relative food consumption and ophthalmoscopic parameters. Injection site observations included nodules, masses, scabs, and/or swelling in 13vPnC treated females and males, starting with the second dose. Histological examination of the injection site revealed microscopic subcutaneous inflammation with minimal necrosis and degeneration as well as degeneration and necrosis of the panniculus muscle at the end of the dosing phase (day 87) observed in some animals treated with 13vPnC. Microscopic subcutaneous inflammation and degeneration/necrosis at the injection site was not resolved at the end of the recovery phase. Draize scoring of the injection sites revealed an increased frequency of irritation in the treated animals compared to control animals, but without increased severity. Clinical pathology changes included increases in fibrinogen and neutrophil counts, increased globulin with concurrent decreased albumin values consistent with inflammatory changes seen microscopically at injection sites. Antibody titers were not fully evaluated in this study.

Thirteen Week SC Toxicity Study In Rats (study #6617-282, RPT-66951)

13vPnC (30.8 µg polysaccharide, 33 µg CRM 197, and 0.125 mg ALP₀₄) and 7vPnC (17.6 µg polysaccharide, 20 µg CRM197, and 0.125 mg ALP₀₄) were administered SC on days 1, 15, 29, 43, 57, 71, and 85 to groups of S-D rats, age 6-7 weeks, (20/gender/group) at a dose of 0.5 ml/injection followed by a 4-week recovery period (10/gender/group). Control groups received 0.5 ml 0.9% sodium chloride for injection (20/gender) or 0.125 mg ALP₀₄ (20/gender). Note that the route of administration (SC) differed from the clinical route (IM). There were no treatment-related mortality or toxicologically relevant changes in clinical signs, relative food consumption and ophthalmoscopic parameters observed in this study. Clinical signs involved the injection site and included slight edema and erythema in animals administered 13vPnC and 7vPnC which were mostly resolved at the end of the recovery phase. Nodules were observed at the injection site which were still present during recovery in the 13vPnC and 7vPnC treated animals and correlated with microscopic inflammation. In the 13vPnC and 7vPnC treated animals nodules correlated with statistically significant increases in neutrophil, monocyte and basophil counts; and decreases in albumin/globulin ratio not deemed

to be of clinical concern. 13vPnC and 7vPnC induced IgG antibody responses to each of the serotypes contained in the vaccines.

In conclusion, 13vPnC administered as repeated doses to rats, rabbits and monkeys produced mild local inflammatory reactions without treatment related systemic toxicity. 13vPnC vaccine induced serologic antibody responses in the test animals. Under the conditions of the nonclinical studies 13vPnC was well tolerated.

Introduction:

Wyeth has developed Prevnar 13 (13vPnC) as a successor to the currently registered Prevnar vaccine. Prevnar is used in infants and young children to prevent pneumococcal disease, especially invasive pneumococcal disease (IPD) and acute otitis media (AOM) caused by the 7 pneumococcal serotypes contained in the vaccine. Prevnar is a 7vPnC that contains serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. In addition to these serotypes, 13vPnC contains serotypes 1, 3, 5, 6A, 7F, and 19A. Prevnar 13 is indicated for the active immunization of infants and toddlers for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; and active immunization of infants and toddlers for the prevention of otitis media caused by serotypes included in the vaccine. Pneumococcal disease accounts for an estimated 3,000 cases of meningitis, 5,000 cases of bacteremia, 500,000 cases of pneumonia and 7 million cases of OM each year in the United States. Since the vaccine introduction with Prevnar, a 98% reduction in IPD caused by these serotypes has been observed among children younger than 5 years of age. The six additional pneumococcal serotypes contained in 13vPnC vaccine were responsible for approximately 62% of IPD cases in children < 5 years of age in 2007. In this same age group, the thirteen serotypes contained in the 13vPnC vaccine were responsible for approximately 64% of IPD cases in 2007.

An IND (b)(4) was submitted on May 26, 2004, but it did not contain toxicological data.

TOXICOLOGY STUDY REVIEW

Title: 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC): EIGHT WEEK (1 DOSE/2 WEEKS) SUBCUTANEOUS TOXICITY STUDY IN JUVENILE RATS (PROTOCOL 05_0405); 900742;

Study number: 900742

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

----- sponsored by Wyeth Research

Study initiation date: 09 Jun 2005

Final Report date: 20 Jun 2007

Test article batch/lot: #7-5064-002B

Animal species and strain: -----(b)(4)----- outbreed

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

Number of animal per group and sex: 40

Age: 7 days

Body weight range: The range of pup weights at the start of dosing on PND 7 was 13.3 to 19.7 g.

Route and site of administration: subcutaneously, left and right scapula.

Volume of injection: 0.15ml/injection on postnatal day (PND) 7; 0.5 ml/injection on subsequent administrations.

Frequency of administration and study duration: 5 doses administered on PNDs 7, 21, 35, 49, and 63; final necropsy on PND 65 .

Dose: 0 or 30.8 µg polysaccharide vaccine, 29 µg CRM197, 0.125 mg aluminum phosphate/0.5mL.

Stability: Stability studies were performed by the sponsor on the same batches of vaccine and adjuvant control as used in this study. Stability studies on formulated bulk materials were performed by the sponsor at ---(b)(4)---- month, the bulk was stable for (b)(4) months. Based on the stability data, -----(b)(4)----- is the assigned expiration date.

Means of administration: needle and syringe

Report status: final

Experimental study design

Group*	Treatment (µg/0.5mL)**	Number of Animals (#/sex/group)	
		Treatment phase (23days)	Treatment phase (65 days)
1 Saline Control	0.9% sodium chloride	20 animals	20 animals
2 13vPnC	30.8 µg polysaccharide, 29 µg CRM197, 0.125 mg aluminum phosphate	20 animals	20 animals

*Dose was delivered subcutaneously on PND 7, 21, 35, 49, and 63.

** Except on PND 7, when the dose volume was approximately 0.15 mL

Methods: Hematology Laboratory (--(b)(4)-- Analyzer), Hematology Laboratory Counter, bone marrow stain, for clinical chemistry -(b)(4)- package was used as a reference.

Randomization procedure: One day prior to initiation of dosing, all litters were weighed and randomly assigned to each of the dosage groups using a computer-based randomization procedure.

Statistical analysis plan:

Statistical testing was performed using two-sided t-test for hematology and clinical chemistry; ANOVA *F*-test for body and organ weight, and exact Wilcoxon for the evaluation of the injection irritation.

Clinical observations

Parameters	Frequency of Testing
Cageside observation ¹	Dams: twice daily prior to birth, once prior to start of dosing and on the days of body weight assessment Pups: once on day of arrival, once before weaning and twice weekly after weaning
Clinical observations ²	Dams: once prior to start of dosing and on the days of body weight assessment Pups: once on day of arrival, once before weaning and twice weekly after weaning
Body weight	Dams: day 4, 7, 14, 21 post partum Pups: daily between day 4 and 28 post partum, twice weekly thereafter, final fasted body weight at day of necropsy
Food consumption	Pups: twice weekly
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry*	Pups: days 23 and 65, food was removed overnight,
Hematology*	Pups: days 23 and 65, food was removed overnight,
Coagulation*	Pups: days 23 and 65, food was removed overnight,
Immunological response	Pups: days 23 and 65
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Pups: days 23 and 65
Necropsy	Pups: days 23 and 65
Tissues for histopathology	Pups: days 23 and 65

*(site of blood collection: abdominal aorta under isoflurane anesthesia); NC: not collected

¹ Cageside observations include mortality, morbidity, general health and signs of toxicity.

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

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

Histology evaluation

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	(thoracic) !	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/pons, and olfactory bulb)	*	
Cervix	!	
Colon, Caecum	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		
Gross lesions (if any)		
Gut associated lymphnodes	!	
Harderian gland (if applicable)	!	
Heart	*!	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	*!	
Lacrimal glands		x
Larynx		x
Liver	*!	
Lung (sample of 2 lobes)	!	
Lymph nodes (axillary)	!	
Lymph nodes (cervical)		
Lymph nodes (inguinal)	!	
Lymph nodes (mandibular)	!	
Lymph nodes (mesenteric)		
Mammary glands		

Naso-oropharyngeal cavity (turbinates, nares, soft palate)		
Ovaries	*!	
Pancreas	!	
Peyer's patch (if applicable)		
Pituitary gland	*!	
Prostate	*!	
Rectum		
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicles	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	*!	
Stomach (squamous and glandular)	!	
Testes	*!	
Thymus	*!	
Thyroid (w/ parathyroid glands)	*!	
Tongue	!	
Trachea	!	
Ureters		
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		

Table of Histology – Tissues examined: All dose group

(*) Organ weight was determined

(!) Histology was performed

Group one and two were evaluated on day 23 and 65

Results:

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

Clinical Chemistry

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein: ND Fibrinogen: ND
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase Fasting triglycerides

(ND): not done

No fold changes between the control group and the treatment group greater than 1.5 have been determined.

Hematology evaluation		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean corp. Hb. (MCH) Mean corp. Hb. Conc. (MCHC), Mean corp. Volume (MCV) Total erythrocyte count (RBC) Reticulocytes
WHITE BLOOD CELLS	Females day 23: Large Unstained Cells (LUC) 2.6 times higher in the 13vPnC ($0.0026 \times 10^9/\mu\text{l}$) compared to the saline control ($0.01 \times 10^9/\mu\text{l}$)	Basophils, eosinophils count lymphocyte count Macrophage/monocyte count Neutrophil count Total leukocytes (WBC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

With the exception of an increase of Large Unstained Cells in females of the treatment group on day 23 no significant differences between control group and treatment group have been determined.

Systemic toxicity:

No treatment-related mortality and no toxicologically relevant changes in body weight (gain), relative food consumption, clinical signs, clinical chemistry, gross anatomy, and organ weight were observed.

A few incidences of statistically significant differences in the clinical chemistry results were observed; however, the fold change was below 1.5 and no meaningful differences were found. Hematologic evaluations revealed Large Unstained Cells (LUC) that were 2.8 times higher in females assigned to the 13VPnC group compared to the saline group. LUCs are large peroxidase-negative cells which can either be large lymphocytes or stem cells. This increase in LUC was observed on day 23 and no longer observed on day 65.

Clinically the formation of nodules at the injection site was observed in 13vPnC treated groups especially after the fourth and fifth subcutaneous injection (days 49 and 53 post partum) which regressed within 4 days.

Organ weight

GROUPS	MALES				FEMALES				
	0 (Control; Day 23)	1 (13vPnC; Day 23)	2 (Control; day 65)	3 (13vPnC; day 65)	0 (Control; day 23)	1 (13vPnC; day 23)	2 (Control; day 65)	3 (13vPnC ; day 65)	
NUMBER OF ANIMALS	20	20	20	20	20	20	20	20	
BODY WEIGHT (gram) ^a	61.3	63.7			58.6	60.2			
BRAIN									
Absolute Weight ^a	gram	1.5657	1.6037	2.0986	2.1305	1.5071	1.5430	1.9285	1.9571
Per Body Weight ^a	%	3.2845	3.1539	0.5652	0.5888	3.2888	3.2931	0.8811	0.9002
ADRENALS									
Absolute Weight ^a	gram	0.0227	0.0242	0.0636	0.0643	0.0222	0.0226	0.0652	0.0616
Per Body Weight ^a	%	0.0475	0.0477	0.0171	0.0178	0.0483	0.0482	0.0297	0.0283
HEART									
Absolute Weight ^a	gram	0.3265	0.3286	1.4612	1.4456	0.3084	0.3199	0.9883	0.9663
Per Body Weight ^a	%	0.6843	0.6465	0.3925	0.3973	0.6708	0.6796	0.4507	0.4430
KIDNEYS									
Absolute Weight ^a	gram	0.6404	0.6677	2.5917	2.5687	0.6239	0.6361	1.6755	1.6676
Per Body Weight ^a	%	1.3401	1.3162	0.6961	0.7086	1.3591	1.3562	0.7617	0.7612
LIVER									
Absolute Weight ^a	gram	1.5940	1.6852	11.6401	11.0388	1.5887	1.5867	6.7973	6.6202
Per Body Weight ^a	%	3.3333	3.3189	3.1217	3.0302	3.4530	3.3754	3.0790	3.0356
SPLEEN									
Absolute Weight ^a	gram	0.1644	0.1846	0.8907	0.8732	0.1755	0.1761	0.5504	0.5308
Per Body Weight ^a	%	0.3439	0.3618	0.2394	0.2394	0.3805	0.3721	0.2498	0.2438
TESTES									
Absolute Weight ^a	gram	0.3334	0.3430	3.2956	3.3680				
Per Body Weight ^a	%	0.6985	0.6763	0.8851	0.9219				
THYROID and PARATHYROID									
Absolute Weight ^a	gram	0.0073	0.0071	0.0214	0.0210	0.0073	0.0067	0.0165	0.0174
Per Body Weight ^a	%	0.0152	0.0139	0.0057	0.0058	0.0158	0.0142	0.0075	0.0080
Per Brain Weight ^a	%								
THYMUS									
Absolute Weight ^a	gram	0.1932	0.1974	0.5996	0.6720	0.2007	0.2014	0.4441	0.5073
Per Body Weight ^a	%	0.4042	0.3886	0.1614	0.1855	0.4364*	0.4280*	0.2029*	0.2328*
OVARIES									
Absolute Weight ^a						0.0199	0.0192	0.0991	0.0994
Per Body Weight ^a						0.0431	0.0410	0.0452	0.0455
Per Brain Weight ^a									
PITUITARY									
Absolute Weight ^a	gram	0.0026	0.0026	0.0114	0.0106	0.0026	0.0027	0.0128	0.0128
Per Body Weight ^a	%	0.0003	0.0003	0.0031	0.0029	0.0057	0.0057	0.0058	0.0059
PROSTATE									
Absolute Weight ^a	gram	0.0641	0.0634	0.8126	0.8446				
Per Body Weight ^a	%	0.0079	0.0083	0.2186	0.2321				

Table of organ weight and their normalization. Absolute weights are expressed as mean(grams) ± standard deviation (sd). *different from controls at P≤0.05; **different from controls at P≤0.01.

The weight of males in the treatment group was statistically significantly higher compared to control males on all evaluated days (SD 7, 8 to 18) (male body weights were between 4.8 and 7.6% higher compared to males in the control). There was no treatment-related difference in female body weights.

No significant differences in the organ weights between control group and treatment group were observed, except for the relative organ weight of the thymus on day 65 which was slightly higher in the treatment group (male and female). No significant differences in absolute thymus weight between control group and treatment group was observed.

Gross Pathology group identification:

M1: male saline control group

M2: male treatment group

F1: female saline control group

F2: female treatment group

Gross pathology on day 23

Groups	Findings
1M	dark area at the axillar lymph node (1M: 1/20 animals)
2M	mass at the I.S. at the left scapula (2M: 1/20 animals), dark foci in the mandibular lymph node (2M: 1/20 animals), dark areas in the thymus (2M: 2/20 animals),.
1F	dilatation ventricle in the brain (1F: 1/20 animals), brain enlargement and one with clear pale fluid in the brain (1F: 1/20 animals), dark foci in the mandibular lymph node (1F: 1/20 animals).
2F	dark area I.S. at the left scapula (2F: 1/20 animals),, dark foci at the I.S. at the left scapula (2F: 1/20 animals),
1M, 2F	Dark area in the eye (1M: 1/20 animals; 2F: 1/20 animals)
2M, 2F	Thickening at I.S. of left scapula (2M: 1/20 animals; 2F: 4/20 animals)
2M, 2F	Thickening at I.S. of right scapula (2M: 1/20 animals; 2F: 2/20 animals)
1M, 2F	Dark area in the lung (1M: 2/20 animals; 2F: 1/20 animals)
1M, 1F, 2F	Dark area at the I.S. at the left scapula (1M: 1/20 animals; 1F: 1/10 animals, 2F: 1/20 animals)

(I.S.= Injection Site, Total number of animals per group was 20.)

Gross pathology on day 65

Groups	Findings
2M, 2F	Dark foci at the I.S. at the left scapula (2M: 1/20 animals; 2F: 3/20 animals)
2M, 2F	Mass at the I.S. at the left scapula (2M: 1/20 animals; 2F:3/20 animals)
2M, 2F	Nodule at the I.S. at the left scapula (2M: 3/20 animals; 2F: 5/20 animals)
1M, 2F	Scab at the I.S. at the left scapula (2M: 3 animals; 2F: 1 animal)
2M, 2F	Thickening at the I.S. at the left scapula (2M: 3/20 animals; 2F:4/20 animals)
1M, 1F, 2M, 2F	Dark area at the I.S. at the right scapula (1M: 3/20 animals; 2M: 2/20 animals; 1F: 2/20 animals; 2F: 2/20 animals)
2M, 2F	Mass at the I.S. at the right scapula (2M: 6/20 animal; 2F:7/20 animals)
2M, 2F	Nodule at the I.S. at the right scapula (2M: 1/20 animals; 2F: 3/20 animals)
1M, 2F	Scab at the I.S. at the right scapula (1M: 1/20 animals; 2F: 3/20 animals)
2M, 2F	Thickening at the I.S. at the right scapula (2M: 3/20 animals; 2F:4/20 animals)
1M, 2F	Dilatation of the pelvis of the kidney (2M: 1/20 animal; 2F: 1/20 animals)
2M, 2F	Dark area in the lung (2M: 1/20 animals; 2F: 2/20 animals)
1M, 1F, 2M, 2F	Enlargement of axillar lymph node (1M: 1/20 animals; 2M: 2/20 animals; 1F: 1/20 animal; 2F: 4/20 animals)
2M, 2F	Enlargement of mandibular lymph node (2M: 1/20 animals; 2F: 1/20 animal)
1F, 2F	Dark area at thymus (1F: 1/20 animals; 2 F: 3/20 animals)
1F, 2F	Dark foci at thymus (1F: 1/20 animals; 2 F: 2/20 animals)
1F, 2F	Thickening of the urinary bladder (1F: 1/20 animals; 2 F: 1/20 animals)
1M	dark foci in the lymph node (not specified which lymphnode) (1M: 1/20 animals)
2M	small epididymis (2M: 1/20 animals), dark area in the stomach(2M: 1/20 animals), enlarged testis (2M: 1/20 animals), small testis (2M: 1/20 animals).
1F	raised area in the liver (1F: 1/20 animals),, dark area in the lymph node (not specified which one) (1F: 2/20 animals), lost one lobe of the thyroid (1F: 1/20 animals).
2F	ulceration at the I.S. at the right scapula (2F: 1/20 animals), pale area in the liver (2F: 1/20 animals),

(I.S.= Injection Site, Total number of animals per group was 20.)

Microscopic finding day 23

Groups	Findings
2M	Two animals had a fold/rosette retina. (2M: 2 animals)
1M, 2M	Epicardial inflammation of the heart (1M: 1 animal; 2M: 2 animals).
2M, 1F	Crust at the I.S. of the left scapula (2M: 1 animal; 1F: 1 animal).
1M, 1F, 2M,	Inflammation of the dermis and epidermis at the I.S. of the left scapula (1M: 1 animal; 2M: 1 animal; 1F: 1 animal).
1M, 1F, 2M, 2F	Acute and subacute subcutaneous inflammation at the I.S. of the left scapula (1M: 1 animal; 2M: 16 animals; 1F: 2 animals; 2F: 16 animals).
1M, 1F, 2M, 2F	Chronic subcutaneous inflammation at the I.S. of the left scapula (1M: 1 animal; 2M: 1 animal; 1F: 1 animal; 2F: 1 animal).
2M, 2F	Subcutaneous degeneration and necrosis at the I.S. of the left scapula (2M: 13 animals; 2F: 15 animals).
1M, 1F, 2F	Hemorrhage at the I.S. of the left scapula (1M: 1 animal; 1F: 1 animal; 2F: 2 animals).
1M, 2M, 1F,	Hyperostosis of the femorotibial joint (1M: 1 animal; 2M: 1 animal; 1F: 2 animals).
2M	Fibrosis of the femorotibial joint (2M: 1 animal).
1M, 1F, 2M, 2F	Interstitial inflammation in the kidney (1M: 3 animals; 2M: 5 animals; 1F: 1 animal; 2F: 2 animals).
1M, 1F, 2M	Tubular basophile in the kidney (1M: 1 animal; 2M: 1 animal; 1F: 1 animal).
2F	Pyelitis/pyelonephritis in the kidney (2F: 1 animal).
1M, 1F, 2M, 2F	Extramedullar hematopoiesis (1M: 20 animals; 2M: 20 animals; 1F: 20 animals; 2F: 19 animals).
1M	Hyperplasia of the bronchial/bronchilar epithelium in the lung (1M: 1 animal).
1F, 2M, 2F	Erythrocytosis/hemorrhage in the mandibular lymph node (2M: 1 animal; 1F: 1 animal; 2F: 1 animal).
1M, 1F, 2M, 2F	Erythrocytosis/hemorrhage in the axillar lymph node (1M: 2 animals; 2M: 1 animal; 1F: 2 animals; 2F: 1 animal).
2M, 2F	Cytoplasmic granules (macrophages) in the axillar lymph node (2M: 2 animals; 2F: 1 animal).
1F, 2F	Partial follicular development in the ovary (1F: 20 animals; 2F: 17 animals).
1M, 2M	Partial glandular development in the prostate (1M: 19 animals; 2M: 20 animals).
1M, 2M	Partial glandular development of the seminal vesicle (1M: 20 animals; 2M: 19 animals).
2M	Capsular inflammation of the spleen (2M: 1 animal).
2M	Hemorrhage in the stomach (2M: 1 animal).
2F	Erosion of the glandular mucosa in the stomach (2F: 1 animal).
1M, 1F, 2M, 2F	Necrosis of the lymphoid tissue in the thymus (1M: 6 animals; 2M:

	6 animals; 1F: 5 animals; 2F: 2 animals).
1M, 2M	Hemorrhage in the thymus (1M: 1 animal; 2M: 3 animals).
1M, 2M	Hypo/aspermatogenesis (1M: 20 animals; 2M: 16 animals).
1F, 2F	Inflammation of the urinary bladder (1F: 1 animal; 2F: 1 animal).
1F, 2F	Partial glandular development of the uterus(2M: 20 animals; 2F: 19 animals).

(I.S.= Injection Site, Total number of animals per group was 20.)

Microscopic findings day 65

Groups	Findings
1F	Ventricle dilation in the brain in one animal.
1M, 2M,	Oligo/aspermia in the epididymis (1M: 2 animals; 2M: 1 animal)
1M	Two animals had a fold/rosette retina.
1M, 1F, 2M, 2F	Mineralization of the cornea (1M: 3 animals; 2M: 3 animals; 1F: 1 animal; 2F: 1 animal).
2M	One animal had fibrosis in the eye.
1M	Two animals had mineralization in the GALT.
1M	One animal had myocardial degeneration/necrosis.
1M, 2F	Crust at the I.S. of the left scapula (1M: 3 animals; 2F: 2 animals)
1M, 2F	Erosion at I.S. of the left scapula (1M: 1 animal; 2F: 1 animal)
1M, 2F,	Inflammation of the dermis and epidermis at the I.S. of the left scapula (1M: 2 animals; 2F: 2 animals)
2M, 2F	Acute and subacute subcutaneous inflammation at the I.S. of the left scapula (2M: 3 animals; 2F: 2 animals)
2M, 2F	Chronic subcutaneous inflammation at the I.S. of the left scapula (2M: 15 animals; 2F: 10 animals)
2M, 2F	Subcutaneous degeneration and necrosis at the I.S. of the left scapula (2M: 2 animals; 2F: 2 animals)
2M, 2F	Degeneration of the panniculus muscle at the I.S. of the left scapula (2M: 1 animal; 2F: 3 animals)
2M, 2F	Hemorrhage at the I.S. of the left scapula (2M: 2 animals; 2F: 3 animals)
1M, 2F	Crust at the I.S. of the right scapula (1M: 1 animal; 2F: 1 animal)
1M, 2M, 2F,	Inflammation of the dermis and epidermis at the I.S. of the right scapula (1M: 1 animal; 2M: 1 animal; 2F: 4 animals)
2M, 2F	Acute and subacute subcutaneous inflammation at the I.S. of the right scapula (2M: 9 animals; 2F: 6 animals)
1M, 2M, 2F	Chronic subcutaneous inflammation at the I.S. of the right scapula (1M: 1 animal; 2M: 7 animals; 2F: 10 animals)
2M, 2F	Subcutaneous degeneration and necrosis at the I.S. of the right scapula (2M: 9 animals; 2F: 8 animals)
1M, 2M, 2F	Degeneration of the panniculus muscle at the I.S. of the right scapula (1M: 1 animal, 2M: 3 animals; 2F: 3 animals)
1M, 2M, 2F	Hemorrhage at the I.S. of the right scapula (1M: 1 animal; 2M: 6 animals; 2F: 1 animal)

2F	Erosion at the I.S. of the right scapula (2F: 1 animal)
2F	Ulceration at the I.S. of the right scapula (2F: 3 animals)
1M, 1F, 2M	Interstitial inflammation in the kidney (1M: 3 animals; 2M: 1 animal; 1F: 2 animals)
1M, 2M, 2F	Tubular basophile in the kidney (1M: 2 animals; 2M: 1 animal; 2F: 2 animals)
1M, 2F	Dilatation of the kidney pelvis (1M: 1 animal; 2F: 1 animal)
1F, 2F	Pyelitis/pyelonephritis in the kidney (1F: 2 animals; 2F: 2 animals)
1M, 2M	Hyaline tubular droplet in the kidney (1M: 1 animal, 2M: 1 animal)
1M, 2M	Extramedullar hematopoiesis in the liver (1M: 5 animals; 2M: 4 animals)
1M, 1F	Necrosis in the liver (1M: 2 animals; 1F: 1 animal)
1M	Capsular inflammation of the liver (1M: 1 animal)
2M	Bronchial hyperplasia of the epithelium (2M: 1 animal)
1M, 2M	Histocytosis in the lung (1M: 1 animal; 2M: 2 animals)
1F, 2M, 2F	Hemorrhage in the lung (2M: 2 animals; 1F: 1 animal; 2F: 2 animals)
1M, 1F	Erythrocytosis/hemorrhage in lymph node (not specified which one) (1M: 1 animal; 1F: 2 animals)
2M, 2F	Cytoplasmic granules (macrophages) in the axillar lymph node (2M: 3 animals; 2F: 8 animals)
1F	Erythrocytosis/hemorrhage in the axillar lymph node (1F: 2 animals)
2F	Sinusal dilatation without congestion in the axillar lymph node (2F: 1 animal)
2M	Lymphoid hyperplasia in the mandibular lymph node (2M: 1 animal)
1M, 2F	Erythrocytosis/hemorrhage in the mesenteric lymph node (1M: 1 animal; 2F: 1 animal)
1M	Pituitary cyst (1M: 1 animal)
2M	Inflammation of the prostate (2M: 1 animal)
1F	Crust at the skin (1F: 1 animal)
1M	Squamous cyst at the lumbar spinal cord (1M: 1 animal)
2M	Hemorrhage in the stomach (2M: 1 animal)
1M	Mineralization in the stomach (1M: 1 animal)
2M, 1F, 2F	Inflammation of the stomach (2M: 4 animals; 1F: 1 animal; 2F: 1 animal)
1M, 1F, 2M, 2F	Hemorrhage in the thymus (1M: 1 animal; 2M: 1 animal; 1F: 2 animals, 2F: 5 animals)
1M, 2M	Hypo/aspermatogenesis in the testis (1M: 1 animal; 2M: 1 animal)
1M, 2M	Tubular dilatation in the testis (1M: 1 animal; 2M: 1 animal)
2M	Single cell necrosis in the testis (2M: 1 animal)
1F, 2F	Inflammation of the urinary bladder (1F: 2 animal; 2F: 2 animals)

(I.S.= Injection Site, Total number of animals per group was 20.)

