

**BLA:** STN 125363

**Sponsor:** Glaxo SmithKline Biologicals

**Product:** Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine

Cross references: BB-IND -----(b)(4)-----

Proposed use: prevention of invasive diseases caused by *Haemophilus influenzae* type b and *Neisseriameningitidis* serogroups C and Y in infants age 6 weeks to 15 months.

**Précis:**

GSK Biologicals' vaccine consists of *H. influenzae* type b and *N. meningitidis* serogroups C and Y-tetanus toxoid conjugate vaccine combined (Hib-MenCY-TT) consists of *N. meningitidis* capsular polysaccharides C (PSC) and Y (PSY) each coupled to a protein carrier (tetanus toxoid, TT), and the *H. influenzae* type b capsular polysaccharide (PRP) coupled to tetanus toxoid as protein carrier by -----(b)(4)-----.

The proposed indication for GSK Biologicals' Hib-MenCY vaccine is immunization of infants and toddlers 6 weeks through 15 months of age for the prevention of invasive diseases caused by *Haemophilus influenzae* type b and *Neisseriameningitidis* serogroups C and Y. It is administered as a 4-dose series by intramuscular injection at 2, 4, 6 and 12 through 15 months of age.

**Introduction:**

GSK Biologicals developed a Hib-MenCY-TT conjugate vaccine using TT as the carrier protein for the PRP and the meningococcal components. In the US, this vaccine will provide protection against serogroups C and Y meningococcal disease without increasing the number of injections required to fully vaccinate a child according to the routine childhood schedule. Serogroups C and Y are two of the most important serogroups causing meningococcal disease in the US, the third being serogroup B. GSK Biologicals' *H. influenzae* type b and *N. meningitidis* serogroups C and Y-tetanus toxoid conjugate vaccine combined (Hib-MenCY-TT) consists of *N. meningitidis* capsular polysaccharides C (PSC) and Y (PSY) each coupled to a protein carrier (tetanus toxoid, TT), and the *H. influenzae* type b capsular polysaccharide (PRP) coupled to tetanus toxoid as protein carrier by a -----(b)(4)-----.

The proposed indication for GSK Biologicals' Hib-MenCY vaccine is active immunization of infants and toddlers 6 weeks through 15 months of age for the prevention of invasive diseases caused by *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroups C and Y. It is administered as a 4-dose series by intramuscular injection at 2, 4, 6 and 12 through 15 months of age. Hib-MenCY-TT refers to the licensure formulation of the vaccine. The treatment group receiving the Hib-MenCY-TT vaccine licensure formulation is identified as the Hib-MenCY group. Two additional formulations [Hib-MenCY 5/10/10 containing (b)(4) of PRP-TT, and (b)(4) each of PSC-TT and PSY-TT; Hib-MenCY 5/5/5 containing (b)(4) each of PRP-TT, PSC-TT and PSY-TT] were evaluated in dose-ranging studies. Based on immunogenicity and safety results from the dose-ranging studies, the Hib-MenCY-TT vaccine containing (b)(4) of PRP-TT, and (b)(4) each of PSC-TT and PSY-TT was selected for further clinical development, and is the candidate vaccine formulation submitted for licensure.

**Proposed clinical study:**

Three formulations of the Hib-MenCY-TT -----(b)(4)----- vaccine were evaluated in clinical trials. Based on immunogenicity and safety results from previous dose-ranging studies, the candidate Hib-MenCY-TT vaccine formulation submitted for licensure in the US is a lyophilized product containing (b)(4) of PRP, and 5  $\mu$ g each of *N. meningitidis* capsular polysaccharides C (PSC) and Y (PSY) each conjugated to tetanus toxoid (TT) per 0.5 mL dose volume after reconstitution with the liquid saline diluent(supplied). The Hib-MenCY-TT vaccine is not adjuvanted and does not contain preservatives. This submission contained the results of the completed clinical studies together with the nonclinical toxicology studies. The BLA submission includes results of the 13 phase II and III clinical studies in the Hib-MenCY-TT development program completed to date.

**Composition of Hib-MenCY-TT vaccine (per human dose [0.5mL] after reconstitution with saline diluent)**

Ingredients	Amount per dose	Function
<b>Active ingredients</b>		
Conjugate of <i>Haemophilus influenzae</i> type b capsular polysaccharide and tetanus toxoid (mean TT/PS ratio: 2.5)	2.5 $\mu$ g Hib ~6.25 $\mu$ g TT	Immunogen
Conjugate of <i>Neisseria meningitidis</i> C capsular polysaccharide and tetanus toxoid (mean TT/PS ratio: 1)	5 $\mu$ g MenC ~5 $\mu$ g TT	Immunogen
Conjugate of <i>Neisseria meningitidis</i> Y capsular polysaccharide and tetanus toxoid (mean TT/PS ratio (b)(4))	5 $\mu$ g MenY (b)(4)	Immunogen
<b>Excipients</b>		
1. Lyophilised with active substance		
Sucrose	12.6 mg	Stabiliser and (b)(4)
Tris (Trometamol)-HCl (b)(4)	96.8 $\mu$ g	(b)(4)
2. In liquid diluent		
NaCl	(b)(4)	(b)(4)
(b)(4)	(b)(4)	(b)(4)