A large number of different tissues were examined for histology. Findings related to the brain, lung, liver, kidney, heart and eye were rare and evenly distributed between treated and control groups. Findings related to the testis, epididymis, prostate, ovary and uterus were related to the young age of the animals and were equally distributed between treated and control groups of animals. The following observations were made in isolated animals (of 20/group/sex) from the 13vPnc group but not the saline control group: fold/rosette retina fibrosis of the femorotibial joint, pyelitis/pyelonephritis in the kidney capsular, inflammation of the spleen, hemorrhage in the stomach, erosion of the glandular mucosa in the stomach on day 23 and fibrosis in the eye, inflammation of the prostate, hemorrhage in the stomach, single cell necrosis in the testis on day 65.

Body temperature: not evaluated

Local toxicity:

Findings at the injection site: In the treatment group, on day 23, an acute inflammation with degeneration and necrosis was observed which developed into a chronic inflammation with decreased degeneration and necrosis on day 65.

Dark areas, a sign of trauma and irritation, were observed at the side of injection on day 23 and 65 in the group receiving the vaccine. Microscopic subcutaneous acute and sub-acute inflammation (♂: 16/20; ♀:16/20) combined with degeneration and necrosis (♂: 13/20; ♀:15/20) was found at the side of injection on day 23 in the treatment group, while the control group showed almost no inflammation (♂: 1/20; ♀:2/20) and degeneration (♂: 0/20; ♀:0/20). On day 65 microscopic acute/sub-acute inflammation (♂: 9/20; ♀:6/20) and degeneration/necrosis (♂: 9/20; ♀:8/20) persisted in the treatment group, moreover, the inflammation spread into the epidermis (♂: 9/20; ♀:8/20) and developed into a chronic inflammation (♂: 7/20; ♀:10/20). Furthermore, degeneration of the panniculus muscle (♂: 3/20; ♀:3/20), subcutaneous hemorrhages (♂: 6/20) and ulceration (♀: 3/20) were found at the side of injection in treated animals.

Draize scoring:

Edema/Erythema, 4h post injection

Treatment group	4h post injection																								
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
control	8	0				8	0				4	0				4	0				4	0			
13vPnC	8	0				8	0				8	0				3	3				3	1	8	1	

Serotype 7F	2672	2090	24551	32032
Serotype 9V cont.	474	559	378	549
Serotype 9V	2076	1531	80218	84973
Serotype 14 cont.	107	69	76	207
Serotype 14	334	340	402	314
Serotype 18 C cont.	50	50	108	82
Serotype 18 C	252	200	6112	8631
Serotype 19A cont.	2299	2858	1229	1345
Serotype 19A	2705	1932	47427	34617
Serotype 19F cont.	646	904	312	147
Serotype 19F	2278	1515	5707	3945
Serotype 23F cont.	519	773	577	665
Serotype 23F	4602	2101	21414	24237

GMT: geometric mean titer

Serology analysis

	GMT (males & females): day 23 (U/ml)	GMT (males & females): day 65 (U/ml)
Serotype 1 cont.	417	400
Serotype 1	2414	7510
Serotype 3 cont.	895	2134 (one very high BG)
Serotype 3	2171	2325
Serotype 4 cont.	300	246
Serotype 4	1481	3657
Serotype 5 cont.	1133	781
Serotype 5	3302	26050
Serotype 6A cont.	2661	792
Serotype 6A	4286	13608
Serotype 6B cont.	881	709
Serotype 6B	3503	10166
Serotype 7F cont.	405	651
Serotype 7F	2381	28292
Serotype 9V cont.	517	414
Serotype 9V	1804	82596
Serotype 14 cont.	88	142
Serotype 14	184	358
Serotype 18 C cont.	50	95
Serotype 18 C	226	7371
Serotype 19A cont.	2579	1287
Serotype 19A	2319	41022

Serotype 19F cont.	775	230
Serotype 19F	1897	4826
Serotype 23F cont.	682	621
Serotype 23F	3352	22826

GMT: geometric mean titer, bolded letters for serotype 3: only a small difference between the antigen titer of the control and the treatment group.

Serology analysis

	day 23: animals* with no titer	day 65: animals* with no titer
Serotype 1	1	0
Serotype 3	4	2
Serotype 4	2	6
Serotype 5	3	0
Serotype 6A	7	0
Serotype 6B	2	0
Serotype 7F	0	0
Serotype 9V	2	0
Serotype 14	6	2
Serotype 18 C	10	0
Serotype 19A	9	0
Serotype 19F	2	0
Serotype 23F	0	0

(*): out of 20 animals

Assessment:

This study was the only juvenile toxicology study performed for this BLA. Given the fact that Prevnar13 is intended mostly for use in infants and toddlers this study is of great relevance. The animals used in the toxicity study were juvenile rats which received their first injection on day 7 after birth. It is difficult to correlate this age to the corresponding age in humans. However it has been described that the immune system of newborn rats is similar to a human fetus in the second trimester. A 21 day old rat is similar to a 2 year old toddler. Therefore, the choice of 7 day old rats seems appropriate. The route of administration (RoA) was subcutaneous which is different from the clinically proposed RoA. After subcutaneous injection the vaccines encounters a higher number of antigen presenting cells, the density of the antigen tends to be higher and a stronger local response is observed. Several clinical reports comparing the intramuscular with the subcutaneous routs of injection described that the intramuscular route showed less local irritation and toxicity; however, it lead to a immunological response measured by antibody titers as least as good as the subcutaneous route

(Mark A. et al., Vaccine, 1998; Cook IF, Vaccine, 2007; Petousis-harris H., vaccine, 2008). Therefore, the subcutaneous injection in the animal experiment, although it is different from the clinically proposed RoA, may present a worse-case-scenario in regard to local toxicity. In this study, the animals received 5 doses of subcutaneous injections every two weeks, after 23 days and 65 days pathology, serology as well hematology and clinical chemistry were performed. The last dose of vaccine was administered on day 63. Notably, only a two day recovery period was allowed prior to last sample collection. A longer recovery period, e.g., 4 weeks would have been more appropriate to evaluate potential reversion of local toxicity.

Microscopically pathological findings of the intestinal system were noted and were as follows: a hemorrhage of the stomach was detected in one male animal on day 23 and on day 65 and an erosion of the glandular mucosa in the stomach was observed in one female animal on day 23 assigned to the treatment group, none of these findings were observed in the saline control group. Additionally, an inflammation of the stomach was observed in four male animals and one female animal of the treatment group, but only in one animal of the control group. These findings were of interest, as similar observations were also observed in two other studies (study number 501297 and 6617-282A). Apart from these findings no adverse gross or microscopic alteration that could be indicative of treatment-related systemic or local toxicity were observed.

The Draize score (max 2) showed only a moderate edema and erythema at the side of injection in the treatment group. However, histological evaluations revealed microscopic acute and subacute subcutaneous inflammation with degeneration and necrosis at the site of injection in a significant number of animals which did not resolve at the latest evaluated time point. At day 65 acute/sub-acute inflammation and degeneration/necrosis could still be found in the treatment group. In addition, inflammation spread into the epidermis and developed into a chronic inflammation. The last sample at day 65 was taken only 2 days after the animal received the last injection, the animal only had a very short recovery phase. It would have been helpful to have allowed a later time point in order to see a potential reversion of toxicity. Of note, the injection interval in the juvenile study was every 2 weeks for 5 times with a volume of 0.5ml/ animal (except at PND7, where the volume was 0.15ml) which can be considered a substantial stress in these young animals. This may explain the observed localized trauma and inflammation. Furthermore, the compressed dosing schedule might further explain localized trauma and inflammation. Also, subcutaneous vaccine administration in general, has been shown to induce a stronger local reaction compared to intramuscular injection which may further explain the local toxicity.

Serology performed in this study verified that a dose was administered that induced an antibody response, as most animals had a positive serum IgG antibody response to each of the 13 serotypes of 13vPnC after 5 doses. However, for serotype 3 (2 out of 20 animals did not show seroconversion), serotype 4 (6 out of 20 animals did not show seroconversion) and serotype 14 (2 out of 20 animals did not show seroconversion) not all animals showed seroconversion. No differences were found between female and male animals.

Title: 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC): THIRTEEN WEEK (1 DOSE/2 WEEKS) SUBCUTANEOUS TOXICITY STUDY IN MONKEYS (PROTOCOL 05_0331)

Study number: 501297

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

Study initiation date: not given

Final Report date: 20.06.2007

Test article batch/lot: lot #7-5064-001A

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

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

Number of animal per group and sex: 6

Age: 2.5 - 3.5 years

Body weight range: males 2.1 to 2.9 kg; females 1.8 to 2.3 kg

Route and site of administration: subcutaneous injection

Volume of injection: 0.5ml

Frequency of administration and study duration: a total of 7 injections were administered on days 1, 15, 29, 43, 57, 71 and 85 followed by a 4 week recovery phase.

Dose: 30.8 µg polysaccharide, 29 µg CRM197, 0.125 mg aluminum phosphate/0.5 mL

Stability: Stability studies were performed by the sponsor on the same batches of vaccine and adjuvant control as used in this study. The final drug product was demonstrated to be stable over a 12-month period (App. 4).

Means of administration: needle and syringe

Report status: final

Experimental study design

Group*	Treatment	Number of Animals (#/sex/group)	
		Treatment phase: Necropsy day 87	Recovery phase: Necropsy day 115
1	0.9% Sodium Chloride (b)(4)/0.5ml	Male:3 Female: 3	Male:3 Female: 3
2	30.8 µg polysaccharide, 29 µg CRM197, 0.125 mg aluminum phosphate/0.5 mL.	Male:3 Female: 3	Male:3 Female: 3

*Dose was delivered subcutaneously on day 1, 15, 29, 43, 57, 71 and 85.

Methods: No details are given regarding the methods used for hematology and clinical chemistry.

Randomization procedure: Approximately 2 weeks prior to initiation of dosing, all animals were weighed and randomly assigned to dose groups using a computer-based randomization procedure. Randomization was by stratification using body weight as the parameter. Males and females were randomized separately.

Statistical analysis plan: For each parameter of interest, if none of the group variances is equal to zero, the group variances were tested for homogeneity using the folded form of the *F*

statistic (computed from the ratio of the group variances). When group variances were not found to be heterogeneous ($p > 0.05$), a two-sided t -test was used to compare both group means. Whenever group variances were found to be heterogeneous ($p \leq 0.05$), the Satterthwaite's approximation was used when performing the two-sided t -test. If one of the group variances is equal to zero, the group comparison was done using the exact Wilcoxon rank-sum test.

Clinical observations: The following parameters were evaluated: clinical signs (animals were examined twice daily following transfer and allocation to the study (except on day of arrival and necropsy) for mortality and signs of ill health or reaction to treatment. A complete detailed physical examination was also performed weekly on all study animals commencing on day - 8.), skin reactions at the subcutaneous site of injection (The injection site was observed for redness and swelling predose, and approximately 4 and 24 hours postdose on each dosing day and daily for 6 days following each dose administration. If signs of irritation were present on the 6th day after each dose, evaluations continued daily for the animal until no signs of irritation were present. The injection site was also evaluated on the day of necropsy), body weights (weekly, commencing on day -8 and day of necropsy), food consumption (daily), body temperature (each day of dosing: prior to dose administration and at 4 and 24 hrs post dose), ophthalmoscopy (once prior to the start of dosing, and during week 12 and week 16 of treatment animals were subjected to fundoscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations, hematology and clinical chemistry (prior to dosing: day -17 and day -7 and on days 3, 31, 74 and 112), gross anatomy at termination, organ weights and histopathology on a selection of tissues. Blood samples for antibody-determination were taken and analyzed on days -7, 87 (main and recovery animals) and 115 (recovery animals) (non-GLP) under the responsibility of the sponsor.

Clinical observations

Parameters	Frequency of Testing
Cageside observation ¹	Twice daily
Clinical observations ²	Weekly commencing day -8
Body weight	Weekly commencing day -8
Food consumption	daily
Body temperature	prior to dose administration and at 4 and 24 hrs post dose
Ophthalmologic exam	Once prior to the start of dosing, and during week 12 and week 16 of treatment
Clinical chemistry*	day -17 and day -7 and on days 3, 31, 74 and 112, femoral vein
Hematology*	day -17 and day -7 and on days 3, 31, 74 and 112, femoral vein
Coagulation*	NC
Immunological response	day -7, 87, 115
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	4 and 24 hours postdose, daily for at least 6 days until no signs of irritation were present,

Necropsy	Day 87 and 115
Tissues for histopathology	Day 87 and 115

*(blood collection site: femoral vein); NC: not collected

¹ Cageside observations include mortality, morbidity, general health and signs of toxicity.

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

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

Histology evaluation

Organ/Tissue	Collected	Not collected
Adrenal glands	!	
Aorta	!	
Bone and joint (distal femur)	!	
Bone marrow (rib)	!	
Bone (sternum & femur)		
Bone marrow (sternum & femur)		
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	!	
cecum	!	
Cervix		
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		
Gall bladder	!	
Gross lesions (if any)		
Harderian gland (if applicable)		
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		
Larynx		
Liver	!*	
Lung (main-stem;	!	

bronchi)		
Lymph nodes (cervical)		
Lymph nodes (mandibular, unilateral)	!	
Lymph nodes (axillar)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		
Optic nerves	!	
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)	!	
Pituitary gland	!*	
Prostate	!*	
Rectum		
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicles	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!	
Stomach (squamous and glandular)	!	
Testes	!*	
Thymus	!	
Thyroid (w/ parathyroid glands)	!*	
Tongue	!	
Trachea	!	
Ureters		
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		

Table of Histology – Tissues examined: All dose groups

(*) Organ weight was determined

(!) Histology was performed

Treatment and control group were examined on day 85 and 115.

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

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM	Females: amyl: higher in the saline group: 1.7	Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase ND Total bile acids
	Males: DBIL: higher in treatment group: 2 nd pretreatment: 1.63; day 112: 2.5; IBIL: higher in treatment group: day 112: 1.6.	Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein: ND Fibrinogen: ND
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Males: LIPA: higher in saline group (only in one case): 2 nd pretreatment: 4.2 Females: LIPA; higher in saline group: day 74: 3.6 Cholinesterase: ND Creatine kinase: ND	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase Fasting triglycerides

Hematology

MEASURE MENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS	<p>Males: Reticulocytes %: higher in the saline group: day 112: 1.7. Reticulocytes #: higher in the saline group: day 112: 1.7.</p> <p>Females: Reticulocytes %: higher in the saline group: day 31: 1.9, day 112: 2.1. Reticulocytes #: higher in the saline group: day 31: 1.9; day 112: 2.0</p>	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean corp. Hb. (MCH) Mean corp. Hb. Conc. (MCHC), Mean corp. Volume (MCV) Total erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	<p>Males: Neutrophile % is always higher in the treatment group compared to the saline group (day 3: 1.8, day 112: 2.1). Neutrophile count: higher in treatment group: day 112: 1.9. Lymphocyte count: higher in saline group: 1.pretreatment: 1.9; 2.pretreatment: 1.8; day3: 1.6; day 31: 1.4; day 74: 1.6; day 112: 1.9. Monocytes %: higher in the saline group: day 112: 1.6. Monocytes #: higher in saline control group: 2nd pre-treatment: 2.2; day 3: 1.6, day 112: 1.7. Eosinophiles %: higher in the treatment group: day 31: 1.9; day Basophiles #: higher in saline control group: 2nd pre-treatment: 1.76; day 3: 1.8, day 31: 2.0, day 74: day 112: 2.1. Leukocytes #: higher in the saline group: 1st pre-treatment: 1.7 2nd pre-treatment: 2.2; day 3: 1.9; day 74: 1.7; day 112: 1.8.</p> <p>Females: Neutrophile count: higher in treatment group 1st pretreatment: 2.2. Eosinophiles %: higher in the saline group: 1st pretreatment: 5, 2nd pretreatment: 1.9. Eosinophiles #: higher in the saline group: 1st pretreatment: 4.4, Basophiles #: higher in treatment group: 2nd pre-treatment: 1.8.</p>	Basophils, Eosinophils count lymphocyte count Macrophage/Monocyte count Neutrophil count Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL	<p>Males: Platelet counts always lower in treatment group (saline around 1.2 times higher)</p>	Activated partial- thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Systemic toxicity:

No treatment-related mortality and no toxicologically relevant changes in body weight (gain), relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, clinical chemistry, gross anatomy or organ weight were found.

There were several incidences of differences in the clinical chemistry and hematology parameters which showed a fold change equal or higher than 1.5 in the treatment groups compared to the control group. Most of the chemical and hematological values were in the normal range or elevations were isolated events. However, in males of the saline control group the lymphocyte count ($8.7-10.4 \times 10^3/\mu\text{l}$; normal range: $4.4-7.2 \times 10^3/\mu\text{l}$) and the basophile count ($0.098 - 0.158 \times 10^3/\mu\text{l}$; normal range: $0-0.084 \times 10^3/\mu\text{l}$) were significantly elevated.

There were no treatment-related effects on organ weights or weight ratios for males or females detected.

An incidence of liquid/soft feces was noted in some animals during the dosing and recovery periods. For some animals, following veterinary examination, these clinical signs were considered due to the presence of *Giardia* and were not treatment related.

Organ Weight

GROUPS		MALES				FEMALES			
		Saline; day 87	Treatment; day 87	Saline; day 115	Treatment; day 87	Saline; day 87	Treatment; day 87	Saline; day 115	Treatment; day 87
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
BODY WEIGHT (gram) ^a		2830	2830	2870	2800	2070	2100	2200	2070
BRAIN									
Absolute Weight ^a	gram	68.0493	69.6870	63.1350	69.7817	55.6090	59.8173	59.4373	59.4397
Per Body Weight ^a	%	2.40200	2.57184	2.19921	2.53719	2.69800	2.86305	2.72162	2.88539
ADRENALS									
Absolute Weight ^a	gram	0.40727	0.40793	0.40803	0.37830	0.34467	0.42737	0.39173	0.36313
Per Body Weight ^a	%	0.01446	0.01498	0.01420	0.01359	0.01649	0.02079	0.01799	0.01776
Per Brain Weight ^a	%	0.60162	0.58541	0.64504	0.55278	0.61381	0.72672	0.66005	0.61155
EPIDIDYMIDES: ND									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
HEART									
Absolute Weight ^a	gram	10.9883	12.0250	12.3830	12.4893	8.8177	9.0380	8.4750	7.6793
Per Body Weight ^a	%	0.39105	0.41940	0.43132	0.44833	0.42602	0.43063	0.38368	0.37300
Per Brain Weight ^a	%	16.28472	17.26373	19.61088	18.25836	15.77678	15.22900	14.22959	12.91749
KIDNEYS									
Absolute Weight ^a	gram	12.2130	11.1507	11.1710	12.3253	10.2873	9.5260	9.3163	9.2250
Per Body Weight ^a	%	0.43428	0.39588	0.38898	0.44569	0.50593	0.45431	0.42845	0.44503

GROUPS		MALES				FEMALES			
		Saline; day 87	Treatment; day 87	Saline; day 115	Treatment; day 87	Saline; day 87	Treatment; day 87	Saline; day 115	Treatment; day 87
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
Per Brain Weight ^a	%	18.07223	16.00620	17.64924	17.72321	18.67052	16.00146	15.69478	15.50834
LIVER									
Absolute Weight ^a	gram	58.0500	60.3943	60.2637	59.0357	47.8030	52.1863	52.8853	44.8533
Per Body Weight ^a	%	2.05871	2.16506	2.10752	2.11600	2.32372	2.47819	2.41639	2.16827
Per Brain Weight ^a	%	85.66006	86.68615	95.96975	86.29475	86.07549	87.62655	88.85795	75.43213
SPLEEN: ND									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
TESTES									
Absolute Weight ^a	gram	4.8560	2.0933	3.5830	4.4873				
Per Body Weight ^a	%	0.17137	0.06885	0.12252	0.14884				
Per Brain Weight ^a	%	7.16899	3.00668	5.39552	7.10006				
Prostate									
Absolute Weight ^a	gram	0.7033	0.4050	0.4043	0.4547				
Per Body Weight	%	0.02454	0.01387	0.01418	0.01571				
Per Brain Weight ^a	%	1.02594	0.58152	0.63965	0.69822				
THYROID and PARATHYROID									
Absolute Weight ^a	gram	0.34313	0.36227	0.30730	0.45830	0.22783	0.25233	0.26877	0.31270
Per Body Weight ^a	%	0.01227	0.01275	0.01073	0.01630	0.01097	0.01215	0.01238	0.01496
Per Brain Weight ^a	%	0.51081	0.52005	0.48688	0.68896	0.40809	0.42946	0.45348	0.52415
THYMUS: NC									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
OVARIES						0.27383	0.17237	0.17527	0.25410
Absolute Weight ^a	gram					0.01346	0.00821	0.00810	0.01248
Per Body Weight ^a	%					0.49384	0.28085	0.29579	0.42883
Per Brain Weight ^a	%								
UTERUS: ND									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
Pituitary									
Absolute Weight ^a	gram	0.04210	0.03063	0.04060	0.04717	0.04220	0.04090	0.04643	0.03847
Per Body Weight ^a	%	0.00148	0.00113	0.00141	0.00169	0.00206	0.00192	0.00214	0.00187
Per Brain Weight ^a	%	0.06154	0.04396	0.06414	0.06856	0.07624	0.06724	0.07827	0.06474

Table of organ weight and their normalization. Absolute weights are expressed as mean(grams) ± standard deviation (sd).

*different from controls at P≤0.05;

**different from controls at P≤0.01. (ND: not determined)

No significant differences in the organ weight between control group and treatment groups could be determined, except in testis, prostate and ovary which were increased in the saline control group on day 87. However, on day 115 none of these differences were observed. On day 115 the thyroid/parathyroid was detected to be 1.5 times increased in the treatment group compared to the control group.

Gross Pathology:

Gross Pathology group identification:

M1: male saline control group

M2: male treatment group

F1: female saline control group

F2: female treatment group

Gross Pathology, day 87

Group	Findings
1M, 2M	Epididymis: small (1M: 1 animal; 2M: 2 animals)
2M	Left lumbar I.S.: dark area (2M: 1 animal)
2M	Right lumbar I.S. : thickening (2M: 1 animal)
2M	Left scapular I.S.: nodule (2M: 1 animal)
2M, 2F	Left scapular I.S: thickening (2M: 2 animals; 2F: 2 animals)
2F	Left scapular I.S: dark area (2F: 1 animal)
2F	Left scapular I.S.: swelling (2F: 2 animals)
2M	Right lumbar I.S.: dark area (2M: 1 animal)
2F	Left dorsal thorax I.S.: swelling (2F: 2 animals)
1F	Colon: nodule (1F: 1 animal)
1F	kidney: pale foci (1F: 1 animal)
1M	Lung: adhesion (2M: 1 animal)
2M, 1F	Lung: thickening (2M: 1 animal, 1F: 1 animal)
1M, 2M	Prostate: small (1M: 1 animal; 2M: 2 animals)
1M, 2M	Seminal vesicle: small (1M: 1 animal; 2M: 2 animals)
1F, 2F	Spleen: enlargement (1F: 1 animal, 2F: 2 animals)
1M	Stomach: pale area (2M: 1 animal)
2F	Subcutaneous tissue: mass not detected (2F: 2 animals)
1M, 2M	Testis: small (1M: 1 animal; 2M: 2 animals)
1M	Thymus: dark foci (1M: 1 animal)
2M	Thyroid: raised area (2M: 1 animal)
2M	Thyroid: cyst (2M: 1 animal)
2F	Urinary bladder: raised area (2F: 1 animal)

I.S.: Injection Site; Total number of animals per group and sex was 3.

Gross Pathology, day 115

Group	Findings
1F	Duodenum: pale areas (1F: 1 animal)
1M, 2M	Epididymis: small (1M: 2 animals; 2M: 2 animals)
1M	Fat: cyst (1M: 1 animal)
1F	Right dorsal thorax I.S.: swelling (1F: 1 animal)
1F, 2F	Right lumbar I.S.: dark area (1F: 1 animal; 2F: 1 animal)
1F	kidney: pale foci (1F: 1 animal)
2F	Liver: area raised (2F: 1 animal)
2M	Liver: area depressed (2M: 1 animal)
1M	Lung: adhesion (1M: 1 animal)
1F, 2F	Lung.: dark area (1F: 1 animal; 2F: 1 animal)
1F	Lung: spongy (1F: 1 animal)
1F	Lymph node: enlargement (1F: 1 animal)
1F	Lymph node axillar: enlargement (1F: 1 animal)
2F	Ovary: dark area (2F: 1 animal)
1M, 2M	Prostate: small (1M: 1 animal; 2M: 2 animals)
1M, 2M	Seminal vesicle: small (1M: 1 animal; 2M: 1 animal)
2M	Spleen: area depressed (2M: 1 animal)
1M, 1F	Spleen: enlargement (1M: 2 animals, 1F: 1 animal)
1M, 2M	Testis: small (1M: 2 animals; 2M: 2 animals)
2M, 1F	Thymus: dark foci (2M: 1 animal, 1F: 1 animal)
2M	Thyroid: enlargement (2M: 2 animals)

Total number of animals per group and sex was 3

Microscopic findings**Microscopic findings group identification:**

M1: male saline control group

M2: male treatment group

F1: female saline control group

F2: female treatment group

Microscopic findings, day 87

Groups	Findings
1M	Adrenal: focal cortical hypertrophy (1M: 1 animal)
1F	Colon: granuloma (1M: 1 animal)
1M, 2M	Epididymis: oligo/aspermia (1M: 2 animals, 2M: 3 animals)
2M, 2F	Left dorsal thorax I.S.: inflammation (2M: 2 animals; 2F: 2 animals)
2M	Left lumbar I.S.: inflammation (2M: 2 animals)
2F	Right lumbar I.S.: inflammation (2F: 1 animal)

2M	Right lumbar I.S.: hemorrhage (2M: 1 animal)
2M, 2F	Left scapular I.S.: inflammation (2M: 2 animals, 2F: 3 animals)
1M, 2M, 1F, 2F	Kidney: interstitial inflammation (1M: 1 animal, 2M: 1 animal, 1F: 1 animal, 2F: 2 animals)
2M, 1F	Lung: fibrosis of the pleura (2M: 1 animal, 1F: 1 animal)
1M, 1F	Lymph node axillar: erythrocytosis/hemorrhage (1M: 1 animal, 1F: 1 animal)
2F	Lymph node axillar: macrophages (2F: 1 animal)
2M	Pancreas: ectopic splenic tissue (2M: 1 animal)
1M, 2M	Prostate: partial glandular development (1M: 3 animals, 2M: 3 animals)
1M, 2M	Seminal vesicle: partial glandular development (1M: 3 animals, 2M: 3 animals)
2M, 2F	Thyroid cyst (2M: 1 animal, 2F: 2 animals)
1M, 2M	Testis: hypo/asprematogenesis (1M: 2 animals, 2M: 3 animals)
1M	Thymus: hemorrhage (1M: 1 animal)

Total number of animals per group and sex was 3.

Microscopic findings, day 115

Groups	Findings
1M, 2M	Epididymis: oligo/aspermia (1M: 3 animals, 2M: 3 animals)
1M	Fat: cyst (1M: 1 animal)
2F	Left dorsal thorax I.S.: inflammation (2F: 1 animal)
2F	Left lumbar I.S.: inflammation (2F: 1 animal)
1F, 2F	Right lumbar I.S.: hemorrhage (1F: 1 animal, 2F: 1 animal)
2M, 2F	Left scapular I.S.: inflammation (2M: 1 animal; 2F: 2 animals)
1M, 2M, 1F, 2F	Kidney: interstitial inflammation (1M: 3 animals, 2M: 3 animals, 1F: 1 animal, 2F: 1 animal)
2F	Liver: necrosis (2F: 1 animal) (multifocal, grade 1)
2F	Liver: capsular fibrosis (2F: 1 animal) (multifocal, grade 1) different animal
1M	Lung: fibrosis of the pleura (1M: 1 animal)
1F	Lung: infiltration of mononuclear cells (1F: 1 animal)
2F	Lung: histocytosis (2F: 1 animal)
2F	Lung: hemorrhage (2F: 1 animal)
2M	Lymph node axillar: macrophages (2M: 1 animal)
2F	Lymph node axillar: erythrocytosis/hemorrhage (2F: 1 animal)
2M	Muscle skeletal: infiltration of mixed cells (2M: 1 animal)
1F	Pancreas: congestion (1F: 1 animal)
1M, 2M	Prostate: partial glandular development (1M: 3 animals, 2M: 3 animals)
1M, 2M	Seminal vesicle: partial glandular development (1M: 3 animals, 2M: 3 animals)
1M, 2M	Testis: hypo/asprematogenesis (1M: 3 animals, 2M: 3 animals)

2M	Testis: infiltration of mononuclear cells (2M: 1 animal)
2M, 1F	Thymus: hemorrhage (2M: 1 animal, 1F: 1 animal)
2M	Thyroid cyst (1M: 1 animal)
1F	Trachea: infiltration of mononuclear cells (1F: 1 animal)

Total number of animals per group and sex was 3.

A large number of different tissues was examined for histology. Findings related to the colon, adrenal, lung, liver, pancreas, thyroid, thymus, trachea and fat were isolated cases and/or evenly distributed between treated and control groups. Findings in the testis, prostate and seminal vesicle were related to the young age of the animals and were equally distributed between treated and control groups. Interstitial inflammation in the kidney was observed in several animals on day 87 and 115 in the control as well as the treatment group. Findings specific to the treatment group were found at the injection site; on days 87 and 115 inflammation and hemorrhage was found at the injection site.

On day 115 in the female treatment group grade 1 necrosis and capsular fibrosis of the liver as well as histiocytosis and hemorrhage in the lung was found (each in one animal). In the male treatment group, infiltration of mononuclear cells in the testis as well as a thyroid cyst was found (each in one animal).

Body temperature

Group	Males	Females
Control		
1	0	0
2	0	0

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

Local toxicity:

At the sites of injection dark area, swelling, thickening and nodule were observed on day 87, in particular in the treatment group. Most of these signs resolved by day 115, except a dark area in two animals at one site.

Draize scoring:

Edema/Erythema, 4h post injection

Treatment group																				
	1 st dose					2 nd dose					3 rd dose					4 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
control	1					1					1					1				
	2					2					2					2				
13vPnC	1					1	1				1					1	1			
	2					1					2					1				

Edema/Erythema, 4h post injection

Treatment group																				
	5 th dose					6 th dose					7 th dose									
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
control	1	1				1					1	2								
	1					2					0									
13vPnC	1					1					1	1								
	2					2					1									

Edema/Erythema, 24h post injection

Treatment group																				
	1 st dose					2 nd dose					3 rd dose					4 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
control	1	1				1					1					1				
	1					2					2					2				
13vPnC	1	2				1	1	1			1	1				1		2		
	0					0					1					0				

Edema/Erythema, 24h post injection

Treatment group																				
	5 th dose					6 th dose					7 th dose									
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
control	1					1					1	1								
	2					2					1									
13vPnC	1	1				9	2	1			8	4								
	1																			

Draize scoring of the injection sites revealed irritation occurring with increased frequency and slightly increased severity in the treated animals compared to control animals.

Histological inflammation was observed at the site of injection in the treatment group, but not in the control group. On day 87 and 115 inflammation was observed in the treatment group; however the number of animals showing inflammation decreased from day 87 to day 115.

Serology:

Serum was collected on study day -7 and 2 days after the final injection from all animals (day 87) and after a 30 day recovery period (day 115) from 3 animals/sex/group for analysis for IgG antibodies to 13 pneumococcal serotypes contained in the 13vPnC formulation and to the CRM197 carrier protein. The assays used were -----(b)(4)-----
-----.

Serology analysis

	GMT (males): day -7 (U/ml)	GMT (females): day -7 (U/ml)	GMT (males): day 87 (U/ml)	GMT (females): day 87 (U/ml)	GMT (males): day 115 (U/ml)	GMT (females): day 115 (U/ml)
Serotype 1 cont.	0.03	0.03	0.02	0.04	0.01	0.01
Serotype 1	0.02	0.04	3.40	7.25	1.90	2.70
Serotype 3 cont.	0.27	0.15	0.20	0.11	0.28	0.07
Serotype 3	0.12	0.15	2.45	4.20	1.10	3.20
Serotype 4 cont.	0.02	0.02	0.21	0.21	0.30	0.01
Serotype 4	0.01	0.01	3.80	6.30	1.60	3.30
Serotype 5 cont.	0.21	0.18	0.25	0.37	0.18	0.19
Serotype 5	0.46	0.18	7.80	12.3	4.60	5.30
Serotype 6A cont.	0.09	0.02	0.24	0.12	0.07	0.04
Serotype 6A	0.02	0.18	4.35	12.4	4.70	5.30
Serotype 6B cont.	0.05	0.04	0.06	0.05	0.02	0.05
Serotype 6B	0.03	0.07	11.6	19.8	8.70	14.8
Serotype 7F cont.	0.32	0.25	0.18	0.18	0.27	0.10
Serotype 7F	0.23	0.17	4.90	7.00	3.03	2.63
Serotype 9V cont.	0.02	0.47	0.03	0.35	0.03	0.07
Serotype 9V	0.02	0.05	3.15	5.37	1.52	3.68
Serotype 14 cont.	0.04	0.13	0.05	0.13	0.04	0.35
Serotype 14	0.08	0.06	3.09	4.72	3.87	3.42
Serotype 18 C cont.	0.01	0.01	0.01	0.01	0.02	0.01
Serotype 18 C	0.02	0.04	7.70	13.08	2.75	5.92
Serotype 19A cont.	0.22	0.11	0.22	0.26	0.43	0.14
Serotype 19A	0.20	0.49	5.24	5.87	3.32	4.17
Serotype 19F cont.	0.06	0.03	0.12	0.04	0.05	0.04
Serotype 19F	0.03	4.3	4.63	7.21	2.72	3.91
Serotype 23F cont.	0.03	0.02	0.03	0.02	0.03	0.03
Serotype 23F	0.02	0.02	8.40	4.72	3.54	3.01

GMT: geometric mean titer

Serology analysis

	GMT (males & females): day -7 (U/ml)	GMT (males & females): day 87 (U/ml)	GMT (males & females): day 115 (U/ml)
Serotype 1 cont.	0.02	0.02	0.01
Serotype 1	0.02	3.9	2.3
Serotype 3 cont.	0.12	0.08	0.09
Serotype 3	0.11	3	1.7
Serotype 4 cont.	0.01	0.02	0.02
Serotype 4	0.01	4.12	1.98
Serotype 5 cont.	0.13	0.14	0.11
Serotype 5	0.08	4.23	1.93
Serotype 6A cont.	0.07	0.09	0.05
Serotype 6A	0.05	7.64	4.3
Serotype 6B cont.	0.04	0.05	0.05
Serotype 6B	0.04	13.4	10.9
Serotype 7F cont.	0.19	0.13	0.11
Serotype 7F	0.12	5.51	2.64
Serotype 9V cont.	0.04	0.05	0.04
Serotype 9V	0.02	3.72	2.17
Serotype 14 cont.	0.05	0.05	0.08
Serotype 14	0.04	2.87	3.50
Serotype 18 C cont.	0.01	0.01	0.01
Serotype 18 C	0.02	9.12	3.98
Serotype 19A cont.	0.10	0.15	0.13
Serotype 19A	0.18	4.90	3.55
Serotype 19F cont.	0.04	0.04	0.04
Serotype 19F	0.03	4.06	2.72
Serotype 23F cont.	0.03	0.02	0.03
Serotype 23F	0.02	5.00	2.77

GMT: geometric mean titer

The serological assays confirmed that the test article, 13vPnC, administered by subcutaneous injection for a total of 7 injections (1 dose/2 weeks) to cynomolgus monkeys was capable of inducing specific serum antibodies to each of the 13 pneumococcal serotypes of 13vPnC. As measured by (b)(4), no animal in the control group and each animal in the vaccine group mounted an immune response to each of the 13 pneumococcal serotypes of 13vPnC.

Assessment:

No treatment-related mortality and no toxicologically relevant changes in body weight (gain), relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight were found.

There were several incidences of differences in the clinical chemical and hematology parameters which showed a fold change equal or higher than 1.5. Many of these differences were of a magnitude or nature not considered clinically significant and remained within the normal range of values established for gender, laboratory or species.

A large number of different tissues were examined for histology. Findings related to the colon, adrenal, lung, liver, pancreas, thymus, trachea and fat were isolated cases and/or evenly distributed between treated and control groups of animals. Findings in the testis, prostate and seminal vesicle were related to the young age of the animals and were equally distributed between treated and control groups of animals. Interstitial inflammation in the kidney was observed in several animals on day 87 and 115 in the control as well as the treatment group.

In the pathology study two out of three female monkeys, and one out of three male monkeys showed thyroid cysts after the subcutaneous injection of the vaccine at day 87. Additionally on day 115, one male animal in the treatment group developed a thyroid cyst; while none of the animals in the control group developed a thyroid cyst.

Draize scoring of the injection sites revealed an increased frequency of irritation in the treated animals compared to control animals, but without increased severity. Histological inflammation was observed at the site of injection in the treatment group, but not in the control group. On day 87 and 115 inflammation was observed in the treatment group; however the number of animals showing inflammation decreased from day 87 to day 115.

The serological assays confirmed that the test article, 13vPnC administered by subcutaneous injection for a total of 7 injections (1 dose/2 weeks) to ---(b)(4)---- monkeys was capable of inducing specific serum antibodies to each of the 13 pneumococcal serotypes of 13vPnC. As measured by (b)(4), no animal in the control group and each animal in the vaccine group mounted an immune response to each of the 13 pneumococcal serotypes of 13vPnC.

**Title: 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC):
5-CYCLE (1 DOSE/3 WEEK CYCLE) INTRAMUSCULAR TOXICITY
STUDY WITH A 4-WEEK OBSERVATION PERIOD IN RABBITS
(PROTOCOL 07_2483)**

Study number: RPT-72055

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

Study initiation date: Protocol approved: 21 Aug 2007, first date of dosing: 11 Sep 2007

Final Report date: 05.30.2008

Test article batch/lot: 7-5095-005A

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

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

Number of animal per group and sex: 10

Age: approximately 5 month

Body weight range: males: 2.5 -2.9 kg; females: 2.7- 3.4 kg

Route and site of administration: intramuscular

Volume of injection: 0.5 ml per injection

Frequency of administration and study duration: 5 intramuscular (IM)

Injections administered on days 1, 22, 43, 64 and 85, 1 dose every 3 weeks; followed by a 4-week observation period after the last dose.

Dose: 31.3 µg polysaccharide and 36.4 µg CRM₁₉₇/dose

Stability: The 13vPnC Vaccine (stored in syringes) was found to be stable for at least 18 months at refrigerated conditions as per expiry dating on release documentation; expiration date: 07-August-2008.

The 13vPnC drug product placebo (stored in syringes) was found to be stable for at least 24 months at refrigerated conditions as per expiry dating on release documentation; expiration date: 31-May-2009.

Saline solution: 31-Oct-2009

Means of administration: needles and syringe

Report status: final

Experimental study design

Group*	Treatment	Number of Animals (#/sex/group)	
		Treatment phase (13 weeks)	Recovery phase (4 weeks)
1 saline control	0.9% sodium chloride	5	5
2. adjuvant control	5 mM succinate buffer at pH ---- (b)(4)----- and 0.02% Polysorbate 80 as excipients and aluminum	5	5

	phosphate at 0.25 mg aluminum/mL		
3. 13vPnC	31.3 µg polysaccharide and 36.4 µg pCRM ₁₉₇ /dose 5 mM succinate containing 0.02% Polysorbate 80 in ----(b)(4)----, as excipients at pH (b)(4) with aluminum phosphate at 0.25 mg aluminum/mL	5	5

*Dose was delivered intramuscular on day 1, 22, 43, 64 and 85.

Methods: Hematology parameters were measured on an -----(b)(4)----- hematology analyzer utilizing (b)(4) reagents. When (b)(4) results warranted, a manual differential was performed. Coagulation parameters were measured on a -----(b)(4)----- coagulation analyzer using reagent systems from -----(b)(4)----- . Reticulocyte counting was performed on a -----(b)(4)----- utilizing -----(b)(4)----- reagents and standards.

Randomization procedure: Animals were weighed and assigned to dosage groups by gender in a manner that minimized mean body weight differences among dosage groups. Dosage groups were assigned to cages in a manner designed to uniformly disperse dosage groups throughout the study room.

Statistical analysis plan: Body weight, injection site evaluation, and body temperature data: nonparametric one-way analysis of variance (ANOVA) to test for potential effects among dosage groups. Analyses were done separately for each sex. A nonparametric one-way ANOVA on ranks utilizing all groups was conducted to test for differences among groups. Average ranks were utilized for ties. If the p-value from the overall test was significant (p-value ≤ 0.05), follow up pair wise comparisons of groups 1 and 3 to group 2 were performed using two-sided least significant difference (LSD) tests.

Clinical observations: The following parameters were evaluated: general clinical observation (daily, beginning 7 days prior to initiation of dosing), detailed clinical examination (twice pretest: 7 days and 1 day prior to initiation of dosing, prior to each dose administration, daily for 6 days following each dose, and once per week during dosing phase weeks 2, 3, 5, 6, 8, 9, 11, 12, and 14-16), body weight (weekly, twice pretest: 7 days and 1 day prior to initiation of dosing), food consumption (daily, starting 7 days prior to dosing), ophthalmologic examinations (once pretest and during week 13 and 17), evaluation of injection sites (day of each dose administration: prior to dosing and approximately 4, 24, 48, and 72 hours following dose administration, day of scheduled necropsy, prior to euthanasia), body temperature (at the day of dose administration: prior to dosing, 4 and 24 hours following dose administration; day of final necropsy); hematology and clinical chemistry (once pretest, dosing phase days 3, 45, 87, 100, and 115), gross anatomy at termination, organ weights and histopathology on a selection of tissues (days 87, and 130), blood samples for antibody-determination were taken and analyzed (non-GLP) under the responsibility of the sponsor (once pretest and on dosing

phase days 42 and 84, on the day of or the day before scheduled necropsy, for those animals scheduled for necropsy).

Clinical observations

Parameters	Frequency of Testing
Cageside observation ¹	daily, beginning 7 days prior to initiation of dosing
Clinical observations ²	twice pretest: 7 days and 1 day prior to initiation of dosing, prior to each dose administration, daily for 6 days following each dose, and once per week during dosing phase weeks 2, 3, 5, 6, 8, 9, 11, 12, and 14-16
Body weight	weekly, twice pretest: 7 days and 1 day prior to imitation of dosing
Food consumption	daily, starting 7 days prior to dosing
Body temperature	at the day of dose administration: prior to dosing, 4 and 24 hours following dose administration; day of final necropsy
Ophthalmologic exam	once pretest and during week 13 an 17
Clinical chemistry*	Once pretest, Dosing Phase Days 3, 45, 87, 100, and 115; Saphenous or ear vein/ear artery
Hematology*	Once pretest, Dosing Phase Days 3, 45, 87, 100, and 115; Saphenous or ear vein/ear artery
Coagulation*	Once pretest, Dosing Phase Days 3, 45, 87, 100, and 115; Saphenous or ear vein/ear artery
Immunological response	once pretest and on dosing phase days 42 and 84
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	on the day of each dose administration prior to dosing and approximately 4, 24, 48, and 72 hours following dose administration
Necropsy	days 87, and 115
Tissues for histopathology	days 87, and 115

*(blood collection site: Saphenous or ear vein/ear artery.)

¹ Cageside observations include mortality, morbidity, general health and signs of toxicity.

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

Postmortem procedures: The following tissues were collected at necropsy.

Histology evaluation

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone (femur)	!	
Bone marrow (rib)	!	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	!*	
Cecum	!	
Cervix	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		!
Gall bladder	!	
Gross lesions (if any)	!	
Gut associated Lymphoid tissue	!	
Harderian gland (if applicable)		
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		!
Larynx		!
Liver	!*	
Lung (main-stem; bonchi)	!	
Lymph nodes (cervical)		!
Lymph nodes (right inguinal) at injection site	!	
Lymph nodes (mandibular)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!

Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)	!	
Pituitary gland	!*	
Prostate	!*	
Rectum		!
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal Vesicles	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach (squamous and glandular)	!	
Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!*	
Tongue	!	
Trachea	!	
Ureters		!
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		!

Table of Histology – Tissues examined: All dose groups

(*) Organ weight was determined

(!) Histology was performed

Results:

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

Clinical Chemistry

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
LIVER FUNCTION:	<p>ALT (IU/L): Females: day 100: saline control (64.8) is 1.8x higher than the placebo control (37.0) and 1.6x higher than 13vPnC (39.8) Day 115: saline control (68.8) is 1.8x higher than the placebo control (37.6) and 1.6x higher than 13vPnC (41.6)</p> <p>AP(IU/L): Males: day 100: placebo control (99.8) is 1.7x higher than 13vPnC (60.6) Day 115: placebo control (103.8) is 1.7x higher than 13vPnC (61.6)</p> <p>TBIL(mg/dL): Males: day 3: placebo control (0.2) is 2x higher than the saline control (0.1) and 13vPnC (0.1) Day 87: saline control (0.2) is 2x higher than the placebo control (0.1) and 13vPnC (0.1) Day 115: 13vPnC (0.2) is 2x higher than the saline control (0.1) and placebo control (0.1) Females: Day 3: saline control (0.2) and 13vPnC (0.2) are 2x higher than the placebo control (0.1) Day 87: placebo control (0.2) and 13vPnC (0.2) are 2x higher than the saline control (0.1) Day 100: saline control (0.2) and placebo control (0.2) are 2x higher than 13vPnC (0.1)</p>	<p>Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase sorbitol dehydrogenase Total bile acids Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin</p>
LIVER FUNCTION:	<p>IBIL (mg/dL): Males: day 3: placebo control (0.2) is 2x higher than the saline control (0.1) and 13vPnC (0.1) Day 87: saline control (0.2) is 2x higher than the placebo control (0.1) and 13vPnC (0.1) Day 115: 13vPnC (0.2) is 2x higher than the saline control (0.1) and</p>	

	<p>placebo control (0.1)</p> <p>Females: Day 3: saline control (0.2) and 13vPnC (0.2) are 2x higher than the placebo control (0.1)</p> <p>Day 87: placebo control (0.2) and 13vPnC (0.2) are 2x higher than the saline control (0.1)</p> <p>Day 100: saline control (0.2) and placebo control (0.2) are 2x higher than 13vPnC (0.1)</p>	
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen ND
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	<p>LDH (IU/L):</p> <p>Male: day3: saline control (91.6) is 1.7x higher than 13vPnC (57.7)</p> <p>CHOL (mg/dL):</p> <p>Males: day 100: 13vPnC (24.0) is 1.6x higher than the adjuvant control (0.1)</p> <p>day 115: 13vPnC (18.8) is 1.6x higher than the adjuvant control (11.6)</p>	<p>Albumin (A)</p> <p>Globulin (G, calculated) or A/G Ratio</p> <p>Total cholesterol</p> <p>Cholinesterase</p> <p>Total protein</p> <p>Creatine kinase</p> <p>Fasting triglycerides</p>

ND: not done

Hematology

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean corp. Hb. (MCH) Mean corp. Hb. Conc. (MCHC), Mean corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
WHITE BLOOD CELLS	<p>Monocytes: Male: pretest day 16: adjuvant control (0.13) 1.6x higher than saline control (0.21) and 1.9x higher 13vPnC (0.25) Day 45: adjuvant control (0.04) 2.5x higher than 13vPnC (0.10) Day 100: adjuvant control (0.28) 1.6x higher than 13vPnC (0.47) Day 115: saline control (0.09) 1.7x higher than adjuvant control (0.15) Females: 13vPnC (0.11) and adjuvant control (0.11) are 1.6x higher than the saline control (0.07)</p> <p>Eosinophils: Male: day 3: saline control (0.21) is 1.6x higher than 13vPnC (0.13) Day 87: adjuvant control (0.12) is 1.7x times higher than 13vPnC (0.07) Day 100: 13vPnC (0.05) is 2.5x higher than the adjuvant control (0.02) and the saline control (0.02) Day 115: 13vPnC (0.22) is 1.7x higher than the adjuvant control (0.13) and 2x higher than the saline control (0.11) Females: day 87: adjuvant control (0.03) is 3x higher and 13vPnC (0.02) is 2x higher than saline control (0.01) Day 115: adjuvant control (0.16) and 13vPnC (0.15) are 2.3x higher than saline control (0.07)</p> <p>Basophils: Male: Day 87: 13vPnC (0.44) is 1.6x higher than the adjuvant control (0.27) Day 115: 13vPnC (0.56) is 3.1x higher than the adjuvant control (0.18) and 1.6x higher than the saline control (0.34)</p> <p>Neutrophils: Female: Day 100: 13vPnC (2.60) is 1.6x higher than the adjuvant control (1.60) and 1.7x higher than the saline control (1.57)</p>	Basophils, eosinophils count lymphocyte count Macrophage/monocyte count Neutrophil count Total leukocytes (WBC) Large unstained Cells (LUC)
OTHERS		Bone marrow cytology

ND: not done

Systemic toxicity:

No treatment-related mortality and no toxicologically relevant changes in body weight (gain), relative food consumption, ophthalmoscopic parameters, clinical chemistry, gross anatomy or organ weight were found.

There were several incidences of differences in the clinical chemical and hematology parameters with a fold change equal or higher than 1.5. ALT was elevated in the saline control group compared to the treatment group. Cholesterol was detected 1.6 higher in the 13vPnC treatment group than in the control groups at day 100 and 115 in the male rabbits. Neutrophils were elevated in males of the treatment group on day 87 and 115; neutrophils were elevated in females of the treatment group on day 100. Eosinophiles were elevated 2.5 fold in males in the 13vPnC group compared to saline control group on day 100 and 2 fold on day 115 in the 13vPnC group. Basophiles were elevated 1.6 fold on days 87 and 115 in males of the 13vPnC group compared to the saline control group.

There were no treatment-related effects on organ weights or weight ratios for males or females detected.

Organ Weight, day 87

		MALES			FEMALES		
GROUPS		saline control	adjuvant control	13vPnC	saline control	adjuvant control	13vPnC
NUMBER OF ANIMALS		5	5	5	5	5	5
BODY WEIGHT (kg) ^a		3.42	3.24	3.30	3.76	3.70	3.64
BRAIN							
Absolute Weight ^a	gram	9.77	9.67	9.69	9.68	9.59	9.57
Per Body Weight ^a	%	0.29	0.30	0.29	0.26	0.26	0.26
ADRENALS							
Absolute Weight ^a	gram	0.438	0.433	0.383	0.355	0.361	0.354
Per Body Weight ^a	%	0.013	0.013	0.012	0.009	0.010	0.010
Per Brain Weight ^a	%	4.495	4.479	3.642	3.662	3.780	3.707
EPIDIDYMIDES							
Absolute Weight ^a	gram						
Per Body Weight ^a	%						
Per Brain Weight ^a	%						
HEART							
Absolute Weight ^a	gram	7.31	7.31	6.86	7.41	7.39	6.99
Per Body Weight ^a	%	0.21	0.23	0.21	0.20	0.20	0.19
Per Brain Weight ^a	%	74.91	75.81	70.87	76.46	77.25	72.85
KIDNEYS							
Absolute Weight ^a	gram	15.40	17.17	15.83	17.31	16.22	16.25
Per Body Weight ^a	%	0.45	0.53	0.48	0.46	0.44	0.45
Per Brain Weight ^a	%	158.28	177.56	163.66	178.61	169.46	169.95
LIVER							
Absolute Weight ^a	gram	78.87	80.02	78.93	77.30	77.34	74.43

		MALES			FEMALES		
GROUPS		saline control	adjuvant control	13vPnC	saline control	adjuvant control	13vPnC
NUMBER OF ANIMALS		5	5	5	5	5	5
Per Body Weight ^a	%	2.30	2.48	2.40	2.05	2.08	2.05
Per Brain Weight ^a	%	810.65	827.55	817.18	796.83	809.60	779.68
Pituitary							
Absolute Weight ^a		0.031	0.026	0.024	0.044	0.036	0.041
Per Body Weight ^a		0.092	0.051	0.073	0.116	0.097	0.113
Per Brain Weight ^a		0.324	0.271	0.249	0.451	0.380	0.430
SPLEEN							
Absolute Weight ^a	gram	1.01	1.28	1.24	1.80	1.62	1.46
Per Body Weight ^a	%	0.03	0.04	0.04	0.05	0.04	0.04
Per Brain Weight ^a	%	10.27	13.22	12.80	18.52	16.87	15.19
TESTES							
Absolute Weight ^a	gram	6.10	6.09	6.23			
Per Body Weight ^a	%	0.18	0.19	0.19			
Per Brain Weight ^a	%	62.94	63.10	64.11			
THYROID and PARATHYROID							
Absolute Weight ^a	gram	0.348	0.336	0.252	0.293	0.296	0.274
Per Body Weight ^a	%	1.014	1.041	0.764	0.788	0.801	0.749
Per Brain Weight ^a	%	3.565	3.472	2.605	3.023	3.090	2.831
THYMUS							
Absolute Weight ^a	gram	3.819	3.247	3.439	3.683	3.512	3.506
Per Body Weight ^a	%	0.112	0.100	0.104	0.098	0.095	0.097
Per Brain Weight ^a	%	38.996	33.575	35.391	37.930	36.734	36.922
OVARIES							
Absolute Weight ^a	gram				0.542	0.398	0.435
Per Body Weight ^a	%				1.432	1.071	1.196
Per Brain Weight ^a	%				5.587	4.182	4.551
UTERUS							
Absolute Weight ^a	gram						
Per Body Weight ^a	%						
Per Brain Weight ^a	%						
Prostate							
Absolute Weight ^a	gram	2.01	2.51	2.13			
Per Body Weight ^a	%	0.06	0.08	0.06			
Per Brain Weight ^a	%	20.54	25.95	21.99			

Table of organ weight and their normalization. Absolute weights are expressed as mean(grams) ± standard deviation (sd).