\* quantum sufficit ad – a sufficient quantity to make

From the submission provided by Glaxo SmithKline Biologicals

**Toxicology Studies****SINGLE-DOSE TOXICITY**

The purpose of study TNO V4726 was to provide data on the local reactogenicity of two Hib-MenCY-TT candidate vaccines after a single intramuscular injection to albino rabbits. This study also included an assessment of clinical signs of toxicity and body weight changes during the three days following intramuscular injection of 0.5ml doses. The reactions of the two vaccines were compared to those of a sham-treated saline group. Groups of 6 -----(b)(4)----- rabbits (3/sex) were administered intramuscular injections in the left and right paravertebral muscle. A full human dose (500 ul) of Hib non-ads MenCY non-ads/10-10-10 was injected in each of two sites in the left paravertebral muscle, while the same dose of Hib non-ads MenCY ads/10-10-10 was injected in each of two sites in the right paravertebral muscle. The effects of these two formulations were compared to a control group composed of 4 rabbits (2/sex) administered intramuscular injections of saline in the left and right paravertebral muscle. The animals were

sacrificed 3 days after injection and the injection sites were examined for gross and microscopic changes. No clinical signs related to the intramuscular injection of the Hib-MenCY-TT candidate vaccines was observed during the 3-day study period. All animals showed body weight gain during the study. No treatment-related gross changes at the injection sites were observed. In conclusion, single intramuscular injection of two Hib-MenCY-TT candidate vaccines (Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10) produced no clinical signs of toxicity or changes in body weight gain relative to the saline control group.

## REPEAT-DOSE TOXICITY

The effects of repeated (five times at two week intervals) intramuscular injection on two Hib MenCY-TT candidate vaccines (Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10) were examined in groups of -----(b)(4)----- rabbits (10/sex) and compared with a saline control group --(b)(4)----- Both the vaccine and control groups were divided into two subgroups of 5 rabbits/sex each, sacrificed 3 and 30 days after the fifth injection. Observations included: clinical signs, ophthalmoscopy, body temperature, body weight, food consumption, haematology, clinical chemistry, gross pathology, organ weights, and histopathology. Neither of the Hib-MenCY-TT candidate vaccines produced distinct treatment-related changes in clinical signs, ophthalmoscopy, body temperature, body weight, or food consumption. A few haematology and clinical chemistry parameters were slightly and affected, however these changes were transient, within pre-dose ranges and considered incidental. Higher absolute and relative right popliteal lymph nodes weights were recorded 3 and 30 days post last dose in the males of the Hib non-ads MenCY non-ads/10-10-10 group. Histopathological examination of the popliteal lymph nodes did not reveal distinct treatment-related changes. Since the right lymph nodes drained the most recently-injected sites, the weight change was considered to be due to the immunization, and of no toxicological significance. Macroscopic observations of the injected muscles were limited to a discolored area at the final (fifth) injection site in one male rabbits in the Hib non-ads MenCY ads/10-10-10 group sacrificed 3 days post last dose. Microscopic examination of the injection sites 3 days post last dose revealed mixed inflammatory cell infiltrate graded as slight in 1/5 male rabbits from the Hib non-ads MenCY non-ads/10-10-10 group. Mixed inflammatory cell infiltrate graded as slight in 1/5 males and 1/5 females, and moderate (with necrosis) in 1/5 males was observed in the Hib non-ads MenCY ads/10-10-10 group 3 days post last dose. The incidence and severity of these injection site observations were similar 30 days post last dose, although necrosis was no longer present. Repeated-dose intramuscular injection of the two Hib-MenCY-TT candidate vaccines (Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10) did not produce adverse systemic reactions compared with the concurrent control group. Treatment-related effects appeared limited to minor local inflammation reactions at the injection site which were slightly more pronounced in Hib non-ads MenCY ads/10-10-10.

## LOCAL TOLERANCE

In a study to evaluate the local reactogenicity of two Hib-MenCY-TT candidate vaccines ----(b)(4)-----, groups of 6 -----(b)(4)----- rabbits (3/sex) were administered intramuscular injections in the left and right paravertebral muscle. A full human dose (500 ul) of Hib non-ads MenCY non-ads/10-10-10 was injected in each of two sites in the left paravertebral muscle, while the same dose of Hib non-ads MenCY ads/10-10-10 was injected in each of two sites in the right paravertebral muscle. The effects of these two formulations were compared to a control group composed of 4 rabbits (2/sex) administered intramuscular injections of saline in the left and right paravertebral muscle. The animals were sacrificed 3 days after injection and the injection sites were examined for gross and microscopic changes. No clinical signs related to the intramuscular injection of the Hib-MenCY-TT candidate vaccines were observed during the 3-day study period. All animals showed body weight gain during the study. No treatment-related gross changes at the