*different from controls at $P \leq 0.05$;

**different from controls at $P \leq 0.01$.

Organ weight, day 115

GROUPS		MALES			FEMALES		
		saline control	adjuvant control	13vPnC	saline control	adjuvant control	13vPnC
NUMBER OF ANIMALS		5	5	5	5	5	5
BODY WEIGHT (kg) ^a		3.46	3.42	3.56	3.86	3.90	4.02
BRAIN							
Absolute Weight ^a	gram	9.96	9.92	9.81	9.62	10.18	9.90
Per Body Weight ^a	%	0.29	0.29	0.28	0.25	0.26	0.25
ADRENALS							
Absolute Weight ^a	gram	0.475	0.384	0.462	0.350	0.388	0.435
Per Body Weight ^a	%	0.014	0.011	0.013	0.009	0.009	0.010
Per Brain Weight ^a	%	4.780	3.887	4.712	3.651	3.831	4.390
EPIDIDYMIDES							
Absolute Weight ^a	gram						
Per Body Weight ^a	%						
Per Brain Weight ^a	%						
HEART							
Absolute Weight ^a	gram	7.24	7.31	7.37	7.15	7.44	7.48
Per Body Weight ^a	%	0.21	0.21	0.21	0.19	0.19	0.19
Per Brain Weight ^a	%	72.58	73.85	75.95	74.36	73.24	75.93
KIDNEYS							
Absolute Weight ^a	gram	16.56	17.28	16.34	15.22	18.05	16.01
Per Body Weight ^a	%	0.48	0.51	0.46	0.39	0.46	0.40
Per Brain Weight ^a	%	166.06	174.57	166.25	158.38	177.31	161.92
LIVER							
Absolute Weight ^a	gram	85.47	86.70	83.01	73.37	80.73	88.63
Per Body Weight ^a	%	2.47	2.54	2.33	1.89	2.08	2.20
Per Brain Weight ^a	%	857.77	877.98	847.48	763.19	794.41	896.67
SPLEEN							
Absolute Weight ^a	gram	1.27	1.19	1.34	1.39	1.56	1.80
Per Body Weight ^a	%	0.04	0.03	0.04	0.04	0.04	0.04
Per Brain Weight ^a	%	12.72	12.01	13.65	14.53	15.37	18.15
TESTES							
Absolute Weight ^a	gram	7.20	7.14	7.11			
Per Body Weight ^a	%	0.21	0.21	0.20			
Per Brain Weight ^a	%	72.22	71.97	72.40			
THYROID and PARATHYROID							
Absolute Weight ^a	gram	0.302	0.288	0.270	0.225	0.280	0.277
Per Body Weight ^a	%	0.873	0.844	0.758	0.584	0.722	0.688
Per Brain Weight ^a	%	3.035	2.930	2.747	2.368	2.753	2.800
THYMUS							
Absolute Weight ^a	gram	3.666	3.673	3.720	4.034	3.622	4.434
Per Body Weight ^a	%	0.105	0.107	0.106	0.104	0.094	0.111

		MALES				FEMALES			
GROUPS		saline control	adjuvant control	13vPnC	saline control	adjuvant control	13vPnC		
NUMBER OF ANIMALS		5	5	5	5	5	5		
Per Brain Weight ^a	%	36.791	37.042	37.954	42.584	35.438	45.188		
OVARIES									
Absolute Weight ^a	gram				0.667	0.566	0.573		
Per Body Weight ^a	%				1.711	1.453	1.417		
Per Brain Weight ^a	%				6.767	5.538	5.756		
UTERUS									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
Pituitary									
Absolute Weight ^a	gram	0.026	0.028	0.026	0.035	0.038	0.037		
Per Body Weight ^a	%	0.074	0.082	0.074	0.089	0.097	0.092		
Per Brain Weight ^a	%	0.256	0.286	0.269	0.358	0.369	0.372		
Prostate									
Absolute Weight ^a	gram	1.82	1.73	1.89					
Per Body Weight ^a	%	0.05	0.05	0.05					
Per Brain Weight ^a	%	18.33	17.44	19.23					

Table of organ weight and their normalization. Absolute weights are expressed as mean (grams) \pm standard deviation (sd).

*different from controls at $P \leq 0.05$;

**different from controls at $P \leq 0.01$.

No significant differences in the in the organ weight between control group and treatment group could be determined.

Gross Pathology:

Group identification:

M1: male saline control group

M2: male adjuvant control group

M3: male treatment group

F1: female saline control group

F2: female adjuvant control group

F3: female treatment group

Gross pathology, day 87

Group	Findings
1F	Discolored Cortex of the kidney's (1F: 1/5 animals)
1M, 2M	Discoloration of the injection site (1M: 1/5 animals; 2M: 2/5 animals)

Gross pathology, day 115

Group	Findings
2F	Discolored medulla of the kidney (2F: 1/5 animals)
3M	Mucosal discoloration of the cecum (3M: 1/5 animals)

In the adjuvant alone group one female showed discoloring of the kidney medulla, in the 13vPnC treatment group this was not observed. One male in the treatment group showed discoloration of the cecum.

Microscopic findings, day 87

Groups	Findings
1F,	Developmental anomaly in the brain (1F: 1/5 animals)
1M, 2M, 3M, 1F, 2F, 3F	Lymphoplasmatic inflammation of the cecum (1M: 2/5 animals, 2M: 1/5 animals, 3M: 5/5 animals, 1F: 5/5 animals, 2F: 5/5 animals, 3F: 3/5 animals)
1M, 2M, 3M, 1F, 2F, 3F	Lymphoplasmatic inflammation of the colon (1M: 1/5 animals, 2M: 1/5 animals, 3M: 2/5 animals, 1F: 5/5 animals, 2F: 4/5 animals, 3F: 3/5 animals)
3M	Inflammation and mixed cells at the epididymides (3M: 1/5 animals)
3F	Mineralization of the gut associated lymphoid tissue (1/5 animals)
2M, 3M, 1M	Mononuclear inflammation of the heart (2M: 1/5 animals, 3M: 1/5 animals, 1F: 2/5 animals)
2F	Mixed cell inflammation of the heart (2F: 1/5 animals)
3M, 1F, 2F	Pigmentation of inguinal lymph node (3M: 1/5 animals, 1F: 2/5 animals, 2F: 1/5 animals)
1M, 2M, 3M, 1F, 3F	Hemorrhage at injection site (1M: 1/5 animals, 2M: 1/5 animals, 3M: 2/5 animals, 1F: 1/5 animals, 3F: 1/5 animals)
2M, 3M, 1F, 2F, 3F	Degeneration/necrosis at injection site (2M: 1/5 animals, 3M: 3/5 animals, 1F: 1/5 animals, 2F: 1/5 animals, 3F: 2/5 animals)
2M, 3M, 2F, 3F	Chronic inflammation at injection site (2M: 2/5 animals, 3M: 4/5 animals, 2F: 3/5 animals, 3F: 5/5 animals)
2M, 3M, 1F, 2F, 3F	Tubular basophilia of the kidney (2M: 3/5 animals, 3M: 2/5 animals, 1F: 1/5 animals, 2F: 3/5 animals, 3F: 4/5 animals)
3M, 1F, 2F, 3F	Mineralization of the kidney (3M: 1/5 animals, 1F: 1/5 animals, 2F: 2/5 animals, 3F: 1/5 animals)

1F, 3F	Tubular degeneration of the kidney (1F: 1/5 animals, 3F: 1/5 animals)
1M, 3M, 2F	Mononuclear cell infiltration of the liver (1M: 1/5 animals, 3M: 1/5 animals, 2F: 2/5 animals)
3M	Heterophilic inflammation of the liver (3M: 1/5 animals)
3M	Heterophilic inflammation of the lung (3M: 1/5 animals)
1M, 3F	Sinus erythrocytosis in the mandibular node (1M: 1/5 animals, 1F: 1/5 animals)
1F	Heterophilic inflammation of the pancreas (1F: 1/5 animals)
2F	Cyst in the pituitary (1F: 1/5 animals)
3M	Metaplasia in the prostate (3M: 1/5 animals)
2M, 1F, 2F	Axonal degeneration of the sciatic nerve (2M: 1/5 animals, 1F: 1/5 animals, 2F: 1/5 animals)
1M, 2M, 3M, 1F, 2F, 3F	Pigment in the spleen (1M: 1/5 animals, 2M: 1/5 animals, 3M: 1/5 animals, 1F: 1/5 animals, 2F: 2/5 animals, 3F: 2/5 animals)
3M	Crypt abscess in the stomach (3M: 1/5 animals)
1F	Glandular dilatation in the stomach (1F: 1/5 animals)
1M	Tubular giant cells in the testes (1M: 1/5 animals)
1M, 2M, 3M, 1F, 2F, 3F	Decreased cellularity in the thymus (1M: 4/5 animals, 2M: 4/5 animals, 3M: 4/5 animals, 1F: 3/5 animals, 2F: 3/5 animals, 3F: 3/5 animals)
3M	Mixed cell inflammation of the thyroid (3M: 1/5 animals)
2M	Ectopic thymic tissue (2M: 1/5 animals)
2M	C-cell hyperplasia of the thyroid (2M: 1/5 animals)
2M	Squamous cyst in the thyroid (2M: 1/5 animals)

Microscopic findings, day 115

Groups	Findings
1M	Congestion of the adrenal cortex (1M: 2/5 animals)
3F	Vascular mineralization of the aorta (3F: 1/5 animals)
3F	Lymphatic inflammation of the brain (3F: 1/5 animals)
1M, 2M, 3M, 1F, 2F, 3F	Lymphoplasmatic inflammation of the cecum (1M: 4/5 animals, 2M: 5/5 animals, 3M: 5/5 animals, 1F: 4/5 animals, 2F: 2/5 animals, 3F: 5/5 animals)
3M	Hemorrhage of the cecum (3M: 1/5 animals)
1M, 2M, 3M, 1F, 2F, 3F	Lymphoplasmatic inflammation of the colon (1M: 4/5 animals, 2M: 5/5 animals, 3M: 3/5 animals, 1F: 4/5 animals, 2F: 2/5 animals, 3F: 5/5 animals)
3F	Lymphatic inflammation of the eye (3F: 1/5 animals)
1M, 2M, 3M, 2F, 3F	Mononuclear inflammation of the heart (1M: 1/5 animals, 2M: 1/5 animals, 3M: 1/5 animals, 2F: 1/5 animals, 3F: 1/5 animals)
3M, 3F	Vascular mineralization of the heart (3M: 1/5 animals, 3F: 1/5 animals)
3M, 1F, 2F, 3F	Pigmentation of inguinal lymph node (3M: 1/5 animals, 1F: 3/5 animals)

	animals, 2F: 3/5 animals, 3F: 2/5 animals)
2M, 3M, 2F, 3F	Chronic inflammation at injection site (2M: 1/5 animals, 3M: 2/5 animals, 2F: 1/5 animals, 3F: 3/5 animals) specific to adjuvant
1M, 3M, 3F	Tubular basophilia of the kidney (1M: 1/5 animals, 3M: 1/5 animals, 3F: 2/5 animals)
1M, 2M, 1F, 2F, 3F	Mineralization of the kidney (1M: 1/5 animals, 2M: 1/5 animals, 1F: 2/5 animals, 2F: 2/5 animals, 3F: 3/5 animals)
1F, 2F, 3F	Tubular degeneration of the kidney (1F: 1/5 animals, 2F: 3/5 animals, 3F: 1/5 animals)
1F, 2F	Mononuclear cell infiltration of the liver (1F: 1/5 animals, 2F: 1/5 animals)
1F	Heterophilic inflammation of the lung (1F: 1/5 animals)
2F	Alveolar macrophages in the lung (2F: 2/5 animals)
3F	Dilatation of mammary gland (3F: 1/5 animals)
3F	Cyst of mandibular node (3F: 1/5 animals)
3M	Ectopic spleen (3M: 2/5 animals)
1M	Heterophilic inflammation of the pancreas (1M: 1/5 animals)
2M	Cyst in parathyroids (2M: 1/4 animals)
2F	Ectopic thymus (2F: 1/4 animals)
1M	Mixed cell inflammation of prostate (1M: 1/5 animals)
2M, 3M	Axonal degeneration of the sciatic nerve (2M: 1/5 animals, 3M: 1/5 animals)
2M, 1F, 2F, 3F	Pigment in the spleen (2M: 1/5 animals, 1F: 4/5 animals, 2F: 1/5 animals, 3F: 1/5 animals)
2F	Glandular dilatation in the stomach (2F: 1/5 animals)
2M	Spontaneous bilateral hypoplasia of the testes (2M: 1/5 animals)
1M, 2M, 3M, 1F, 3F	Decreased cellularity in the thymus (1M: 3/5 animals, 2M: 5/5 animals, 3M: 2/5 animals, 1F: 5/5 animals, 3F: 2/5 animals)
2M	Ectopic thymic tissue (2M: 1/5 animals)
2M, 1F, 2F, 3F	Lymphohistiocytic inflammation of the thyroid (2M: 1/5 animals, 1F: 1/5 animals, 2F: 2/5 animals, 3F: 1/5 animals)

A large number of different tissues were examined for histology. Findings related to the brain, heart, spleen, kidney, liver were isolated cases and evenly distributed between treated and control groups of animals; except a heterophilic inflammation of the liver which was only seen on day 87 in one male animal from the treatment group. No elevation in the liver clinical chemistry was detected in these animals.

Lymphoplasmatic inflammation of the cecum and colon as well as decreased cellularity was frequently found on day 87 and 115 in animals across all groups including the saline control group, and thus, not considered vaccine-related.

Tubular basophilia was found on day 87 in all groups that had received adjuvant (adjuvant control group and 13vPnC group). Between 2 and 4 animals were affected compared to only one female in the saline group that was diagnosed with tubular basophilia on day 87. However, on day 115 the incidence of tubular basophilia was evenly distributed between control groups and treatment groups.

The following findings were only found in males of the treatment group: inflammation of the epididymis, mineralization of the gut associated lymphoid tissue, heterophilic inflammation of the liver and lung, metaplasie in the prostate, crypt abscess in the stomach, mixed cell inflammation of the thyroid, ectopic spleen, and hemorrhage of the cecum. The following observations were only found in females of the treatment group: vascular mineralization of the aorta, lymphatic inflammation of the brain and eye, dilatation of the mammary gland, and cyst of the mandibular gland. However, these findings were rare and not associated with any other symptoms.

Body temperature

Group	Males	Females
Saline Control	7	2
Adjuvant Control	2	4
13vPnC	7	2

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

Local toxicity: Chronic inflammation was found in the treatment group and adjuvant at day 87 and 115 at the injection site.

Histological hemorrhage and degeneration/necrosis was observed at the injection site in some of the treated animals and control animals on day 87. Chronic inflammation at the injection site was observed in the adjuvant control group as well as the treatment group; it was seen on day 87 and did not resolve by day 115.

Draize scoring of the injection site revealed the following as presented in the table below:

Erythema, pre injection

Treatment group	Erythema, pre injection																													
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4		1	2	3	4		1	2	3	4		1	2	3	4					
Saline control	2					2					2					2					2					2				
	0					0					0					0					0					0				
Adjuvant control	2					2					2					2					2					2				
	0					0					0					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Erythema, 4h post injection

Treatment group																									
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	1	7			1	2					2					1	2		1		1	2			
	3			8							0					7					8				
Adjuvant control	1	6			1	1					1	1				1	5				1	1			
	4			9							9					5					9				
13vPnC	1	2	1		1	2					1	2				1	4				1	2			
	7			8							8					6					8				

Erythema, 24h post injection

Treatment group																									
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	1	1				1	2	2			1	1				1	2	2			1	2	3		
	9					6					9					6					5				
Adjuvant control	1	3				1	3	2			1	1				1	3	2			1	2			
	7					5					9					5					8				
13vPnC	1	4	1			1	3				1	1				1	3				1	1			
	5					7					9					7					9				

Erythema, 48 hours post injection

Treatment group																									
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	1	1				1	1				2					1	1				1	2			
	9					9					0					9					8				
Adjuvant control	1	1				2					1	1				2					2				
	9					0					9					0					0				
13vPnC	1		1			2					1	1				2					1	1			
	9					0					9					0					9				

Erythema, 72 hours post injection

Treatment group	Erythema, 72 hours post injection																													
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	2					1	1				2					1					2					2				
	0					9					0					9					0					0				
Adjuvant control	2					2					1	2				2					2					2				
	0					0					8					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Edema, pre injection

Treatment group	Edema, pre injection																													
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2					2					2					2				
	0					0					0					0					0					0				
Adjuvant control	2					2					2					2					2					2				
	0					0					0					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Edema, 4 hours post injection

Treatment group	Edema, 4 hours post injection																													
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	1	2				1	1				2					1	1				1	2				1	2			
	8					9					0					9					8					8				
Adjuvant control	1	1				1	1				1	1				1	2				1	1				1	1			
	9					9					9					8					9					9				
13vPnC	1		1			1	5				2					1	4				1	1				1	1			
	9					5					0					6					9					9				

Edema, 24 hours post injection

Treatment group	Edema, 24 hours post injection																								
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	1 9	1				1 8	2				2 0					1 9	1				1 9	1			
Adjuvant control	1 9	1				1 9	1				2 0					1 9	1				1 8	2			
13vPnC	1 6	2	2			1 9	1				2 0					1 9	1				1 7	3			

Edema, 48 hours post injection

Treatment group	Edema, 48 hours post injection																								
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	2 0					2 0					2 0					2 0					1 9	1			
Adjuvant control	2 0					1 9	1				2 0					2 0					2 0				
13vPnC	2 0					2 0					2 0					2 0					2 0				

Edema, 72 hours post injection

Treatment group	Edema, 72 hours post injection																								
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	2 0					2 0					2 0					2 0					2 0				
Adjuvant control	2 0					2 0					1 9					2 0					2 0				
13vPnC	2 0					2 0					1 9					2 0					2 0				

Draize scoring of the injection sites revealed an increased frequency of irritation in the treated animals compared to control animals, but without increased severity. A Draize score of 1 or below was observed, only in three occasions was a Draize score of 2 seen in the 13vPnC group.

Serology:

10 male and 10 female (b)(4) rabbits per group were given 13vPnC, placebo control, or saline control intramuscular at a dose volume of 0.5 ml per injection (approximately 30.8 µg polysaccharide and 29 µg pCRM₁₉₇/dose) for a total of 5 doses. Serum samples collected at predose and dose days 84 (prior to the 5th dose) were analyzed for pneumococcal serotype-specific IgG antibody responses to each of the serotypes of 13vPnC. The predose and dose day 84 specimens from the saline control group, placebo control group, and 13vPnC group were evaluated for serotype-specific IgG concentrations for each of the 13 serotypes of 13vPnC using a -----(b)(4)-----

Serology analysis

	GMT (males): day -6 (U/ml)	GMT (females): day -6 (U/ml)	GMT (males): day 84 (U/ml)	GMT (females): day 84 (U/ml)
Serotype 1 saline cont.	0.17	0.20	0.17	0.17
Serotype 1 adjuvant cont.	0.17	0.17	0.17	0.17
Serotype 1	0.17	0.21	225.00	452.37
Serotype 3 saline cont.	0.64	1.25	0.64	0.75
Serotype 3 adjuvant cont.	0.64	0.64	0.74	0.64
Serotype 3	0.95	0.64	376.97	659.81
Serotype 4 saline cont.	0.29	0.35	0.29	0.32
Serotype 4 adjuvant cont.	0.29	0.29	0.37	0.29
Serotype 4	0.29	0.29	1035.68	7002.99
Serotype 5 saline cont.	0.17	0.79	0.17	0.77
Serotype 5 adjuvant cont.	0.17	0.17	0.17	0.17
Serotype 5	0.17	2.44	570.15	790.21
Serotype 6A saline cont.	0.10	0.10	0.10	0.10
Serotype 6A adjuvant cont.	0.10	0.10	0.10	0.10
Serotype 6A	0.10	0.10	401.26	1125.20
Serotype 6B saline cont.	0.26	0.26	0.26	0.26
Serotype 6B adjuvant cont.	0.26	0.26	0.26	0.26
Serotype 6B	0.26	0.26	851.67	2567.60
Serotype 7F saline cont.	4.7	0.31	0.70	0.42
Serotype 7F adjuvant cont.	1.35	2.12	0.34	1.66
Serotype 7F	0.28	0.33	960.00	2732.03
Serotype 9V saline cont.	39.47	3.11	22.45	0.20
Serotype 9V adjuvant cont.	310.03	4.13	36.5	222.70
Serotype 9V	5.86	10.83	1541.81	4040.45
Serotype 14 saline cont.	95.74	8.73	85.29	12.13
Serotype 14 adjuvant cont.	16.00	108.10	27.85	28.64
Serotype 14	244.90	77.72	2090.76	2666.44

Serotype 18 C saline cont.	2.53	0.10	0.60	0.08
Serotype 18 C adjuvant cont.	0.07	0.15	0.09	0.10
Serotype 18 C	0.07	0.07	859.99	1867.88
Serotype 19A saline cont.	0.11	0.11	0.11	0.11
Serotype 19A adjuvant cont.	0.11	0.11	0.11	0.28
Serotype 19A	0.11	0.11	605.06	1090.50
Serotype 19F saline cont.	0.98	0.09	0.37	0.25
Serotype 19F adjuvant cont.	0.07	0.07	0.07	0.07
Serotype 19F	0.007	0.07	258.91	1517.00
Serotype 23F saline cont.	0.32	0.32	0.32	0.35
Serotype 23F adjuvant cont.	0.32	0.32	0.32	0.32
Serotype 23F	0.32	0.32	1097.40	1574.84

GMT: geometric mean titer

Serology analysis

	GMT (males & females): day -6 (U/ml)	GMT (males & females): day 84 (U/ml)
Serotype 1 saline cont.	0.18	0.17
Serotype 1 adjuvant cont.	0.17	0.17
Serotype 1	0.18	223.25
Serotype 3 saline cont.	0.76	0.67
Serotype 3 adjuvant cont.	0.64	0.67
Serotype 3	0.72	357.40
Serotype 4 saline cont.	0.30	0.30
Serotype 4 adjuvant cont.	0.29	0.31
Serotype 4	0.29	4031.47
Serotype 5 saline cont.	0.20	0.21
Serotype 5 adjuvant cont.	0.17	0.17
Serotype 5	0.21	518.10
Serotype 6A saline cont.	0.10	0.10
Serotype 6A adjuvant cont.	0.10	0.10
Serotype 6A	0.10	388.75
Serotype 6B saline cont.	0.26	0.26
Serotype 6B adjuvant cont.	0.26	0.26
Serotype 6B	0.26	1119.86
Serotype 7F saline cont.	0.38	0.31
Serotype 7F adjuvant cont.	0.43	0.40
Serotype 7F	0.25	1253.39
Serotype 9V saline cont.	1.22	1.13
Serotype 9V adjuvant cont.	1.71	1.28
Serotype 9V	0.80	1653.10

Serotype 14 saline cont.	2.35	5.66
Serotype 14 adjuvant cont.	2.09	3.16
Serotype 14	4.47	1214.07
Serotype 18 C saline cont.	0.13	0.11
Serotype 18 C adjuvant cont.	0.09	0.08
Serotype 18 C	0.07	649.93
Serotype 19A saline cont.	0.11	0.11
Serotype 19A adjuvant cont.	0.11	0.12
Serotype 19A	0.11	585.91
Serotype 19F saline cont.	0.09	0.10
Serotype 19F adjuvant cont.	0.07	0.07
Serotype 19F	0.08	566.16
Serotype 23F saline cont.	0.32	0.33
Serotype 23F adjuvant cont.	0.32	0.32
Serotype 23F	0.32	1023.03

GMT: geometric mean titer

The serological assays confirmed that the test article, 13vPnC administered by intramuscular injection of 4 injections (1 dose/2 weeks) to -----(b)(4)----White rabbits was capable of inducing specific serum antibodies to each of the 13 pneumococcal serotypes of 13vPnC. As measured by ---(b)(4)--, no animal in the control group and each animal in the vaccine group mounted an immune response to most of the 13 pneumococcal serotypes of 13vPnC with the exception of PN14; in the PN14 group were two animals identified that did not produced specific antigen titers at day 84.

Assessment:

No treatment-related mortality and notoxicologically relevant changes in relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight were found.

A Draize score of 1 or below was observed most frequently, only in rare occasions a Draize score of 2 was seen in the 13vPnC group. Histological hemorrhage and degeneration/necrosis was observed at the injection site in some of the treated animals and control animals at day 87. Chronic inflammation at the injection site was observed in the adjuvant control group as well as the treatment group. It was seen on day 87 and did not resolve by day 115.

The serological assays confirmed that the test article, 13vPnC administered by intramuscular injection of 4 injections (1 dose/2 weeks) to -----(b)(4)---- White rabbits was capable of inducing specific serum antibodies to each of the 13 pneumococcal serotypes of 13vPnC. As measured by ----(b)(4)--- no animal in the control group and each animal in the vaccine group mounted an immune response to most of the 13 pneumococcal serotypes of 13vPnC with the exception of serotype 14. For serotype 14 two animals were identified that did not produced specific antigen titers at day 84.

**Title: 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC):
SINGLE DOSE INTRAMUSCULAR IRRITATION STUDY IN MALE
RABBITS (PROTOCOL 05_1934)**

Study number: 501549

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

Study initiation date: 24 Oct 2005

Final Report date: 09 May 2006

Test article batch/lot: 13vPnC; 13-Valent Pneumococcal Conjugate in Succinate Buffer with Aluminum Phosphate: 75064002B

13vPnC; 13-Valent Pneumococcal Conjugate in Succinate Buffer: 75088002A

Animal species and strain: male ----(b)(4)----White rabbits

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

Number of animal per group and sex: 3 male animals per group

Age: 5 months

Body weight range: 2.8 to 3.4 kg

Route and site of administration: intramuscular

Volume of injection: 0.5 ml

Frequency of administration and study duration: one intramuscular injection followed by a 2 day observation period.

Dose: 13vPnC with adjuvant: 30.8 µg polysaccharide, 29 µg CRM197, 0.125 mg aluminum phosphate; 13vPnC without adjuvant: 30.8 µg polysaccharide, 29 µg CRM197

Stability: Expiration date: Nov 2005

Means of administration: needle and syringes

Report status: final report

Experimental study design

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1. Control*	0.9% sodium chloride	3 males	
2. 13vPnC without adjuvant	30.8 µg polysaccharide, 29 µg CRM197	3 males	
3. 13vPnC with adjuvant ^a	30.8 µg polysaccharide, 29 µg CRM197, 0.125 mg aluminum phosphate	3 males	

a: Clinical Dosage Formulation; *Dose was delivered intramuscular on day 1.

Methods: In this study no clinical chemistry, hematology, coagulation were performed.

Randomization procedure: Prior to initiation of dosing, all animals were weighed and randomly assigned to dosage groups using a computer-based randomization procedure. Randomization was by stratification using body weight as the parameter.

Statistical analysis plan: Numerical data obtained during the conduct of the study were subjected to calculation of group means and standard deviations.

The following parameters were evaluated: Clinical signs (twice daily, except on day of arrival and necropsy only once), detailed physical examination (once prior to randomization on day -1 and daily thereafter), skin reactions at the intramuscular site of injection (immediately prior to dosing and approximately 5 minutes, 1, 3, 6, 24 and 48 hours after dosing), body weights (twice pretreatment (including day -1) and on day 2, last observation day; final body weight was taken at scheduled euthanasia), food consumption (daily, beginning 14 days prior to dosing) gross anatomy at termination, and histopathology on a selection of tissues.

Clinical observations

Parameters	Frequency of Testing
Cageside observation ¹	daily, except on day of arrival and necropsy only once
Clinical observations ²	once prior to randomization on day -1 and daily thereafter
Body weight	twice pretreatment (including day -1) and on day 2, last observation day
Food consumption	daily, beginning 14 days prior to dosing
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry	NC
Hematology	NC
Coagulation	NC
Immunological response	NC
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	immediately prior to dosing and approximately 5 minutes, 1, 3, 6, 24 and 48 hours after dosing
Necropsy	One at day 2
Tissues for histopathology	One at day 2

(NC = not collected)

¹ Cageside observations include mortality, morbidity, general health and signs of toxicity.

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

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

Histology evaluation

Organ/Tissue	Collected	Not collected
Adrenal glands	!	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	!	
Cervix*	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		
Gall bladder	!	
Gross lesions (if any)		
Gut associated lymphoid tissue	!	
Harderian gland (if applicable)		
Heart	!	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!	
Lacrimal glands		
Larynx		
Liver	!	
Lung (main-stem; bonchi)	!	
Lymph nodes (mandibular, unilateral; and mesenteric)	!	
Lymph nodes (inguinal)	!	
Lymph nodes (cervical)		!
Lymph nodes (mandibular)		!

Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!
Optic nerves	!	
Ovaries*	!	
Pancreas	!	
Peyer's patch (if applicable)		
Pituitary gland	!	
Prostate	!	
Rectum		!
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicles	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar)	!	
Spleen	!	
Stomach (squamous and glandular)	!	
Testes	!	
Thymus	!	
Thyroid (w/ parathyroid glands)	!	
Tongue	!	
Trachea	!	
Ureters	!	
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		

Table of Histology – Tissues examined: All dose group
 (!) Histology was performed; * males only study

Results:

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

Systemic toxicity:

No treatment-related mortality and no toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, gross anatomy or organ weight were found, except dark foci in the thymus of one (out of three) animals in the 13vPnC plus adjuvant group.

Gross Pathology:

Groups:

1: saline control

2: 13vPnC without adjuvant

3: 13vPnC with adjuvant title table

Gross Pathology

Group	Findings
1, 2, 3	Dark area at the injection site (1: 2/3 animals, 2: 1/3 animals, 3: 2/3 animals)
3	Dark foci in the thymus (3: 1/3 animals)

Microscopic finding

Groups	Findings
1,2	Injection site: myofiber degeneration/necrosis (1: 1/3 animals, 2: 1/3 animals)
1,2,3	Injection site: Hemorrhage (1: 2/3 animals, 2: 1/3 animals, 3: 2/3 animals)
1,2	Injection site: inflammation (1: 1/3 animals, 2: 1/3 animals)
3	Injection site :infiltration of mononuclear cells (3: 1/3 animals)
3	Hemorrhage in the thymus (3: 1/3 animals)

A number of tissues were examined for histology. The only finding reported was a hemorrhage in the thymus in one animal of the 13vPnC adjuvant group.

Local toxicity: Dark areas at the injection site were described for all groups probably representing trauma as a result of the injection. Microscopically, degeneration and necrosis as well as inflammation were observed at the injection site in group 1 (saline control) and 2 (13vPnC without adjuvant). Hemorrhage was detected in all three groups.

Draize scoring of the injection site revealed the following as presented in the table below¹.

Erythema

Treatment group																				
	5min post dose					1h post dose					3h post dose					6h post dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	2	1				2	1				2	1				2	1			
2	3					3					3					3				
3	3					3					3					3				

Erythema

Treatment group																				
	24h post dose					48h post dose														
	0	1	2	3	4	0	1	2	3	4										
1	3					3														
2	3					3														
3	3					3														

Edema

Treatment group																				
	5min post dose					1h post dose					3h post dose					6h post dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4		1	2	3	4
1	2			1		2		1			2		1			3				
2	3					3					3					3				
3	3					3					3					3				

Edema

Treatment group																				
	24h post dose					48h post dose														
	0	1	2	3	4	0	1	2	3	4										
1	3					3														
2	3					3														
3	3					3														

¹ 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.

Draize scoring of the injection sites revealed only irritation in one animal in the saline control group; the 13vPnC peptide group and the 13vPnC peptide adjuvant group did not show any measurable irritation.

Microscopically, degeneration and necrosis as well as inflammation were observed at the injection site in animals in group 1 (saline control) and 2 (13vPnC without adjuvant). Hemorrhage was detected in all three groups.

Assessment:

No treatment-related mortality and no toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, gross anatomy or organ weight were found.

Draize scoring of the injection sites revealed only irritation in one animal in the saline control group, the 13vPnC peptide group and the 13vPnC peptide adjuvant group did not show any measurable irritation. Microscopically hemorrhages were detected in all three groups (saline control, 13vPnC without and without adjuvant), degeneration and necrosis as well as inflammation were observed at the injection site of the saline control and the 13vPnC without adjuvant group.

This study compared the potential toxicity of the adjuvant in the presence 13vPnC and/or saline. No difference between saline control, 13vPnC with and without adjuvant was observed. However, only one injection was given in this study while in all other animal studies at least 5 injections were given (in humans four injections are planned). In the other animal studies local toxicity was not observed after the first injection, only after multiple injections the treatment group (13vPnC plus adjuvant) showed local toxicity. Therefore, it is possible that the difference in local toxicity between 13vPnC and 13vPnC with adjuvant will only be observed after multiple injections and a single injection is not sufficient to show this difference.

**Title 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC):
THIRTEEN WEEK (1 DOSE/2 WEEKS) SUBCUTANEOUS INJECTION
TOXICITY STUDY IN RATS (PROTOCOL 05_0330)**

Study number: 501296

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

Study initiation date: 20 May 2005

Final Report date: 07.03.2007

Test article batch/lot: 7-5064-001A

Animal species and strain: -----(b)(4)----- rat, outbred

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

Number of animal per group and sex: 20

Age: 56 days

Body weight range: males: 266 to 317 g; females: 187 to 222 g

Route and site of administration: subcutaneous

Volume of injection: 0.5 mL/injection

Frequency of administration and study duration: once every 2 weeks for 13 weeks on days 1, 15, 29, 43, 57, 71, and 85 (a total of seven injections); treatment phase: 13 weeks; recovery phase: 4 weeks.

Dose 30.8 µg polysaccharide, 29 µg CRM₁₉₇, 0.125 mg aluminum phosphate/0.5 mL

Stability: The stability of the capsular saccharide protein conjugate (13 valent) vaccine Lot # 7-5064-001A was tested over a period of 12 months, and was found to be stable.. The expiration date was determined as January 1, 2006 when stored at 5± 3°C

Means of administration: needles and syringes

Report status: final

Experimental study design

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1 Saline Control	0.9% sodium chloride	10	10
2 13 vPnCa	30.8 µg polysaccharide, 29 µg CRM ₁₉₇ , 0.125 mg aluminum phosphate	10	10

a: Clinical dosage formulation; *Dose was delivered subcutaneously on day 1, 15, 29, 43 57, 71 and 85.

Methods: Hematology: --(b)(4)-- Analyzer, light scatter, WBC differential stain: -----(b)(4)---
----- stained, microscopic enumeration; Coagulation: -----(b)(4)-----
----- Clinical chemistry: -----(b)(4)-----
-----.

Randomization procedure: Prior to dose initiation, all animals were weighed and assigned to dose groups using a randomization procedure. Randomization was by stratification using body weight as the parameter. Males and females were randomized separately. Animals in poor health or at the extremes of the body weight range were not assigned to groups.

Statistical analysis plan: For organ weight data, statistical analysis was conducted on absolute weight, organ-to-body weight ratio, and organ-to-brain weight ratio. For each parameter of interest, if none of the group variances were equal to zero, the group variances were tested for homogeneity using the folded form of the *F* statistic (computed from the ratio of the group variances). When group variances were not found to be heterogeneous, a two-sided *t*-test was used to compare both group means. Whenever group variances were found to be heterogeneous ($p \leq 0.05$), the Satterthwaite's approximation was used when performing the two-sided *t*-test. If one of the group variances was equal to zero, the group comparison was done using the exact Wilcoxon rank-sum test. For each group comparison with the *t*-test or the exact Wilcoxon rank-sum test, significance was reported at the 0.05, 0.01, and 0.001 levels.

Clinical observations: The following parameters were evaluated: clinical signs (twice daily, except of arrival and necropsy), detailed physical examination (day -6, -1 and weekly thereafter), injection site evaluation (pre dose, 4 and 24 hours post injection, daily for 6 days, day of necropsy), body weight (weekly, starting at the last week of acclimation), food consumption (weekly, starting at the last week of acclimation), ophthalmology (once prior to start, during week 12 and 16), hematology and biochemistry (day 3 and 31), hematology, biochemistry and coagulation (day 74 and 112), antibody evaluation (day -5, 87, 115) necropsy and histopathology (day 87, 115)

Clinical observations

Parameters	Frequency of Testing
Cageside observation ¹	twice daily, except of arrival and necropsy
Clinical observations ²	day -6, -1 and weekly thereafter
Body weight	weekly, starting at the last week of acclimation
Food consumption	weekly, starting at the last week of acclimation
Body temperature	NC
Ophthalmologic exam	once prior to start, during week 12 and 16
Clinical chemistry*	day 3, 31, 74 and 112
Hematology*	day 3, 31, 74 and 112
Coagulation*	day 74 and 112
Immunological response	day -5, 87, 115
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	predose, 4 and 24 hours post injection, daily for 6 days, day of necropsy
Necropsy	day 87, 115
Tissues for histopathology	day 87, 115

*(indicate blood collection site: jugular vein of unanesthetized animals) (NC = not collected)

¹ Cageside observations include mortality, morbidity, general health and signs of toxicity.

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

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

Histology evaluation

Organ/Tissue	Collected	Not collected
Abnormalities	!	
Adrenal glands	!*	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/pons, and olfactory bulb)	!*	
Cervix	!	
Cecum	!	
Colon	!	
Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		
Gall bladder		!
Gross lesions (if any)	!	
Gut associated lymphoid tissue	!	
Harderian gland (if applicable)		!
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		!
Larynx		!
Liver	!*	
Lung (main-stem; bronchi)	!	
Lymph nodes (cervical)		
Lymph nodes (mandibular and mesenteric)	!	
Lymph nodes (right and left axillary)	!	
Mammary glands	!	

(inguinal)		
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		!
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)		
Pituitary gland	!*	
Prostate	!*	
Rectum		!
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Seminal vesicles	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar)	!	
Spleen	!*	
Stomach (squamous and glandular)	!	
Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!*	
Tongue	!	
Trachea	!	
Ureters		!
Uterus (w/ cervix)	!	
Urinary bladder	!	
Vagina	!	
Zymbal's gland (if applicable)		!

Table of Histology – Tissues examined: All dose group

(*) Organ weight was determined

(!) Histology was performed

Results:

Morbidity and mortality:

Two unscheduled deaths occurred during the study. One control female (animal no. 1502) and one 13vPnC-treated female (animal no. 2507) were found dead on day 74. Because of the lack of previous relevant clinical history and the occurrence of the deaths during or shortly after

blood collection for clinical pathology these deaths were considered related to the blood collection procedure. All other animals survived to scheduled termination.

Clinical Chemistry

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase sorbitol dehydrogenase Total bile acids
		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen ND
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Males: TRIG (mg/dL), day 31: saline control (229.4) is increased, 1.9x higher than the 13vPnC (123.4) group. Females: TRIG (mg/dL), day 31: saline control (153.1) is increased, 2.2 x higher than the 13vPnC (69.4) group. Day 74: saline control (100.3) is increased, 1.8 x higher than the 13vPnC (55.8) group.	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase Fasting triglycerides

Hematology

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean corp. Hb. (MCH) Mean corp. Hb. Conc. (MCHC), Mean corp. Volume (MCV) Total erythrocyte count (RBC) Reticulocytes
WHITE BLOOD CELLS	<p>Females: % Neutrophils: day 31: 13vPnC (15.89) is 1.8x higher than the saline control (8.64) Day 74: 13vPnC (18.04) is 1.6x higher than the saline control (11.11) # Neutrophils: day 31: 13vPnC (1.342) is 1.8x higher than the saline control (0.760) Day 74: 13vPnC (1.043) is 1.6x higher than the saline control (0.659) # Monocytes: day 31: 13vPnC (0.209) is 1.6x higher than the saline control (0.133) % Monocytes: day 31: 13vPnC (2.49) is 1.6x higher than the saline control (1.53) # Monocytes: day 31: 13vPnC (0.209) is 1.6x higher than the saline control (0.133) # Basophils: day 112: saline control (0.049) is 1.6x higher than the 13vPnC (0.031)</p>	Basophils, eosinophils count lymphocyte count Macrophage/monocyte count Neutrophil count Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Systemic toxicity:

No treatment-related mortality (except the two females mentioned above) and no toxicologically relevant changes in clinical signs, relative food consumption and ophthalmoscopic parameters were found.

The 13vPnC group showed a 10% lower weight gain up to day -1, resulting in a 0.8% lower starting group mean body weight difference at day -1 compared to the saline group (note that at randomization there was no difference in the group mean body weights). On day 84 (end of dosing phase) the difference was 7% between the 13vPnC-treated females and controls. The rate of body weight gain for the females in the 13vPnC group from the time of randomization to day -1 was 10% less than those assigned to the control group. At the end of the dosing phase, the difference was 17% for the 13vPnC-treated females compared to controls. There was no corresponding effect on food consumption. At the end of the recovery period (day 112), the mean body weight of the females in the 13vPnC group were 5.5% less than controls; however, the rate of body weight gain was increased compared to controls.

Hematologic evaluation revealed an increase in the percent and number of neutrophils (1.8x) and monocytes (1.6x) in females of the treatment group on day 31; all other hematologic and biochemistry parameters were without pathologic findings.

Histologic examination revealed an adrenal cortical hypertrophy in one female in the treatment group and cytoplasmic granules containing macrophages in the axillary lymph node on day 87 in another female in the treatment group. In females in the treatment group on day 115 one female was diagnosed with a hyaline cast in the kidney, one with cytoplasmic granules containing macrophages in the axillary lymph node, one with a hemorrhage and one with subcutaneous inflammation of the skeletal muscle. However, these findings were rare and not associated with any other symptoms. In the treatment group 4 males out of 10 were diagnosed with inflammation of the prostate on day 115, on day 87 one animal in the control group showed inflammation of the prostate.

There were no treatment-related effects on organ weights or weight ratios for males receiving the 13vPnC vaccine, except a slight but statistically significant increase in adrenal weight in the females assigned to the treatment group on day 87 (absolute weight and per body weight).

Organ Weight

GROUPS		MALES				FEMALES			
		Saline control (day 87)	13vPnC (day 87)	Saline control (day 115)	13vPnC (day 115)	Saline control (day 87)	13vPnC (day 87)	Saline control (day 115)	13vPnC (day 115)
NUMBER OF ANIMALS		10	10	10	10	10	10	10	10
BODY WEIGHT (gram) ^a		570.748	556.646	590.150	582.751	306.219	289.573	317.538	301.279
BRAIN									
Absolute Weight ^a	gram	2.2571	2.2500	2.2645	2.2296	2.0042	2.0159	2.0140	1.9735
Per Body Weight ^a	%	0.39644	0.40768	0.38689	0.38681	0.65910	0.69901	0.63853	0.66341
ADRENALS									
Absolute Weight ^a	gram	0.06180	0.06639	0.05769	0.05589	0.05918	0.07100 **	0.06849	0.05958
Per Body Weight ^a	%	0.01084	0.01195	0.00979	0.00964	0.01950	0.02468 **	0.02161	0.02009
Per Brain Weight ^a	%	2.74420	2.95011	2.55162	2.50372	2.95353	3.53187	3.39549	3.02325
EPIDIDYMIDES									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
HEART									
Absolute Weight ^a	gram	1.6487	1.6985	1.6604	1.6682	1.0556	1.0603	1.1467	1.0903
Per Body Weight ^a	%	0.28915	0.30452	0.28167	0.28750	0.34589	0.36768	0.36348	0.36444
Per Brain Weight ^a	%	73.11839	75.69861	73.46808	74.83307	52.77326	52.58465	57.08796	55.25238
KIDNEYS									
Absolute Weight ^a	gram	3.2170	3.0174	3.3065	3.3682	1.8130	1.8978	1.9618	1.9264
Per Body Weight ^a	%	0.56274	0.54426	0.55982	0.58154	0.59284*	0.65828*	0.61844	0.64522
Per Brain Weight ^a	%	142.53357	134.3530	146.4570	151.1167	90.48239	94.24493	97.64594	97.61848
LIVER									
Absolute Weight ^a	gram	13.9827	13.2558	14.7156	13.9931	7.6037	7.5528	8.1996	7.5621
Per Body Weight ^a	%	2.44424	2.37941	2.48825	2.39127	2.48344	2.60944*	2.58296	2.51499
Per Brain Weight ^a	%	620.06809	591.5417	652.4261	626.6945	379.7171	375.30350	407.32495	383.34562
SPLEEN									
Absolute Weight ^a	gram	1.0242	0.8926	1.0170	0.8701	0.5357	0.5662	0.5901	0.5579
Per Body Weight ^a	%	0.17898	0.16103	0.17169*	0.15070*	0.17483	0.19661	0.18723	0.18537
Per Brain Weight ^a	%	45.38106	39.61330	45.00415	39.02269	26.73042	28.23685	29.36534	28.32848
TESTES									
Absolute Weight ^a	gram	3.5719	3.6107	3.7271	3.5752				
Per Body Weight ^a	%	0.62664	0.65412	0.63602	0.61933				
Per Brain Weight ^a	%	158.34613	160.8993	164.8596	160.5301				
THYROID and PARATHYROID									
Absolute Weight ^a	gram	0.02657	0.02581	0.03139	0.02752	0.02083	0.02146	0.02112	0.02240
Per Body Weight ^a	%	0.00466	0.00466	0.04227	0.04227	0.00679	0.00740	0.00668	0.00745
Per Brain Weight ^a	%	1.18099	1.14083	1.38383	1.22942	1.04093	1.06387	1.04906	1.13456

GROUPS		MALES				FEMALES			
		Saline control (day 87)	13vPnC (day 87)	Saline control (day 115)	13vPnC (day 115)	Saline control (day 87)	13vPnC (day 87)	Saline control (day 115)	13vPnC (day 115)
NUMBER OF ANIMALS		10	10	10	10	10	10	10	10
THYMUS									
Absolute Weight ^a	gram	0.25594	0.25243	0.24801	0.22866	0.21422	0.21002	0.18316	0.19186
Per Body Weight ^a	%	0.04495	0.04578	0.00539	0.00473	0.07047	0.07304	0.05753	0.06459
Per Brain Weight ^a	%	11.33285	11.26240	11.06135	10.26454	10.67082	10.43859	9.08888	9.75944
OVARIES									
Absolute Weight ^a	gram					0.09699	0.10207	0.10279	0.08871
Per Body Weight ^a	%					0.03179	0.03544	0.03255	0.02971
Per Brain Weight ^a	%					4.84431	5.08580	5.10375	4.50226
UTERUS									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
Pituitary									
Absolute Weight ^a	gram	0.01269	0.01314	0.01357	0.01401	0.01584	0.01580	0.01756	0.01668
Per Body Weight ^a	%	0.00223	0.00236	0.00231	0.00242	0.00521	0.00546	0.00550	0.00557
Per Brain Weight ^a	%	0.56334	0.56334	0.60130	0.62841	0.78999	0.78474	0.87228	0.84532
Prostate									
Absolute Weight ^a	gram	1.3291	1.4734	1.5338	1.6004				
Per Body Weight ^a	%	0.23318	0.26787	0.26240	0.27694				
Per Brain Weight ^a	%	58.94825	65.72118	67.85840	71.69165				

Table of organ weight and their normalization. Absolute weights are expressed as mean(grams) ± standard deviation (sd).

*different from saline control at $p \leq 0.05$;

**different from saline control at $P \leq 0.01$.

No significant differences in the organ weight between control group and treatment group was observed, except for the absolute and relative (per body weight, but not per brain weight) organ weight of the adrenals on day 87 which was slightly higher in the treatment group (female, $p \leq 0.01$). Furthermore, the relative (per body weight, but not per brain weight) weight for kidney and liver was slightly higher in females assigned to the treatment group compared to the control group at day 87 ($p \leq 0.05$). The absolute organ weight was not statistically significantly different. In males assigned to the control group the relative weight (per body weight, but not per brain weight) of the spleen was slightly higher compared to the spleens of the treatment group on day 115.

Gross Pathology:**Group identification:**

M1: male saline control group

M2: male treatment group

F1: female saline control group

F2: female treatment group

Gross pathology, day 87

Group	Findings
1M, 1F, 2F	Dark area at the injection site (1M: 3/10 animals, 1F: 4/10 animals, 2F: 1/10 animals)
2M, 2F	Dark foci at the injection site (2M: 4/10 animals, 2F: 4/10 animals)
2M, 2F	Mass at injection site (2M: 8/10 animals, 2F: 8/10 animals)
2M, 2F	Nodule at injection site (2M: 4/10 animals, 2F: 6/10 animals)
2M, 2F	Scab at injection site (2M: 1/10 animals, 2F: 1/10 animals)
2M, 2F	Thickening at injection site (2M: 3/10 animals, 2F: 1/10 animals)
2M, 1F	Dilatation of the kidney pelvis (2M: 1/10 animals, 1F: 1/10 animals)
1F	Pale foci in the kidney(1F: 2/10 animals)
1M	Dark area in the lung (1M: 1/10 animals)
1F	Pale area in the liver (1F: 1/10 animals)
2F	Pale discoloration in the liver (2F: 1/10 animals)
1M	Dark area in the auxiliary lymph node (1M: 1/10 animals)
2F	Enlargement of the auxiliary lymph node (2F: 1/10 animals)
1M	Enlargement of the mandibular lymph node (1M: 1/10 animals)
1M	Clot in muscle skeletal misc (1M: 1/10 animals)
1M	Enlargement of the pituitary gland (1M: 1/10 animals)
2M	Cyst in the spleen (2M: 1/10 animals)
1F, 2F	Depressed area in the stomach (1F: 1/10 animals, 2F: 1/10 animals)
1F, 2F	Dark foci in the stomach (1F: 1/10 animals)
1M, 2M, 1F, 2F	Dark foci in the thymus (1M: 1/10 animals, 2M: 1/10 animals, 1F: 1/10 animals, 2F: 3/10 animals)

Gross pathology, day 115

Group	Findings
2M	Dark adrenal discoloration (2M: 1/10 animals)
2M	Small adrenal (2M: 1/10 animals)
2M, 1F	Thickening of the cecum (2M: 1/10 animals, 1F: 1/10 animals)
2M, 2F	Nodule at injection site (2M: 9/10 animals, 2F: 10/10 animals)
2M	Thickening at injection site (2M: 1/10 animals)
1M, 2M	Dilatation of the kidney pelvis (1M: 1/10 animals, 2M: 1/10 animals)
1M	Pale material of the kidney pelvis (1M: 1/10 animals)

1F	Small kidney (1F: 1/10 animals)
1F	Irregular surface of the kidney (1F: 1/10 animals)
1M, 2M, 2F	Pale area of the liver (1M: 1/10 animals, 2M: 3/10 animals, 2F: 1/10 animals)
2M	Dark area in the lung (2M: 1/10 animals)
1M	Cyst in the spleen (2M: 1/10 animals)
2F	Clot in axilliar lymph node (2F: 1/10 animals)
2F	Dark are in mandibular lymph node (2F: 1/10 animals)
2F	Clot in muscle skeletal misc (2F: 1/10 animals)
1F	Ovary cyst (1F: 1/10 animals)
1F, 2F	Enlargement of pituitary gland (1F: 1/10 animals, 2M: 3/10 animals, 2F: 1/10 animals)
1M	Pale testis (1M: 1/10 animals)
1M	Small testis (1M: 1/10 animals)
1M, 2F	Dark foci in thymus (1M: 1/10 animals; 2F: 1/10 animals)
2M	Small thymus (2M: 1/10 animals)
2F	Clot in thymus (2F: 1/10 animals)
2M	Missing thyroid (2M: 1/10 animals)
2F	Clot in subcutaneous tissue (2F: 1/10 animals)

Microscopic findings, day 87

Groups	Findings
1M	Adrenal cortical focal vacuolation (1M: 1/10 animals)
2F	Adrenal cortical focal hypertrophy (2F: 1/10 animals)
1M, 1F	Infiltration of mononuclear cells in the Harderian gland (1M: 1/10 animals; 2F: 2/10 animals)
1M, 2M	Cardiomyopathy (1M: 2/10 animals; 2M: 3/10 animals)
2M, 2F	Acute/subacute subcutaneous inflammation at the injection site (2M: 6/10 animals; 2F: 6/10 animals)
2M, 2F	Chronic subcutaneous inflammation at the injection site (2M: 4/10 animals; 2F: 4/10 animals)
2M, 2F	Subcutaneous degeneration/necrosis at the injection site (2M: 9/10 animals; 2F: 9/10 animals)
2M, 2F	Degeneration/necrosis of the panniculus muscle at the injection site (2M: 9/10 animals; 2F: 9/10 animals)
1M, 2M, 1F, 2F	Hemorrhage at the injection site (1M: 1/10 animals; 2M: 3/10 animals; 1F: 4/10 animals; 2F: 7/10 animals)
2M	Inflammation at the injection site (1M: 1/10 animals)
1M, 1F, 2F	Pyelitis/pyelonephritis (1M: 1/10 animals; 1F: 2/10 animals; 2F: 2/10 animals)
1M	Tubular basophilia (1M: 2/10 animals)
1M	Kidney cyst (1M: 1/10 animals)
1M, 2M, 1F	Dilatation of the renal pelvis (1M: 1/10 animals; 2M: 1/10 animals; 1F: 1/10 animals)

1F, 2F	Infiltration of mixed cells in the liver (1F: 1/10 animals, 2F: 1/10 animals)
1F	Tension lipidosis of the liver (1F: 1/10 animals)
2M	Hemorrhage in the lung (2M: 1/10 animals)
1F	Histocytosis in the lung (1F: 1/10 animals)
1M, 1F	Erythrocytosis /hemorrhage in the axillary lymph node (1M: 1/10 animals, 1F: 1/10 animals)
2F	Cytoplasmic granules with macrophages in the axillary lymph node (2F: 1/10 animals)
1M, 2M	Lymphoid hyperplasia in the mandibular lymph node (1M: 1/10 animals, 2M: 1/10 animals)
1F	Hemorrhage in the muscle skeletal misc (1F: 1/10 animals)
1F	Follicular cyst at the ovary (1F: 1/10 animals)
1M, 2M	Inflammation of the pancreas (1M: 1/10 animals, 2M: 1/10 animals)
1M, 2M	Inflammation of the prostate (1M: 2/10 animals, 2M: 1/10 animals)
1M, 2M	Infiltration of mononuclear cells in the prostate (1M: 3/10 animals, 2M: 1/10 animals)
1M, 2M, 1F, 2F	Hemorrhage in the thymus (1M: 2/10 animals; 2M: 4/10 animals; 1F: 2/10 animals; 2F: 2/10 animals)
2M	Cyst in the thymus (2M: 1/10 animals)
1M, 1F, 2F	Hyperplasia with transitional cells in the urinary bladder (1M: 1/10 animals; 1F: 2/10 animals; 2F: 1/10 animals)

Microscopic findings, day 115

Groups	Findings
1M	Oligo/aspermia in the epididymis (1M: 1/10 animals)
1M, 2M, 1F	Infiltration of mononuclear cells in the Harderian gland (1M: 1/10 animals; 2M: 1/10 animals; 2F: 1/10 animals)
1M, 2M, 1F, 2F	Cardiomyopathy (1M: 3/10 animals; 2M: 3/10 animals, 1F: 1/10 animals; 2F: 1/10 animals)
2M, 2F	Chronic subcutaneous inflammation at the injection site (2M: 10/10 animals; 2F: 10/10 animals)
2M, 2F	Degeneration/necrosis of the panniculus muscle at the injection site (2M: 9/10 animals; 2F: 7/10 animals)
1F	Squamous cyst in the jejunum (1F: 1/10 animals)
1M, 2M, 1F	Tubular basophilia (1M: 1/10 animals; 2M: 1/10 animals; 1F: 1/10 animals)
2M	Kidney cyst (1M: 1/10 animals)
1M, 2M	Dilatation of the renal pelvis (1M: 1/10 animals; 2M: 1/10 animals)
2M	Hyaline cast in the kidney (2M: 1/10 animals)
1F	Pyelitis/pyelonephritis (1F: 1/10 animals)
2M	Tension lipidosis of the liver (2M: 4/10 animals)

2M, 1F, 2F	Erythrocytosis /hemorrhage in the axillary lymph node (1M: 1/10 animals, 1F: 1/10 animals; 2F: 1/10 animals)
2F	Cytoplasmic granules with macrophages in the axillary lymph node (2F: 1/10 animals)
2F	Hemorrhage in the skeletal muscle misc (2F: 1/10 animals)
2F	Subacute inflammation of the skeletal muscle misc (2F: 1/10 animals)
1M, 2M, 1F	Infiltration of mononuclear cells in the pancreas (1M: 1/10 animals; 2M: 3/10 animals; 1F: 2/10 animals)
2M	Inflammation of the prostate (2M: 4/10 animals)
1M, 2M	Infiltration of mononuclear cells in the prostate (1M: 2/10 animals, 2M: 1/10 animals)
2F	Subcutaneous hemorrhage (2F: 1/10 animals)
1M	Atrophy of the seminiferous epithelium in the testis (1M: 1/10 animals)
1M	Tubular dilatation in the testis (1M: 1/10 animals)
1M, 2M, 1F, 2F	Hemorrhage in the thymus (1M: 1/10 animals; 2M: 1/10 animals, 1F: 2/10 animals; 2F: 2/10 animals)
1F	Hyperplasia of transitional cells in the urinary bladder (1F: 1/10 animals)

A large number of different tissues were examined for histology. Findings related to the spleen, kidney, liver, lung, stomach, Harderian gland, pituitary gland, pancreas and prostate were isolated cases and evenly distributed between treated and control groups of animals.

On day 87 subacute, acute and chronic subcutaneous inflammation with necrosis and degeneration was seen in the majority of the treatment group animals at the injection site; as well as degeneration and necrosis of the panniculus muscle at the injection site. On day 115 (end of recovery phase) chronic subcutaneous inflammation was still observed in all the treatment group animals and degeneration and necrosis at the injection site was still observed in the majority of the treatment group animals.

Local toxicity: On day 87 dark foci, mass, thickening, nodule and scab were found at the injection site in the treatment group; while on day 115 only thickening and nodule were found on the injection site of the treatment group.

Systemic toxicity: On day 87 one female in the treatment group showed pale discoloration in the liver and one male was diagnosed with a cyst in the spleen. On day 115 a dark area in the lung as well as clot in the skeletal misc was found in one male of the treatment group. One female in the treatment group showed a clot in the thymus. However, these findings were rare and not associated with any other symptoms.

Draize scoring:

Edema, pre injection

Treatment group	Edema, pre injection																								
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	2					2					2					2					2				
	0					0					0					0					0				
13vPnC	2					2					2					2					2				
	0					0					0					0					0				

Edema, pre injection

Treatment group	Edema, pre injection									
	6 th dose					7 th dose				
	0	1	2	3	4	0	1	2	3	4
Saline control	2					2				
	0					0				
13vPnC	2					2				
	0					0				

Edema, 4h post injection

Treatment group	Edema, 4h post injection																								
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	2					2					2					2					2				
	0					0					0					0					0				
13vPnC	2					2					2					2					2				
	0					0					0					0					0				

Edema, 4h post injection

Treatment group	Edema, 4h post injection									
	6 th dose					7 th dose				
	0	1	2	3	4	0	1	2	3	4
Saline control	2					2				
	0					0				
13vPnC	2					2				
	0					0				

Edema, 24h post injection

Treatment group																																			
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose														
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4										
Saline control	2					2					2					1	1				2					0	9	+			2				
13vPnC	2					1	1				2					2					2					2					0				

Edema, 24h post injection

Treatment group																				
	6 th dose					7 th dose														
	0	1	2	3	4	0	1	2	3	4										
Saline control	2					2					0									
13vPnC	1	7				1	1	1			3					8				

Edema, 48 hours post injection

Treatment group																																			
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose														
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4										
Saline control	2					2					2					2					1	1				0	9								
13vPnC	2					1	2	2			1	1				2					2					2					0	6			

Edema, 48 hours post injection

Treatment group																				
	6 th dose					7 th dose														
	0	1	2	3	4	0	1	2	3	4										
Saline control	2					2					0									
13vPnC	1	5				2					5					0				

Edema, 72 hours post injection

Treatment group																									
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	2					2					2					1	1				2				
	0					0					0					9					0				
13vPnC	2					1	3	3			2					2					2				
	0					7					0					0					0				

Edema, 72 hours post injection

Treatment group										
	6 th dose					7 th dose				
	0	1	2	3	4	0	1	2	3	4
Saline control	2					2				
	0					0				
13vPnC	1	2				2				
	8					0				

Erythema, pre injection

Treatment group																									
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose				
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Saline control	2					2					2					2					2				
	0					0					0					0					0				
13vPnC	2					2					2					2					2				
	0					0					0					0					0				

Erythema, pre injection

Treatment group										
	6 th dose					7 th dose				
	0	1	2	3	4	0	1	2	3	4
Saline control	2					2				
	0					0				
13vPnC	2					2				
	0					0				

Erythema, 4h post injection

Treatment group																														
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2					2					2					2				
	0					0					0					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Erythema, 4h post injection

Treatment group										
	6 th dose					7 th dose				
	0	1	2	3	4	0	1	2	3	4
Saline control	2					2				
	0					0				
13vPnC	2					2				
	0					0				

Erythema, 24h post injection

Treatment group																														
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2					2					2					2				
	0					0					0					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Erythema, 24h post injection

Treatment group										
	6 th dose					7 th dose				
	0	1	2	3	4	0	1	2	3	4
Saline control	2					2				
	0					0				
13vPnC	2					2				
	0					0				

Erythema, 48h post injection

Treatment group																														
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2					2					2					2				
	0					0					0					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Erythema, 48h post injection

Treatment group															
	6 th dose					7 th dose									
	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2				
	0					0					0				
13vPnC	2					2					2				
	0					0					0				

Erythema, 72h post injection

Treatment group																														
	1 st dose					2 nd dose					3 rd dose					4 th dose					5 th dose									
		1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2					2					2					2				
	0					0					0					0					0					0				
13vPnC	2					2					2					2					2					2				
	0					0					0					0					0					0				

Erythema, 72h post injection

Treatment group															
	6 th dose					7 th dose									
	0	1	2	3	4	0	1	2	3	4					
Saline control	2					2					2				
	0					0					0				
13vPnC	2					2					2				
	0					0					0				

Draize scoring of the injection sites revealed edema with a slightly increase in frequency and severity observed in the treated animals compared to control animals. Maximal, a mild edema was observed. No erythema was observed in the control or treatment group.

At the injection site, in treated animals at the end of the treatment phase (day 87), subacute, acute and chronic subcutaneous inflammation with necrosis and degeneration as well as degeneration and necrosis of the panniculus muscle was observed upon histologic examination. At the end of the recovery phase (day 115) chronic subcutaneous inflammation and degeneration/necrosis was still observed at the site of injection; however, this did not result in a high Draize score.

Serology:

Serum was collected pretest and on days 87 or 115 for analysis of antibodies to one serotype, PnPs18C. The assay used was an -----(b)(4)-----

Serology analysis

	GMT (males): day -5 (U/ml)	GMT (females): day -5 (U/ml)	GMT (males): day 87 (U/ml)	GMT (females): day 87 (U/ml)	GMT (males): day 115 (U/ml)	GMT (females): day 115 (U/ml)
Serotype 18 C cont.	363	570	155	384	330	362
Serotype 18 C	264	489	78634	222880	53126	1179905

GMT: geometric mean titer

Serology analysis

	GMT (males & females): day -7 (U/ml)	GMT (males & females): day 87 (U/ml)	GMT (males & females): day 115 (U/ml)
Serotype 18 cont.	467	270	346
Serotype 18	377	150757	616515

GMT: geometric mean titer

A/G ratio: Administration of 13vPnC was associated with decreased albumin and increased globulin. There was a decrease in mean albumin in males (7% to 10%) on days 31 and 74 and in females (4% to 11%) on days 3, 31, and 74 compared to controls. There was an increase in mean globulin in males (6% to 11%) and females (13% to 17%) on days 31 and 74. These differences in albumin and globulin were associated with a decrease in the A/G ratio and were interpreted to be related to the microscopic findings at the injection site.

Assessment:

Neither treatment-related mortality nor toxicologically relevant changes in clinical signs, relative food consumption and ophthalmoscopic parameters and were found.

Body weight gain decreased in females in treatment groups during the dosing phase, but there was also slower body weight gain between randomization and day -1. A higher body weight gain rate during the recovery phase was observed in the treatment group.

Hematologically the percent and number of neutrophils (1.8x) and monocytes (1.6x) were slightly increased in females assigned to the treatment group on day 31 but not of clinical concern. All other parameters in hematology and biochemistry were without pathologic findings.

Histological, the injection site in treated animals showed subacute, acute and chronic subcutaneous inflammation with necrosis and degeneration as well as degeneration and necrosis of the panniculus muscle at the end of the dosing phase (day 87). After the recovery phase (day 115) chronic subcutaneous inflammation and degeneration/necrosis was still observed at the injection. Draize scoring of the injection sites revealed an increased frequency of irritation in the treated animals compared to control animals, but without increased severity

For the antibody titers large parts of the data are missing, only the antibody titers for serotype 18C were determined, there was no reason given why the other serotypes were not analyzed. However, there was a second subcutaneous study performed in rats (study number 501549, see below) which was identically designed to this one, in this study all serotypes were evaluated.

Title: PREVNAR AND 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (13vPnC): THIRTEEN WEEK (1 DOSE/2 WEEKS) SUBCUTANEOUS TOXICITY STUDY IN RATS (PROTOCOL 06_2408)

Study number: 6617-282

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

Study initiation date: 13 Sep 2006

Final Report date: 22 June 2007

Test article batch/lot: Pneumococcal 7-valent conjugate vaccine: 7-5092-005A;
 Pneumococcal 13-valent conjugate vaccine: 7-5095-001A;
 Vaccine Placebo: 7-8035-001A

Animal species and strain -----(b)(4)----- rat), outbred

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

Number of animal per group and sex: 20

Age: 6 to 7 weeks old

Body weight range: males: 196 to 273 g, females: 143 to 181 g

Route and site of administration: subcutaneous injection into the dorsal thoracic region

Volume of injection: 0.5 ml

Frequency of administration and study duration: once every 2 weeks for 13 weeks on days 1, 15, 29, 43, 57, 71, and 85 (a total of seven injections); treatment phase: 13 weeks; recovery phase: 4 weeks.

Dose: 30.8 µg polysaccharide (4.4 µg/mL of each of the serotypes except for serotype 6B at 8.8 µg/mL); 66.05 µg/mL CRM197 carrier protein.

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.

Expiration date: Pneumococcal 7-valent conjugate vaccine: 31 JUL 2007;
 Pneumococcal 13-valent conjugate vaccine: MAY 2007;
 0.9 % Sodium Chloride for Injection: 01 MAR 2008;
 Vehicle control article (placebo): 28 FEB 2007

Means of administration: syringe and needle

Report status: final

Experimental study design

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase (13 weeks)	Recovery phase (4 weeks)
1 Saline Control	0.9 % Sodium Chloride for Injection, (b)(4) (sterile saline)	10	10
2 Vehicle Control	5 mM succinate buffer at pH ---- --(b)(4)----- 0.025% Polysorbate 80, aluminumphosphate at 0.25 mg aluminum/mL	10	10
3 Prevnar	4.4 µg/mL of each of the serotypes except for serotype 6B at 8.8 µg/mL, 40 µg/mL CRM197 carrier protein,	10	10

	and is formulated with (b)(4) as an excipient at pH (b)(4) with aluminum phosphate at 0.25 mg aluminum/mL as an adjuvant		
4 13vPnC	30.8 µg polysaccharide (4.4 µg/mL of each of the serotypes except for serotype 6B at 8.8 µg/mL) 66.05 µg/mL CRM197 carrier protein, 5mM succinate buffer containing 0.02% Polysorbate 80 in (b)(4) at pH (b)(4) aluminum phosphate at 0.25 mg aluminum/mL	10	10

*Dose was delivered subcutaneously on day 1, 15, 29, 43, 57, 71 and 85.

Methods: The anticoagulants were sodium citrate for coagulation tests and potassium EDTA for hematology tests.

Randomization procedure: Computerized procedure designed to achieve body weight balance with respect to groups. After group assignment, the mean body weight for each group/sex will not be statistically different at the 5.0% probability level, as indicated by analysis of variance F probability.

Statistical analysis plan: Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at $p < 0.05$, transformation was used to stabilize the variance. Comparison tests took variance heterogeneity into consideration.

One-way analysis of variance (ANOVA) was used (if applicable) to analyze continuous clinical pathology values, organ weight data, food consumption, body weights, and body weight changes. If the ANOVA was significant, Dunnett's t-test was used for pair wise comparisons between treated and control groups.

When the ANOVA showed significance for body weights at week 1 of the dosing phase, one-way analysis of covariance (ANCOVA) was used to analyze body weights, with initial body weights as the covariate. When the ANCOVA was significant, covariate-adjusted means was used for control versus treated group comparisons.

The following parameters were evaluated: clinical signs (twice daily); cageside observation (once daily, 1 hour pre dosing); detailed clinical observations (once during predose phase, day 1, and weekly thereafter and on the day of scheduled euthanasia). Draize scoring (predose and approximately 4 and 24 hours postdose; prior to necropsy); body weight (predose days 1 and 8, first day of dosing, and weekly thereafter); food consumption (days 1 to 8 of the predose phase; weekly beginning on day 1); ophthalmic examinations (once during the predose phase and during weeks 12 and 16 of the dosing phase); antibody determination (predose phase (7 days prior to start of dose administration) from all animals and on days 87 and 115 of the dosing phase from animals scheduled for necropsy); hematology and clinical chemistry (day 3, 31, 73 and 112); coagulation test (day 73 and 112); necropsy (day 87 and 115).

Clinical observations

Parameters	Frequency of Testing
Cageside observation ¹	once daily, 1 hour pre dosing
Clinical observations ²	once during predose phase, day 1, and weekly thereafter and on the day of scheduled euthanasia
Body weight	predose days 1 and 8, first day of dosing, and weekly thereafter
Food consumption	days 1 to 8 of the predose phase; weekly beginning on day 1
Body temperature	NC
Ophthalmologic exam	once during the predose phase and during weeks 12 and 16 of the dosing phase
Clinical chemistry*	day 3, 31, 73 and 112
Hematology**	day 3, 31, 73 and 112
Coagulation***	day 73 and 112
Immunological response	predose phase (7 days prior to start of dose administration) from all animals and on days 87 and 115 of the dosing phase from animals scheduled for necropsy
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	predose and approximately 4 and 24 hours postdose; prior to necropsy
Necropsy	day 87 and 115
Tissues for histopathology	day 87 and 115

*(blood collection site: jugular vein) (NC = not collected)

¹ Cageside observations include mortality, morbidity, general health and signs of toxicity.

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

Postmortem procedures:**Histology evaluation**

Organ/Tissue	Collected	Not collected
Adrenal glands	!*	
Aorta	!	
Bone (sternum & femur)	!	
Bone marrow (sternum & femur)	!	
Brain (cerebrum, cerebellum, medulla/pons, and olfactory bulb)	!*	
Cervix		
Cecum	!	
Colon	!	

Duodenum	!	
Epididymides	!	
Esophagus	!	
Eyes (optic nerve)	!	
Fallopian tubes (oviduct)		
Gall bladder		
Gross lesions (if any)		
Harderian gland (if applicable)	!	
Heart	!*	
Ileum	!	
Injection site(s)	!	
Jejunum	!	
Kidneys	!*	
Lacrimal glands		
Larynx		
Liver	!*	
Lung (main-stem; bonchi)	!	
Lymph nodes (cervical)		
Lymph nodes (mandibular)	!	
Lymph nodes (mesenteric)	!	
Mammary glands	!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		
Ovaries	!*	
Pancreas	!	
Peyer's patch (if applicable)		
Pituitary gland	!*	
Prostate	!*	
Rectum		
Salivary glands (mandibular)	!	
Sciatic nerve	!	
Semina Vesicle	!	
Skeletal muscle	!	
Skin	!	
Spinal cord (cervical, lumbar, thoracic)	!	
Spleen	!*	
Stomach (squamous and glandular)	!	

Testes	!*	
Thymus	!*	
Thyroid (w/ parathyroid glands)	!*	
Tongue	!	
Trachea	!	
Ureters		
Uterus (w/ cervix)	!	
Urinary bladder		
Vagina	!	
Zymbal's gland (if applicable)		

Table of Histology – Tissues examined: All dose groups

(*) Organ weight was determined

(!) Histology was performed

Results:

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

Clinical Chemistry

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase sorbitol dehydrogenase Total bile acids

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
	<p>Male: TBIL (mg/dL) DSNG 73: the saline control (0.2) is 2x higher than 13PnC (0.1) RECO 26: the saline control (0.2) is 2x higher than Prevnar (0.1) DBIL (mg/dL) DSNG 73: the saline control (0.3) is 3x higher than 13PnC (0.1) ND: Prevnar: DSGN 3 and 73 IBIL (mg/dL) DSNG 31: 13PnC 0.1, all other groups are measured as 0. RECO 26: 13PnC (0.2) is 2x higher than the saline control (0.1) ND: Prevnar DSGN 3 and 73</p> <p>Females: TBIL (mg/dL) DSNG 73: the saline control (0.2) is 2x higher than Prevnar (0.1) and 13PnC (0.1) DBIL: DSGN 3: ND IBIL (mg/dL) DSGN 3: ND ALT (U/L): DSNG 31: the saline control (27) is 2x higher than 13PnC (48) DSNG 73: the saline control (39) is 1.9x higher than Prevnar (74) and 1.7 higher than 13PnC (65) RECO 26: the vehicle control (76) is 1.6x higher than the saline control (49)</p>	Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen ND
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Creatine kinase Fasting triglycerides

DSNG: dosing day; RECO: recovery day; ND: not determined

Hematology

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean corp. Hb. (MCH) Mean corp. Hb. Conc. (MCHC), Mean corp. Volume (MCV) Total erythrocyte count (RBC) Reticulocytes
WHITE BLOOD CELLS	<p>Males: Leukocytes (E3/μl): RECO 26: the saline control (0.14) is 1.6x higher than 13vPnC (0.09)</p> <p>Females: Neutrophils (E3/μl): DSNG 31: Prevnar (2.29) and 13vPnC (1.79) are 2.9x and 2.3x higher than the saline control (0.79) DSNG 73: Prevnar (1.91) and 13vPnC (1.66) are 2x and 1.8x higher than the saline control (0.94) Monocytes (E3/μl): DSNG 31: Prevnar (0.33) and 13vPnC (0.29) are 2.5x and 2.2x higher than the saline control (0.13) DSNG 73: Prevnar (0.28) and 13vPnC (0.24) are 2.3x and 2x higher than the saline control (0.94) Basophiles (E3/μl): DSNG 73: the saline control (0.2) is 2x higher than Prevnar (0.02) Leukocytes (E3/μl): DSNG 31: Prevnar (0.08) and 13vPnC (0.07) are 2x and 1.8x higher than the saline control (0.04) PMON (%) DSNG 31: Prevnar (3.6) and 13vPnC (3.3) are 1.8x and 1.6x higher than the saline control (2.0) DSNG 73: Prevnar (3.9) is 1.9x higher than the saline control (2.1) PBAS (%) RECO 26: Prevnar (0.2) and 13vPnC (0.2) are 2x higher than the saline control (0.1)</p>	Basophils, eosinophils count lymphocyte count Macrophage/monocyte count Neutrophil count Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL	<p>Females: FIB mg/dL DSNG 73: Prevnar (350) is 1.7x higher than the saline control (211)</p>	Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

DSNG: dosing day; RECO: recovery day;

Systemic toxicity:

No treatment-related mortality and no toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption or organ weight were found. In the female Prevnar and 13vPnC treated group neutrophils were elevated on dosing day 31 (Prenvar 2.9x, 13vPnC 2.3x higher than the saline control) and day 73 (Prenvar 2x, 13vPnC 1.8x higher than the saline control). Furthermore, in females treated with 13vPnC the absolute number of monocytes were elevated on day 31 (Prenvar 2.5x, 13vPnC 2.2x higher than the saline control) and day 73 (Prenvar 2.3x, 13vPnC 2x higher than the saline control) and leukocytes were elevated on dosing day 31 (Prenvar 2x, 13vPnC 1.8x higher than the saline control). Also the percentage of monocytes and basophiles was elevated in females assigned to the treatment groups: monocytes on dosing day 31 (Prenvar 1.6x, 13vPnC 1.8x higher than the saline control) and 73 (Prenvar 1.8x higher than the saline control); basophiles on recovery day 26 (Prenvar and 13vPnC are 2x higher than the saline control).

Histological the following observations were made in isolated animals of the 13vPnC group but not the control group: focal hyperplasia of the adrenal cortex, mineralization of the gut associated lymphoid tissue, granulomatous inflammation of the heart, fibrosis of the liver, mixed cell inflammation with eosinophilic crystals, follicular cyst on the ovary, squamous stomach cyst, hyperplasia of the urinary bladder, lymphocytic inflammation of the urinary bladder, increased mucification in the vagina, necrosis in the liver, decreased cellularity in the mesenteric lymph node, mixed cell inflammation in the stomach, spontaneous unilateral atrophy, hemorrhage in the urinary bladder, necrosis of the urinary bladder. However, these findings were rare and not associated with any other symptoms.

Organ weight, dosing phase

GROUPS		MALES				FEMALES			
		1	2	3	4	1	2	3	4
NUMBER OF ANIMALS		10	9	10	10	9	9	9	10
BODY WEIGHT (gram) ^a		527.2	548.6	556.1	531.4	274.8	263.7	275.4	274.8
BRAIN									
Absolute Weight ^a	gram	2.329	2.363	2.353	2.291	2.077	2.057	2.111	2.103
Per Body Weight ^a	%	0.445	0.433	0.427	0.435	0.765	0.785	0.768	0.769
ADRENALS									
Absolute Weight ^a	gram	0.085	0.077	0.081	0.080	0.075	0.068	0.078	0.076
Per Body Weight ^a	%	1.620	1.397	1.451	1.514	2.777	2.606	2.844	2.778
Per Brain Weight ^a	%	3.640	3.244	3.426	3.489	3.626	3.293	3.706	3.616
EPIDIDYMIDES									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
HEART									
Absolute Weight ^a	gram	1.637	1.676	1.774	1.706	1.081	1.028	1.099	1.097
Per Body Weight ^a	%	0.312	0.306	0.318	0.323	0.394	0.394	0.400	0.400
Per Brain Weight ^a	%	70.236	70.974	75.374	74.424	52.254	50.124	52.075	52.270

		MALES				FEMALES			
GROUPS		1	2	3	4	1	2	3	4
NUMBER OF ANIMALS		10	9	10	10	9	9	9	10
KIDNEYS									
Absolute Weight ^a	gram	3.335	3.454	3.456	3.220	1.819	1.803	1.943	1.819
Per Body Weight ^a	%	0.635	0.630	0.626	0.610	0.664	0.686	0.708	0.664
Per Brain Weight ^a	%	143.306	146.097	147.147	140.551	87.927	87.747	92.391	86.703
LIVER									
Absolute Weight ^a	gram	13.529	14.409	14.731	14.038	7.738	7.472	8.195	7.583
Per Body Weight ^a	%	2.571	2.619	2.652	2.635	2.803	2.841	2.980	2.768
Per Brain Weight ^a	%	580.727	609.220	625.916	610.702	374.812	363.821	389.456	361.428
SPLEEN									
Absolute Weight ^a	gram	0.971	0.982	1.094	1.011	0.595	0.573	0.611	0.617
Per Body Weight ^a	%	0.183	0.179	0.197	0.191	0.217	0.218	0.221	0.226
Per Brain Weight ^a	%	41.575	41.611	46.462	43.975	28.564	27.813	28.957	29.392
TESTES									
Absolute Weight ^a	gram	3.634	3.837	3.789	3.743				
Per Body Weight ^a	%	0.694	0.701	0.688	0.711				
Per Brain Weight ^a	%	156.230	162.459	161.233	163.685				
THYROID and PARATHYROID									
Absolute Weight ^a	gram	0.036	0.033	0.040	0.030	0.029	0.022	0.024	0.027
Per Body Weight ^a	%	0.685	0.601	0.696	0.558	1.045	0.837	0.872	1.002
Per Brain Weight ^a	%	1.551	1.393	1.667	1.295	1.379	1.080	1.135	1.295
THYMUS									
Absolute Weight ^a	gram	0.359	0.453	0.373	0.326	0.351	0.300	0.334	0.378
Per Body Weight ^a	%	0.068	0.081	0.067	0.062	0.127	0.113	0.121	0.138
Per Brain Weight ^a	%	15.371	19.040	15.928	14.272	16.898	14.616	15.766	17.992
OVARIES									
Absolute Weight ^a	gram					0.162	0.150	0.163	0.177
Per Body Weight ^a	%					5.980	5.692	5.895	6.471
Per Brain Weight ^a	%					7.758	7.263	7.675	8.456*
UTERUS									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
Pituitary									
Absolute Weight ^a	gram	0.017	0.018	0.016	0.016	0.021	0.019	0.024*	0.023
Per Body Weight ^a	%	0.324	0.319	0.297	0.310	0.777	0.741	0.877	0.826
Per Brain Weight ^a	%	0.732	0.742	0.695	0.710	1.029	0.945	1.146	1.074
Prostate									
Absolute Weight ^a	gram	1.424	1.507	1.491	1.566				
Per Body Weight ^a	%	0.270	0.276	0.273	0.304				
Per Brain Weight ^a	%	60.997	63.942	63.763	68.823				

Table of organ weight and their normalization. Absolute weights are expressed as mean(grams) \pm standard deviation (sd); *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$

Organ weight, recovery phase

		MALES				FEMALES			
GROUPS		1	2	3	4	1	2	3	4
NUMBER OF ANIMALS		10	10	10	10	9	10	9	9
BODY WEIGHT (gram) ^a		565.7	552.6	542.9	570.3	289.9	294.4	297.5	294.9
BRAIN									
Absolute Weight ^a	gram	2.287	2.301	2.246	2.270	2.073	2.067	2.052	2.138
Per Body Weight ^a	%	0.405	0.419	0.415	0.403	0.717	0.709	0.693	0.731
ADRENALS									
Absolute Weight ^a	gram	0.071	0.072	0.072	0.071	0.066	0.074	0.069	0.078
Per Body Weight ^a	%	1.243	1.316	1.323	1.246	2.284	2.572	2.335	2.680
Per Brain Weight ^a	%	3.096	3.148	3.200	3.119	3.182	3.588	3.357	3.657
EPIDIDYMIDES									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
HEART									
Absolute Weight ^a	gram	1.897	1.853	1.71*	1.925*	1.118	1.192	1.216	1.153
Per Body Weight ^a	%	0.335	0.336	0.318	0.339	0.386	0.408	0.410	0.395
Per Brain Weight ^a	%	83.136	80.519	76.566	84.790	54.008	57.712	59.192	54.180
KIDNEYS									
Absolute Weight ^a	gram	3.702	3.351	3.267	3.578	1.877	1.883	1.877	1.937
Per Body Weight ^a	%	0.652	0.608	0.603	0.634	0.649	0.643	0.632	0.662
Per Brain Weight ^a	%	162.482	145.626	145.598	157.906	90.796	91.008	91.640	90.782
LIVER									
Absolute Weight ^a	gram	14.548	14.009	13.665	14.495	7.722	8.025	7.738	8.007
Per Body Weight ^a	%	2.569	2.521	2.517	2.549	2.665	2.735	2.600	2.716
Per Brain Weight ^a	%	637.566	608.430	609.696	638.551	373.179	388.285	377.863	374.851
SPLEEN									
Absolute Weight ^a	gram	1.034	0.955	0.974	1.015	0.585	0.628	0.586	0.586
Per Body Weight ^a	%	0.183	0.173	0.179	0.177	0.203	0.214	0.197	0.201
Per Brain Weight ^a	%	45.377	41.627	43.410	44.609	28.180	30.330	28.513	27.389
TESTES									
Absolute Weight ^a	gram	3.909	3.823	3.701	3.764				
Per Body Weight ^a	%	0.692	0.693	0.683	0.666				
Per Brain Weight ^a	%	171.327	165.795	164.934	165.629				
THYROID and PARATHYROID									
Absolute Weight ^a	gram	0.034	0.034	0.030	0.030	0.026	0.024	0.025	0.028
Per Body Weight ^a	%	0.595	0.617	0.549	0.540	0.885	0.823	0.848	0.944
Per Brain Weight ^a	%	1.471	1.468	1.336	1.345	1.232	1.170	1.220	1.298
THYMUS									
Absolute Weight ^a	gram	0.279	0.225	0.235	0.226	0.236	0.277	0.284	0.248
Per Body Weight ^a	%	0.049	0.040	0.043	0.040	0.082	0.095	0.095	0.084
Per Brain Weight ^a	%	12.245	9.793	10.481	9.940	11.442	13.434	13.864	11.483

		MALES				FEMALES			
GROUPS		1	2	3	4	1	2	3	4
NUMBER OF ANIMALS		10	10	10	10	9	10	9	9
OVARIES									
Absolute Weight ^a	gram					0.153	0.146	0.148	0.144
Per Body Weight ^a	%					5.310	4.960	4.997	4.909
Per Brain Weight ^a	%					7.383	7.076	7.182	6.695
UTERUS									
Absolute Weight ^a	gram								
Per Body Weight ^a	%								
Per Brain Weight ^a	%								
Pituitary									
Absolute Weight ^a	gram	0.016	0.017	0.014	0.016	0.021*	0.027*	0.024	0.028
Per Body Weight ^a	%	0.290	0.301	0.265	0.273	0.733*	0.932*	0.812	0.957
Per Brain Weight ^a	%	0.719	0.722	0.640	0.683	1.025*	1.303*	1.182	1.332
Prostate									
Absolute Weight ^a	gram	1.609	1.394	1.440	1.548				
Per Body Weight ^a	%	0.280	0.255	0.267	0.277				
Per Brain Weight ^a	%	70.751	60.794	64.137	68.414				

Table of organ weight and their normalization. Absolute weights are expressed as mean(grams) \pm standard deviation (sd). *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$.

Gross Pathology:

M1: male saline control group
M2: male vehicle control group
M3: male Prevnar treatment group
M4: male 13vPnC treatment group
F1: female saline control group
F2: female vehicle control group
F3: female Prevnar treatment group
F4: female 13vPnC treatment group

Gross pathology, dosing phase

Group	Findings
1M	Discolored eyes (1M: 1/10 animals)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Thickened injection site (1M: 1/10 animals; 2M: 7/10 animals; 3M: 9/10 animals; 4M: 10/10 animals; 2F: 5/10 animals; 3F: 7/10 animals; 4F: 9/10 animals)
2M, 2F, 3F	Firm injection site (2M: 1/10 animals; 2F: 1/10 animals; 3F: 2/10 animals)
3M	Discolored lung (3M: 1/10 animals)
1M, 3M, 2F, 3F	Large mandibular node (1M: 2/10 animals; 3M: 1/10 animals; 2F: 1/10 animals; 3F: 1/10 animals)
1F, 4F	Crusted skin (1F: 1/10 animals; 4F: 2/10 animals)
2F	Alopecia (2F: 1/10 animals)

Gross pathology, dosing phase

Group	Findings
2M, 3M, 4M, 2F, 3F, 4F	Thickened injection site (2M: 8/10 animals; 3M: 8/10 animals; 4M: 6/10 animals; 2F: 6/10 animals; 3F: 8/10 animals; 4F: 8/10 animals)
4F	Crusted injection site (4F: 2/10 animals)
3M	Kidney cyst (3M: 1/10 animals)
2M, 1F,	Large mandibular node (2M: 1/10 animals; 2F: 1/10 animals)
1M,	Gelatinous prostate (1M: 1/10 animals)
1M,	Gelatinous seminal vesicle (1M: 1/10 animals)
4F	Crusted skin (4F: 1/10 animals)
2F	Alopecia (2F: 1/10 animals)
2M	Distended urinary bladder (2M: 1/10 animals)
1M, 2M	Urinary bladder contains fluid (1M: 1/10 animals; 2M: 1/10 animals)
4M	Discolored urinary bladder (4M: 1/10 animals)
4F	Uterus contains fluid (4F: 1/10 animals)

Microscopic findings, dosing phase

Groups	Findings
1M, 2M, 4M	Vacuolation of adrenal cortex (1M: 1/10 animals; 2M: 2/9 animals; 4M: 1/10 animals)
4M	Focal hyperplasia of the adrenal cortex (4M: 1/10 animals)
1M, 2M, 3M, 4M, 1F	Cyst of bone/joint (1M: 1/10 animals; 2M: 2/9 animals; 3M: 3/10 animals; 4M: 1/10 animals; 1F: 1/9 animals)
1M	Cataract (1M: 1/10 animals)
1M, 4M, 2F	Retinal dysplasia ((1M: 1/10 animals; 4M: 1/10 animals; 2F: 2/9 animals)
1M	Hemorrhage in the (1M: 1/10 animals)
4M	Mineralization of the gut associated lymphoid tissue (4M: 1/10 animals)
3M, 4M, 1F, 3F	Lymphohistocytic inflammation of the Harderian gland (3M: 2/10 animals; 4M: 3/10 animals; 1F: 4/10 animals; 3F: 1/9 animals)
3M	Dilatation of Harderian gland (3M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Spontaneous rat cardiomyopathy (1M: 4/10 animals; 2M: 4/9 animals; 3M: 5/10 animals; 4M: 5/10 animals; 1F: 3/9 animals; 2F: 2/9 animals, 3F: 1/9 animals; 4F: 2/10 animals)
1F	Pigmented macrophage infiltrated heart (1F: 1/9 animals)
4M	Granulomatous inflammation of the heart (4M: 1/10 animals)
2M	Vascular cyst of the heart (2M: 1/9 animals)
2M, 3M, 4M, 1F, 2F, 3F, 4F	Acute subcutaneous inflammation at the injection site (2M: 3/9 animals; 3M: 8/10 animals; 4M: 5/10 animals; 1F: 1/9 animals; 2F: 7/9 animals, 3F: 9/9 animals; 4F: 9/10 animals)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Chronic subcutaneous inflammation at the injection site (1M: 1/10 animals; 2M: 9/9 animals; 3M: 9/10 animals; 4M: 10/10 animals; 2F: 8/9 animals, 3F: 9/9 animals; 4F: 10/10 animals)
1M, 2M, 3M, 4M, 3F, 4F	Tubular basophilia in the kidney (1M: 6/10 animals; 2M: 4/9 animals; 3M: 5/10 animals; 4M: 3/10 animals; 3F: 1/9 animals; 4F: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Lymphocytic inflammation of the kidney (1M: 3/10 animals; 2M: 5/9 animals; 3M: 4/10 animals; 4M: 6/10 animals; 1F: 4/9 animals; 2F: 1/9 animals, 3F: 1/9 animals; 4F: 2/10 animals)
1M, 4M, 3F, 4F	Pelvic inflammation of the kidney (1M: 1/10 animals; 4M: 2/10 animals; 3F: 1/9 animals; 4F: 1/10 animals)
2M, 3M, 4M, 1F, 4F	Tubular cast of the kidney (2M: 1/9 animals; 3M: 1/10 animals; 4M: 1/10 animals; 1F: 2/9 animals; 4F: 1/10 animals)
3M	Tubular mineralization of the kidney (3M: 1/10 animals)
2F, 3F, 4F	Corticomedullary mineralization of the kidney (2F: 1/9 animals, 3F: 2/9 animals; 4F: 2/10 animals)
3M, 4M	Tubular ectasia of kidney (3M: 1/10 animals; 4M: 1/10 animals)
1F	Capsular fibrosis of the kidney (1F: 1/9 animals)
2F	Focal interstitial fibrosis of the kidney (2F: 1/9 animals)
3F	Transitional cell hyperplasia of the kidney (3F: 1/10 animals)

1M	Hemorrhage in the kidney (1M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Mononuclear cell inflammation in the liver (1M: 10/10 animals; 2M: 9/9 animals; 3M: 10/10 animals; 4M: 10/10 animals; 1F: 9/9 animals; 2F: 9/9 animals, 3F: 9/9 animals; 4F: 10/10 animals)
1M, 2M, 3M, 4M,	Vacuolation of the liver (1M: 2/10 animals; 2M: 3/9 animals; 3M: 1/10 animals; 4M: 2/10 animals)
1M, 4M	Mixed cell inflammation in the liver (1M: 1/10 animals; 4M: 1/10 animals)
2M	Eosinic cell focus in the liver (2M: 1/9 animals)
4M	Fibrosis of the liver (4M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Alveolar macrophages in the lung (1M: 2/10 animals; 2M: 2/9 animals; 3M: 2/10 animals; 4M: 6/10 animals; 1F: 4/9 animals; 2F: 2/9 animals, 3F: 4/9 animals; 4F: 1/10 animals)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Lymphohistocytic inflammation in the lung (1M: 2/10 animals; 2M: 2/9 animals; 3M: 8/10 animals; 4M: 4/10 animals; 2F: 1/9 animals, 3F: 1/9 animals; 4F: 4/10 animals)
1M, 2M, 4M, 2F, 4F	Vascular mineralization in the lung (1M: 3/10 animals; 2M: 1/9 animals; 4M: 1/10 animals; 2F: 1/9 animals, 4F: 1/10 animals)
4M, 4F,	Mixed cell inflammation with eosinophilic crystals(4M: 1/10 animals; 4F: 1/10 animals)
2M, 3M, 3F, 4F	Perivascular inflammation in the lung (2M: 1/9 animals; 3M: 1/10 animals; 3F: 2/9 animals; 4F: 1/10 animals)
1F	Lobular hyperplasia of the mammary gland (1F: 1/9 animals)
1M, 2M, 3M,	Edema of the mandibular lymph node (1M: 1/10 animals; 2M: 3/9 animals; 3M: 1/10 animals)
1M, 3M, 4M, 1F, 2F, 3F	Sinus erythrocytosis in the mandibular lymph node(1M: 2/10 animals; 3M: 1/10 animals; 4M: 4/10 animals; 1F: 3/9 animals; 2F: 2/9 animals, 3F: 3/9 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Plasmacytosis in the mandibular lymph node (1M: 5/10 animals; 2M: 3/9 animals; 3M: 5/10 animals; 4M: 4/10 animals; 1F: 1/9 animals; 2F: 1/9 animals, 3F: 2/9 animals; 4F: 5/10 animals)
1M, 2M, 3M, 2F, 3F, 4F	Sinus histocytosis in the mandibular lymph node (1M: 2/10 animals; 2M: 4/9 animals; 3M: 1/10 animals; 2F: 1/9 animals, 3F: 2/9 animals; 4F: 3/10 animals)
3F	Cyst in the mandibular lymph node (3F: 1/9 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Edema in the mesenteric lymph node (1M: 7/10 animals; 2M: 6/9 animals; 3M: 5/10 animals; 4M: 5/10 animals; 1F: 5/9 animals; 2F: 5/9 animals, 3F: 6/9 animals; 4F: 3/10 animals)
1M, 4M, 3F	Sinus erythrocytosis in the mesenteric lymph node (1M: 1/10 animals; 4M: 2/10 animals; 3F:1/9 animals)
4M, 1F, 2F, 3F, 4F	Sinus histocytosis in the mesenteric lymph node (4M: 2/10 animals; 1F: 3/9 animals; 2F: 3/9 animals, 3F: 1/9 animals; 4F: 4/10 animals)
2F	Plasmacytosis in the mesenteric lymph node (2F: 1/9 animals)
4F	Follicular cyst on the ovary (4F: 1/10 animals)
2M, 3M, 4M, 1F, 2F, 3F	Lymphohistocytic inflammation of the pancreas (2M: 3/9 animals; 3M: 3/10 animals; 4M: 1/10 animals; 1F: 2/9 animals; 2F: 3/9

	animals, 3F: 1/9 animals)
2M, 3M, 1F	Acinar cell atrophy in the pancreas (2M: 1/9 animals; 3M: 1/10 animals; 1F: 1/9 animals)
3M, 1F, 2F, 3F	Cyst in the pituitary gland (3M: 1/10 animals; 1F: 1/9 animals; 2F: 1/9 animals, 3F: 2/9 animals)
1M, 3M, 4M,	Lymphohistocytic inflammation of the prostate (1M: 4/10 animals; 3M: 5/10 animals; 4M: 3/10 animals)
1F	Necrosis of the skeletal muscle (1F: 1/9 animals)
1F, 4F	Hyperkeratosis of the skin (1F: 1/9 animals; 4F: 1/10 animals)
2F	Atrophy of the skin (2F: 1/9 animals)
1F, 4F	Sero cellular crust on the skin (1F: 1/9 animals; 4F: 1/10 animals)
4F	Squamous stomach cyst (4F: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 3F, 4F	Glandular dilatation in the stomach (1M: 3/10 animals; 2M: 3/9 animals; 3M: 2/10 animals; 4M: 2/10 animals; 1F: 1/9 animals; 3F: 1/9 animals; 4F: 3/10 animals)
3F	Cyst in stomach (3F: 1/9 animals)
1M, 2M, 3M, 4M, 2F, 3F, 4F	Hemorrhage in thymus (1M: 4/10 animals; 2M: 1/9 animals; 3M: 4/10 animals; 4M: 2/10 animals; 2F: 2/9 animals; 3F: 2/9 animals; 4F: 3/10 animals)
1M, 2F, 3F	Cyst in thymus (1M: 1/10 animals; 2F: 2/9 animals; 3F: 2/9 animals)
1F	Pigmented macrophage infiltrate in the thymus (1F: 1/9 animals)
1M, 3M, 4M, 1F, 2F, 3F	Edema in the mesenteric lymph node (1M: 3/10 animals; 3M: 3/10 animals; 4M: 1/10 animals; 1F: 2/9 animals; 2F: 1/9 animals, 3F: 3/9 animals)
2M, 3M, 3F	Ectopic thymic tissue (2M: 2/9 animals; 3M: 1/10 animals; 3F: 2/9 animals)
3M	C-cell hyperplasia (3M: 1/10 animals)
4F	Hyperplasia of the urinary bladder (4F: 1/10 animals)
4M	Lymphocytic inflammation of the urinary bladder (4M: 1/10 animals)
1F, 3F, 4F	Dilatation of the uterus (1F: 1/9 animals; 3F: 2/9 animals; 4F: 3/10 animals)
4M	Increased mucification in the vagina(4M: 1/10 animals)

(NF = no findings)

Microscopic findings, recovery phase

Groups	Findings
1M, 4M	Vacuolation of adrenal cortex (1M: 2/10 animals; 4M: 1/10 animals)
1M, 2M, 3M, 4M, 2F, 3F	Cyst of bone/joint (1M: 1/10 animals; 2M: 2/9 animals; 3M: 3/10 animals; 4M: 3/10 animals; 2F: 1/9 animals; 3F: 1/9 animals)
2M, 3F	Mixed cell infiltration of the cecum (2M: 1/10 animals; 3F: 1/9 animals)
3M, 4M, 1F	Parasitism in the colon (3M: 1/10 animals; 4M: 1/10 animals; 1F: 1/9 animals)
4M, 1F	Retinal dysplasia (4M: 1/10 animals; 1F: 1/9 animals)
1M	Cyst in the gut associated lymphoid tissue (4M: 1/10 animals)
1M, 2M, 4M	Lymphohistocytic inflammation of the Harderian gland (1M: 3/10 animals; 2M: 1/10 animals; 4M: 2/10 animals)
1M, 2M, 3M, 4M, 1F, 3F, 4F	Spontaneous rat cardiomyopathy (1M: 5/10 animals; 2M: 7/9 animals; 3M: 4/10 animals; 4M: 6/10 animals; 1F: 3/9 animals; 3F: 1/9 animals; 4F: 1/9 animals)
2M, 3M, 4M, 1F, 2F, 3F, 4F	Chronic subcutaneous inflammation at the injection site (2M: 9/9 animals; 3M: 9/10 animals; 4M: 7/10 animals; 1F: 1/10 animals; 2F: 10/10 animals, 3F: 9/9 animals; 4F: 8/9 animals)
1M, 2M, 3M, 4M	Tubular basophilia in the kidney (1M: 3/10 animals; 2M: 1/10 animals; 3M: 1/10 animals; 4M: 4/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Lymphocytic inflammation of the kidney (1M: 6/10 animals; 2M: 5/10 animals; 3M: 6/10 animals; 4M: 4/10 animals; 1F: 2/9 animals; 2F: 1/10 animals, 3F: 2/9 animals; 4F: 1/9 animals)
3M, 4M, 2F	Pelvic inflammation of the kidney (3M: 1/10 animals; 4M: 1/10 animals; 2F: 1/9 animals)
1M,	Tubular cast of the kidney (1M: 1/10 animals)
1F, 3F, 4F	Corticomedullary mineralization of the kidney (1F: 1/9 animals, 3F: 1/9 animals; 4F: 1/10 animals)
1M, 4M	Tubular ectasia of kidney (1M: 1/10 animals; 4M: 1/10 animals)
1M, 2M, 1F	Tubular cyst in the kidney (1M: 1/10 animals; 1M: 1/10 animals, 1F: 1/10 animals)
1M, 4F	Tubular fibrosis in the kidney (1M: 1/10 animals; 4F: 1/9 animals)
1M	Focal interstitial fibrosis of the kidney (1M: 1/10 animals)
2F	Hemorrhage in the kidney (2F: 1/10 animals)
1M, 4M	Papillary necrosis (1M: 1/10 animals; 4M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Mononuclear cell inflammation in the liver (1M: 9/10 animals; 2M: 10/10 animals; 3M: 10/10 animals; 4M: 9/10 animals; 1F: 9/9 animals; 2F: 10/10 animals, 3F: 9/9 animals; 4F: 8/9 animals)
1M, 2M, 4M, 2F	Vacuolation in the kidney (1M: 1/10 animals, 2M: 1/10 animals; 4M: 3/10 animals; 2F: 1/10 animals)
3M, 4M	Necrosis in the liver (3M: 1/10 animals; 4M: 2/10 animals)
2F	Bile duct hyperplasia (2F: 1/10 animals)
2F	Focal fatty changes in the liver (2F: 1/10 animals)

1M, 2M, 3M, 1F, 2F, 3F	Alveolar macrophages in the lung (1M: 2/10 animals; 2M: 1/10 animals; 3M: 3/10 animals; 1F: 2/9 animals; 2F: 4/10 animals, 3F: 1/9 animals)
1M, 2M, 3M, 1F, 4F	Lymphohistocytic inflammation in the lung (1M: 1/10 animals; 2M: 2/10 animals; 3M: 1/10 animals; 1F: 1/9 animals, 4F: 1/9 animals)
1M, 2M, 4M, 2F, 3F	Vascular mineralization in the lung (1M: 2/10 animals; 1M: 1/10 animals; 4M: 2/10 animals; 2F: 1/10 animals, 3F: 1/9 animals)
2F,	Mixed cell inflammation with eosinophilic crystals(2F: 1/10 animals)
2F, 4F	Perivascular inflammation in the lung (2F: 3/10 animals; 4F: 2/9 animals)
2F, 4F	Lobular hyperplasia of the mammary gland (2F: 2/10 animals; 4F: 2/9 animals)
1M, 2M, 3M, 4M, 1F	Edema of the mandibular lymph node (1M: 1/10 animals; 2M: 1/9 animals; 3M: 1/10 animals; 4M: 1/10 animals; 1F: 2/9 animals)
1M, 2M, 3M,	Sinus erythrocytosis in the mandibular lymph node(1M: 1/10 animals; 2M: 2/10 animals; 3M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Plasmacytosis in the mandibular lymph node (1M: 4/10 animals; 2M: 4/10 animals; 3M: 5/10 animals; 4M: 6/10 animals; 1F: 2/9 animals; 2F: 2/10 animals, 3F: 1/9 animals; 4F: 2/9 animals)
1M, 3M	Sinus histocytosis in the mandibular lymph node (1M: 1/10 animals; 3M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F	Edema in the mesenteric lymph node (1M: 5/10 animals; 2M: 2/10 animals; 3M: 4/10 animals; 4M: 2/10 animals; 1F: 1/9 animals; 2F: 6/10 animals, 3F: 5/9 animals)
1M	Sinus erythrocytosis in the mesenteric lymph node (1M: 1/10 animals)
1M, 3M, 1F, 2F, 3F, 4F	Sinus histocytosis in the mesenteric lymph node (1M: 1/10 animals; 3M: 2/10 animals; 1F: 1/9 animals; 2F: 1/10 animals, 3F: 2/9 animals; 4F: 3/9 animals)
4M	Decreased cellularity in the mesenteric lymph node (4M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Lymphohistocytic inflammation of the pancreas (1M: 2/10 animals; 2M: 3/10 animals; 3M: 4/10 animals; 4M: 2/10 animals; 1F: 1/9 animals; 2F: 4/10 animals, 3F: 3/9 animals; 4F: 4/9 animals)
2M, 3M, 3F	Acinar cell atrophy in the pancreas (2M: 1/9 animals; 3M: 1/10 animals; 3F: 2/9 animals)
1M	Fibrosis in the pancreas (1M: 1/10 animals)
1M, 2M, 3M, 4M, 2F, 3F	Cyst in the pituitary gland (1M: 3/10 animals; 2M: 1/10 animals; 3M: 1/10 animals; 4M: 2/10 animals; 2F: 2/10 animals, 3F: 1/9 animals)
1M, 2M, 3M, 4M,	Lymphohistocytic inflammation of the prostate (1M: 4/10 animals; 2M: 3/10 animals; 3M: 3/10 animals; 4M: 2/10 animals)
1M	Fibrinopurulent inflammation of the seminal vesicle (1M: 1/10 animals)

2F	Sero-cellular crust on the skin (2F: 1/10 animals)
1M, 4M	Decreased cellularity in the spleen (1M: 3/10 animals; 4M: 1/10 animals)
1M, 3M, 4M, 3F	Glandular dilatation in the stomach (1M: 1/10 animals; 3M: 2/10 animals; 4M: 1/10 animals; 3F: 1/9 animals)
4M	Mixed cell inflammation in the stomach (4M: 1/10 animals)
1M	Edema in the stomach (1M: 1/10 animals)
4M	Spontaneous unilateral atrophy (4M: 1/10 animals)
2M, 4M, 3F, 4F	Hemorrhage in thymus (2M: 1/10 animals; 4M: 1/10 animals; 3F: 1/9 animals; 4F: 1/9 animals)
1M, 2F	Cyst in thymus (1M: 1/10 animals; 2F: 2/10 animals)
1M, 4M	Decreased cellularity in the thymus (1M: 1/10 animals; 4M: 1/10 animals)
1M, 2M, 3M, 4M, 1F, 2F, 3F, 4F	Squamous cyst in the thyroid (1M: 2/10 animals; 2M: 3/10 animals; 3M: 6/10 animals; 4M: 2/10 animals; 1F: 1/9 animals; 2F: 4/10 animals, 3F: 5/9 animals; 4F: 3/9 animals)
1M, 2M, 3F	Ectopic thymic tissue (1M: 1/10 animals; 2M: 1/10 animals; 3F: 1/9 animals)
1F	Histocytic inflammation of the thyroid (1F: 1/9 animals)
4M, 3F	Glandular dilatation in the trachea (4M: 2/10 animals, 3F: 1/9 animals)
1M	Hyperplasia of the urinary bladder (1M: 1/10 animals)
1M	Lymphocytic inflammation of the urinary bladder (1M: 1/10 animals)
4M	Hemorrhage in the urinary bladder (4M: 1/10 animals)
4M	Necrosis of the urinary bladder (4M: 1/10 animals)
1F, 3F, 4F	Dilatation of the uterus (1F: 1/9 animals; 3F: 2/9 animals; 4F: 3/9 animals)

Histology evaluation revealed the following observations in isolated animals in the 13vPnc group but not the control group: focal hyperplasia of the adrenal cortex, mineralization of the gut associated lymphoid tissue, granulomatous inflammation of the heart, fibrosis of the liver, mixed cell inflammation with eosinophilic crystals, follicular cyst on the ovary, squamous stomach cyst, hyperplasia of the urinary bladder, lymphocytic inflammation of the urinary bladder, increased mucification in the vagina, necrosis in the liver, decreased cellularity in the mesenteric lymph node, mixed cell inflammation in the stomach, spontaneous unilateral atrophy, hemorrhage in the urinary bladder, necrosis of the urinary bladder. However, these findings were rare and not associated with any other symptoms and seem not to be treatment related.

Local toxicity:

At the injection site an acute and chronic subcutaneous inflammation was observed following the dosing phase and a subcutaneous inflammation persisted by the end of the recovery phase.

It was stated in the procedure that Draize scoring was performed at 4h and 24h post injection. If findings were noted in specific animals, additional observations were conducted daily on the specific animals until the irritation scores returned to normal. From the data provided it is not evident that such evaluation occurred.

Draize scoring:

Edema

Treatment group	Edema																			
	1 st dose (Test site A)				2 nd dose (Test site B)				3 rd dose (Test site C)				4 th dose (Test site D)							
		1	2	3	4		1	2	3	4		1	2	3	4		1	2	3	4
1		1	1																	
2		2					3													
3		1					3				9	1	2			1	2	4		
		2										0				5	0			
4		9					7				1	1				1	2	6		
											1	4				5	7			

Edema

Treatment group	Edema																			
	5 th dose (Test site E)				6 th dose (Test site F)				7 th dose (Test site G)											
		1	2	3	4		1	2	3	4		1	2	3	4		1	2	3	4
1																				
2	1						2				1									
3	1	1				3	2				7	2								
	1					6														
4	1					3	5				9	2								
	9					4														

Erythema

Treatment group	Erythema																			
	1 st dose (Test site A)				2 nd dose (Test site B)				3 rd dose (Test site C)				4 th dose (Test site D)							
		1	2	3	4		1	2	3	4		1	2	3	4		1	2	3	4
1		1									4					1	1			
2											3									
3											6					5				
4											5					2				

Erythema

Treatment group	Erythema																			
	5 th dose (Test site E)				6 th dose (Test site F)				7 th dose (Test site G)											
		1	2	3	4		1	2	3	4		1	2	3	4		1	2	3	4
1																				
2																				
3		3	1																	
4		2																		

Draize scoring of the injection sites revealed an increased frequency of irritation in the treated animals compared to control animals, but without increased severity.

Serology:

Blood sample was collected pretest from a jugular vein (approximately 0.5 mL) and on days 87 and 115 of the dosing phase from the abdominal aorta of animals anesthetized in preparation for scheduled necropsy. Blood samples were collected into tubes containing no anticoagulant, held at room temperature for up to 1 hour, and allowed to clot; samples were centrifuged, and serum was harvested. Serum samples were stored in a freezer, set to maintain -20°C or lower.

Serology analysis, day 87

	GMT (males): day -7 (U/ml)	GMT (males): day 87 (U/ml)	GMT (females): day -7 (U/ml)	GMT (females): day 87 (U/ml)
Serotype 4 saline	305.7	55.9	310.9	156.2
Serotype 4 vehicle	221.7	60.1	358.5	146.8
Serotype 4 13vPnC	291.1	96698.0	249.2	54635.6
Serotype 4 Prevnar	234	264394.1	358.6	158648.5
Serotype 6B saline	751.1	804.1	1284.4	1514.4
Serotype 6B vehicle	739.9	276.7	1508.8	586.3
Serotype 6B 13vPnC	1046.0	88527.9	798.0	26677.8
Serotype 6B Prevnar	1170.9	193852.6	1331.5	119494.2
Serotype 9V saline	877.6	312.8	744.5	717.1
Serotype 9V vehicle	622.7	246.7	878.0	435.8
Serotype 9V 13vPnC	624.7	39435.4	637.6	104741.4
Serotype 9V Prevnar	993.8	93887.0	966.9	372636.9
Serotype 14 saline	66.2	252.5	94.9	163.4
Serotype 14 vehicle	64.2	443.3	50	259
Serotype 14 13vPnC	74.8	8608.6	50	15525.1
Serotype 14 Prevnar	71.8	3954.9	79.4	92350.8
Serotype 18C saline	318.9	699.0	407.5	378.2
Serotype 18C vehicle	399.9	126.2	530.8	273.2

Serotype 18C 13vPnC	500.5	89686.1	407.0	155422.7
Serotype 18C Prevnar	489.1	200172	716.3	893089.1
Serotype 19F saline	504.2	195.6	481.5	775.2
Serotype 19F vehicle	354.5	196.9	491.9	233.1
Serotype 19F 13vPnC	445.2	13645.4	421.1	13426.2
Serotype 19F Prevnar	352.7	707091	466.3	286033.9
Serotype 23F saline	548.8	330.6	892.4	6767.3
Serotype 23F vehicle	714.0	292.3	1044.8	549.9
Serotype 23F 13vPnC	1031.5	114440.6	848.9	129983.0
Serotype 23F Prevnar	832.9	347927.5	1208.3	35082.8

Serology analysis, day 115

	GMT (males): day -7 (U/ml)	GMT (males): day 115 (U/ml)	GMT (females): day -7 (U/ml)	GMT (females): day 115 (U/ml)
Serotype 4 saline	268.5	88.2	273.8	114.9
Serotype 4 vehicle	332.1	118.3	318.0	133.9
Serotype 4 13vPnC	287.8	29822.5	347.6	13587.0
Serotype 4 Prevnar	190.1	158648.5	379.6	307958.9
Serotype 6B saline	732.3	393.9	1435.7	1071.1
Serotype 6B vehicle	651.5	613.7	923.7	559.0
Serotype 6B 13vPnC	974.0	15822.1	949.9	1455.1
Serotype 6B Prevnar	879.0	59316.0	1803.1	61476.2
Serotype 9V saline	758.3	311.0	1024.5	644.0
Serotype 9V vehicle	565.7	541.9	700.9	604.6
Serotype 9V 13vPnC	920.4	22918.7	1139.6	115703
Serotype 9V Prevnar	736.6	6429.8	1367.8	145335.4
Serotype 14 saline	73	151.1	69.6	156.3
Serotype 14 vehicle	73.4	294.6	50	56.3
Serotype 14 13vPnC	72.2	1697.2	86.8	11919.1
Serotype 14 Prevnar	55.4	26769.7	64.8	36089.2
Serotype 18C saline	324.7	146.6	582.7	235.8
Serotype 18C vehicle	368.5	121.4	403.1	259.0
Serotype 18C 13vPnC	492.0	27216.8	506.2	8986.5
Serotype 18C Prevnar	438.5	40231.9	668.3	297243.4
Serotype 19F saline	477.1	261.9	427.3	4710.3
Serotype 19F vehicle	369.9	1031.8	418.5	381.0
Serotype 19F 13vPnC	406.0	12647.4	546.5	29229.2
Serotype 19F Prevnar	350.3	35284.1	623.9	170877.3
Serotype 23F saline	680.3	290.6	1028.3	582.4
Serotype 23F vehicle	437.7	558.1	1044.8	549.9
Serotype 23F 13vPnC	1099.6	69583.9	1548.7	207880
Serotype 23F Prevnar	424.4	71598.9	1322.2	384504.8

Serotypes only in 13vPnC, day 87

	GMT (males): day -7 (U/ml)	GMT (males): day 87 (U/ml)	GMT (females): day -7 (U/ml)	GMT (females): day 87 (U/ml)
Serotype 1 13vPnC	309.6	51421.6	90.6	18707.9
Serotype 3 13vPnC	692.7	8019.7	1195.5	18465.0
Serotype 5 13vPnC	749.8	72652.4	534.8	89369.6
Serotype 6A 13vPnC	669.8	148400.2	570.3	118442.0
Serotype 7F 13vPnC	637.0	138249.4	615.9	126293.1
Serotype 19A 13vPnC	1235.0	122033.4	991.9	122618.1

Serotypes only in 13vPnC, day 115

	GMT (males): day -7 (U/ml)	GMT (males): day 115 (U/ml)	GMT (females): day -7 (U/ml)	GMT (females): day 115 (U/ml)
Serotype 1 13vPnC	211.2	182198.5	522.4	32073.8
Serotype 3 13vPnC	623.5	4858.3	878.8	1414.2
Serotype 5 13vPnC	700.3	35107.6	910.8	68171.6
Serotype 6A 13vPnC	697.5	69920.5	993.2	53556.0
Serotype 7F 13vPnC	1120.1	31249.0	7907	9827.4
Serotype 19A 13vPnC	1664.4	78092.8	1161.9	110820.1

Serology analysis, day 87

	GMT (males & females): day -7 (U/ml)	GMT (males & females): day 87 (U/ml)
Serotype 1 saline	ND	ND
Serotype 1 13vPnC	119.7	21942.4
Serotype 3 saline	ND	ND
Serotype 3 13vPnC	651.6	8833.2
Serotype 4 saline	265.3	79.2
Serotype 4 vehicle	271.9	83.2
Serotype 4 13vPnC	232.1	49051.5
Serotype 4 Prevnar	240.6	253976.0
Serotype 5 saline	ND	ND
Serotype 5 13vPnC	444.7	62323.2
Serotype 6A saline	ND	ND

Serotype 6A 13vPnC	474.2	94373.1
Serotype 6B saline	642.9	491.9
Serotype 6B vehicle	935.1	327.9
Serotype 6B 13vPnC	768	39487.1
Serotype 6B Prevnar	1166.4	89015.2
Serotype 7F saline	ND	ND
Serotype 7F 13vPnC	555.7	60692.0
Serotype 9V saline	692.2	376.0
Serotype 9V vehicle	658.5	300.7
Serotype 9V 13vPnC	499.4	36471.6
Serotype 9V Prevnar	833.5	146459.6
Serotype 14 saline	66.3	170.9
Serotype 14 vehicle	55.1	247.4
Serotype 14 13vPnC	56.2	5294.0
Serotype 14 Prevnar	66.1	9849.8
Serotype 18C saline	311.7	205.0
Serotype 18C vehicle	451.3	157.4
Serotype 18C 13vPnC	430.5	93608.4
Serotype 18C Prevnar	517.1	315807.9
Serotype 19A saline	ND	ND
Serotype 19A 13vPnC	989.9	96498.3
Serotype 19F saline	395.0	193.1
Serotype 19F vehicle	392.6	187.2
Serotype 19F 13vPnC	380.0	11002.6
Serotype 19F Prevnar	351.5	63324.0
Serotype 23F saline	600.7	559.4
Serotype 23F vehicle	566.5	748.8
Serotype 23F 13vPnC	823.7	77757.6
Serotype 23F Prevnar	892.7	300750.0

GMT: geometric mean titer

Serology analysis, day 115

	GMT (males & females): day -7 (U/ml)	GMT (males & females): day 87 (U/ml)
Serotype 1 saline	ND	ND
Serotype 1 13vPnC	189.5	12827.9
Serotype 3 saline	ND	ND
Serotype 3 13vPnC	675.7	1986.1
Serotype 4 saline	275.5	88.2
Serotype 4 vehicle	275.2	98.4
Serotype 4 13vPnC	259.2	31315.2
Serotype 4 Prevnar	212.4	93657.9
Serotype 5 saline	ND	ND

Serotype 5 13vPnC	544.0	40378.6
Serotype 6A saline	ND	ND
Serotype 6A 13vPnC	66.7	35854.0
Serotype 6B saline	832.0	368.0
Serotype 6B vehicle	716.4	450.0
Serotype 6B 13vPnC	803.9	11901.4
Serotype 6B Prevnar	1106.3	45342.7
Serotype 7F saline	ND	ND
Serotype 7F 13vPnC	683.5	11562.4
Serotype 9V saline	721.0	27361.6
Serotype 9V vehicle	573.4	384.3
Serotype 9V 13vPnC	721.0	27361.6
Serotype 9V Prevnar	791.5	57891.2
Serotype 14 saline	60.9	122.1
Serotype 14 vehicle	58.6	238.0
Serotype 14 13vPnC	54.7	2900.8
Serotype 14 Prevnar	57.2	10243.9
Serotype 18C saline	390.4	123.4
Serotype 18C vehicle	370.4	136.6
Serotype 18C 13vPnC	433.0	35642.2
Serotype 18C Prevnar	479.8	45825.8
Serotype 19A saline	ND	ND
Serotype 19A 13vPnC	1160.5	41104.8
Serotype 19F saline	432.2	213.5
Serotype 19F vehicle	375.9	337.1
Serotype 19F 13vPnC	375.6	14439.7
Serotype 19F Prevnar	405.0	42999.2
Serotype 23F saline	700.4	296.4
Serotype 23F vehicle	621.2	422.1
Serotype 23F 13vPnC	942.8	72967.3
Serotype 23F Prevnar	866.9	136532.0

GMT: geometric mean titer

The samples were analyzed for antibodies to all 7 pneumococcal serotypes (Prevnar) in the saline group, vehicle group and Prevnar group; and to all 13 pneumococcal serotypes in the 13vPnC group. Analysis was conducted for an IgG response to each serotype using a qualified -----(b)(4)----- method. For the saline control group specimens, the vehicle control group specimens, and the Prevnar group specimens from dose days -7, 87, and 115, IgG antibody titers specific for each of the 7 serotypes of Prevnar were determined by (b)(4) using serotype-specific capsular pneumococcal polysaccharides (PnPs) as the coating antigens. For the 13vPnC group specimens from dose days -7, 87, and 115, IgG antibody titers specific for each of the 13 serotypes of 13vPnC were determined by (b)(4) using serotype-specific capsular PnPs as the coating antigens. For serotype 1, 3, 5, 6A, 7F and 19A which are the added serotypes in 13vPnC neither a vehicle control nor a saline control was performed, therefore there is no control included and the only comparison possible is between pre-dose and post-dose.

IgG responses determined from the (b)(4) indicated that none of the saline control group or vehicle control group animals mounted an immune response to any of the 7 serotypes of Prevnar, the animals given Prevnar mounted a positive serum IgG antibody response to each of the 7 serotypes of Prevnar after 7 doses, and the animals given 13vPnC mounted a positive serum IgG antibody response to each of the 13 serotypes of 13vPnC after 7 doses. It has been noted that Prevnar leads to higher titers than 13vPnC on day 87 and 115.

Assessment:

Neither treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption or organ weight were found.

Prevnar and Prevnar 13 led to similar hematological changes and the level of the hematological changes were not of concern.

Draize scoring of the injection sites revealed an increased frequency of irritation in the treated animals compared to control animals, but without increased severity. At the injection site was an acute as well as chronic subcutaneous inflammation observed after the dosing phase and a subcutaneous inflammation after the recovery phase.

For the serological evaluation the samples were analyzed for antibodies against all 7 pneumococcal serotypes covered by Prevnar in the saline group, vehicle group and Prevnar group; and against all 13 pneumococcal serotypes in the 13vPnC group. The animals given Prevnar made a positive serum IgG antibody response to each of the 7 serotypes of Prevnar after 7 doses, and the animals given 13vPnC made a positive serum IgG antibody response to each of the 13 serotypes of 13vPnC after 7 doses. It has been noted that Prevnar leads to higher titers than 13vPnC on day 87 and 115.

Overall assessment:

For the Pneumococcal 13-valent Conjugate Vaccine five toxicology studies were submitted. One subcutaneous developmental toxicity study was performed in juvenile rats (study number: 900742) receiving 5 doses every two weeks followed by a 2 day recovery dose, mostly local toxicities were observed. For repeated-dose toxicity study a subcutaneous toxicity study in monkeys (7 doses, dosing every 2 weeks, study number 501297), intramuscular toxicity study in rabbits (5 doses, dosing every 2 weeks, protocol number 07_2483) and two subcutaneous toxicity studies in rats 1 (7 doses, dosing every 2 weeks, study number 501296 and study number 6617-282) were performed. Additionally a local tolerance study with single dose intramuscular irritation in male rabbits (study number 501549) was presented.

When comparing study results, local toxicity was most prominent in the juvenile rat study (study number 900742). Acute and subacute inflammation with degeneration and necroses at the site of injection in a significant number of animals was seen after two injections. These symptoms did not resolve by the latest evaluated time point, i.e., day 65. Acute/sub-acute inflammation and degeneration/necrosis was still observed in the treatment group, additionally the inflammation spread into the epidermis and developed into a chronic inflammation. However, the last sample taken at day 65 was collected only 2 days after the animal received the last treatment, therefore, the animals only had a very short recovery phase, it would have been more meaningful to allow later time points (e.g., four weeks later) in order to see a potential reversion of local toxicity.

Taken all studies in consideration the most frequent finding was microscopic acute and subacute inflammation combined with necrosis and degeneration at the injection site during the dosing phase of the studies. Most symptoms resolved by the end of the recovery phase, and there was a marked improvement in local toxicity when comparing the dosing phase to the recovery phase, except in the juvenile toxicity study. In that study, chronic inflammation could still be observed in a substantial number of animals by the end of the recovery phase. However, the recovery period in this study consisted of only 2 days and thus, it is possibly that reversion of local toxicity would have been noted if a longer recovery period would have been allowed.

Overall, observed Draize scores at the injection site were rarely higher than 2. A Draize score higher than 2 was observed for edema (slight) and erythema (well defined). Edema and erythema at the site of injection occurred with increased frequency and slightly increased severity in the treatment groups compared to control groups; overall, local toxicity was not concerning.

Common local injection site reactions in clinical studies conducted in human infants and toddlers consistent of tenderness, mild to moderate erythema as well as mild to moderate indurations/swelling. This is very similar to the observations made in the animal studies were erythema and edema of a Draize score of ≤ 2 were commonly observed, only in isolated cases, erythema of a Draize score of ≤ 3 was detected.

Treatment of animals with 13vPnc did not result in systemic toxicities. However, some pathological findings of the intestinal systems occurred with greater incidence in the vaccine treated groups relative to the control group across species. In study # 900742, the following was noted after 13vPnC administration to juvenile rats (20/group): hemorrhage of the stomach in one male animal on days 23 and 65, erosion of the glandular mucosa of the stomach in one female on day 23, and inflammation of the stomach in four males and one female animal compared to one animal in the control group. In study # 501297, the following was noted after 13vPnC administration to nonhuman primates: hemorrhage of the cecum in one of three animals compared to none in the control groups. In study # 6617-282A the following was noted after 13vPnC administration to rats: squamous stomach cyst, mixed cell infiltration of the cecum or a mixed cell inflammation in the stomach in one of 10 animals. We also noted sporadic thyroid pathologies occurring with greater incidence in the vaccine or adjuvant treated groups relative to the control group: In study # 501297, the following was noted after 13vPnC administration (SC) to nonhuman primates: two of three female monkeys, and one of three male monkeys showed thyroid cysts on day 87. On day 115, one male monkey developed a thyroid cyst. In study # 07_2483, the following was noted after 13vPnC administration (IM) to rabbits: mixed cell inflammation of the thyroid in one of five male rabbits and C-cell hyperplasia and a squamous cyst of the thyroid in one male rabbit in the adjuvant group. In study number 6617-282, the following was noted after 13vPnC administration (SC) to rats: C-cell hyperplasia in one of ten male rats. None of these findings occurred in the control group. Notably, findings were not accompanied by any clinical symptoms in the animals. CBER requested for the sponsor to comment on these findings and the sponsor submitted a response on August 12, 2009 (DATS log number 470718) by providing historical background data for animals of the same species of comparable age and weight. Based on the historical background data it can be concluded that the observed pathological findings of the intestinal system and the thyroid are coincidental and not deemed of clinical concern.

Recommendation: In the BLA (125324) adequate nonclinical toxicology data have been presented to support the preclinical safety of 13vPnC. The 13vPnC administered as repeat doses to rats, rabbits and monkeys using either the subcutaneous or intramuscular route produced mild local inflammatory reactions without systemic toxicity. 13vPnC vaccine induced serologic antibody responses to most of the serotypes in the test species. Under the conditions of the preclinical studies performed, 13vPnC was well tolerated. Approval of the BLA is recommended.

Communications:

CBER sent comments to the sponsor regarding pathological findings in the stomach/intestine as well as in the thyroid which had been seen with greater incidence in the vaccine treatment group relative to the saline control group in several independent toxicity studies in various species. Although these concerns are not critical for the approval, they were communicated to the sponsor and we asked for any available background information including historic reference data or comment on these findings.

The following comments have been sent to the sponsor:

We noted that the following pathological findings of the intestinal system were observed with greater incidence in the vaccine treatment group relative to the saline control group in 3 independent toxicity studies in various species using the subcutaneous route of injection for the investigational vaccine. In toxicity study number 900742 with group sizes of 20 animals per group, juvenile rats given vaccine and adjuvant (30.8 µg polysaccharide (4.4 µg/mL of each of the serotypes except for serotype 6B at 8.8 µg/mL) 66.05 µg/mL CRM197 carrier protein, 5mM succinate buffer containing 0.02% Polysorbate 80 in-----(b)(4)----- at pH(b)(4) aluminum phosphate at 0.25 mg aluminum/mL), two animals experienced a hemorrhage of the stomach: one male animal on study day 23 and on study day 65; an erosion of the glandular mucosa in the stomach in one female animal was also observed on study day 23. Additionally, an inflammation of the stomach was observed in four male animals and one female animal of the treatment group given vaccine and adjuvant, but only in one animal of the control group. In another toxicity study numbered 501297 which used nonhuman primates, hemorrhage of the cecum was observed in one out of three animals in the male treatment group, but in none of the control groups. Lastly, in toxicity study number 6617-282A which was conducted using rats one out of 10 animals was diagnosed with either a squamous stomach cyst, mixed cell infiltration of the cecum or a mixed cell inflammation in the stomach. These observations are suggestive a non-specific stress response which might be increased by the vaccine application. Please provide any available background information or comment on these findings. If you consider these findings to be incidental, please provide some historic reference data for animals of the same species of comparable age and weight.

We also noted that the following thyroid pathologies were observed with greater incidence in the vaccine/adjuvant ((4.4 µg/mL of each of the serotypes except for serotype 6B at 8.8 µg/mL) 66.05 µg/mL CRM197 carrier protein, 5mM succinate buffer containing 0.02% Polysorbate 80 -----(b)(4)----- at pH (b)(4) aluminum phosphate at 0.25 mg aluminum/mL) and adjuvant alone (5 mM succinate buffer at pH-----(b)(4)----- and 0.02% Polysorbate 80 as excipients and aluminum phosphate at 0.25 mg aluminum/mL) group relative to the saline (0.9% sodium chloride) control group in 3 independent toxicity studies in various species using the intramuscular or subcutaneous route of injection for the investigational vaccine. In the toxicity study number 501297, two out of three female monkeys, and one out of three male monkeys showed thyroid cysts after the subcutaneous injection of the vaccine at day 87. Additionally on day 115, one male animal in the treatment group developed a thyroid cyst. After intramuscular injection of the vaccine in the toxicity study number 07_2483, mixed cell inflammation of the thyroid was seen in one out of five rabbits of the male treatment group as well as C-cell hyperplasia and a squamous cyst in the thyroid in one male rabbit in the adjuvant group. In the toxicity study number 6617-282 (subcutaneous injection), C-cell hyperplasia was also observed in one out of ten male rats in the treatment group. None of the observations described in the vaccine group were made in the saline control group. Please provide any available background information or comment on these findings. If you consider these findings to be incidental, please provide some historic reference data for animals of the same species of comparable age and weight

BLA 125324

The sponsor responded to these comments with an information amendment (BLA Amendment DATS Log Number 470718, CBER receipt date 12-Aug-2009). In this response the sponsor provided historical reference data and the findings were considered incidental.

Concurrence: Martin D. Green