injection sites were observed. Very slight to slight inflammation was observed microscopically in 2 male and 1 female rabbits injected with Hib non-ads MenCY non-ads/10-10-10. The inflammation consisted of a mixture of macrophages and granulocytes, with fibre regeneration in one of the males. In the Hib non-ads MenCY ads/10-10-10 group, slight to moderate inflammation, consisting of a mixture of macrophages, monocytes and granulocytes, with dystrophic calcification or necrosis, was observed microscopically in 1 male and 2 females rabbits. Very slight to slight inflammation consisting of a few macrophages, monocytes and granulocytes was observed in 1 male and 2 female rabbits in the saline control group. In conclusion, single intramuscular injection of two Hib-MenCY-TT candidate vaccines (Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10) produced no clinical signs of toxicity or changes in body weight gain relative to the saline control group. Based on macroscopic and microscopic examination of the injection sites, the two Hib-MenCY-TT candidate vaccines produced similar mild local inflammatory responses after a single injection in the rabbit.

## OTHER TOXICITY STUDIES

### Toxicology data on -----(b)(4)-----

*N. meningitidis* C and Y polysaccharides are coupled to tetanus toxoid using -----  
----- (b)(4) ----- chemistry. According to the reaction scheme, polysaccharides react with (b)(4) to give activated polysaccharide and (b)(4), which is liberated as a by-product. In the subsequent -(b)(4)- reaction, activated polysaccharide reacts with the (b)(4) groups of the carrier proteins to yield ----(b)(4)----. During the course of this vaccine development, special attention has been paid to the safety of the conjugate vaccine with respect to (b)(4) residuals. Residual (b)(4) content in GSK Biologicals' MenC-TT and MenY-TT conjugate bulks has been measured and found ----(b)(4)-- MenC and ----(b)(4)----MenY respectively, resulting in a maximal level of about --(b)(4)--- of Hib-MenCY-TT vaccine (see m3.2.S.3.2- MenC-TT and m3.2.S.3.2- MenY-TT). The acute systemic toxicity, *in vitro* mutagenicity, and skin sensitisation potential of (b)(4) has been investigated according to relevant (b)(4) test guidelines, in GLP studies conducted by -----(b)(4)----- and -----(b)(4)-----, sponsored by GSK. In summary, no issues of concern were found, based on the following studies.

#### *Determination of the no-observed effect level (NOEL) following a single oral administration to the rat -----(b)(4)-----*

----- (b)(4) ----- rats, weighing 152-162g (males) and 150-152g (females) were dosed once only, by oral gavage, and 3 dose levels were sequentially tested:

Dose of (b)(4)

50 mg/kg; N= 3m, 3f

200 mg/kg; N= 1m, 1f

500 mg/kg; N= 0m, 1f

Animals dosed at 50 and 200 mg/kg bodyweight showed no clinical signs of toxicity during the 14-day observation period. All animals showed expected gains in bodyweight. No abnormalities were noted at necropsy. The female rat dosed at 500 mg/kg died 1 hour 35 minutes after dosing. Abnormally red lungs, dark liver and kidneys were noted at necropsy of this animal. Therefore, the NOEL for (b)(4), following a single oral administration to the rat, was set at 200 mg/kg bodyweight.

#### *Single Dose Toxicity by Intramuscular Administration to (b)(4) Rats- (b)(4)---*

In the absence of any acute toxicity data for (b)(4) via the IM route, GSK Biologicals performed an acute IM toxicity study --- (b)(4) --- in ----- (b)(4) ----- rats. Groups of 5 males and 5 females, 6 week-old, weighing 212-250g (males) or 143-190g (females), received single IM

doses of 1 or 10 mg/kg bodyweight (the maximum feasible dose based on solubility data) in the biceps femoris of the hindleg on Day 0. There was no sign of toxicity (i.e. deaths, clinical signs, effects on bodyweight gain) during the 14 days following dosing. At macroscopic examination at necropsy of animals on Day 14, enlarged right lumbar lymph nodes were seen in 2 males dosed at 10 mg/kg.

----- (b)(4) ----- This test aims at assessing the contact sensitisation potential of (b)(4) in ----- (b)(4) ----- male albino guinea pigs, 8-12 week-old and weighing 389-450g. Following dose-range studies (0.1% - 1 % w/v in distilled water), the formulations of (b)(4) for the induction and challenge phase were selected as follows: Intradermal induction: 0.1% w/v in a mixture of ----- (b)(4) ----- or 0.1% w/v in distilled water; topical induction: 10% w/w in distilled water; topical challenge: 10% and 5% w/w in distilled water. (b)(4) produced a 30 % (3 out of 10 animals) sensitisation rate and was therefore classified as a moderate sensitizer to guinea pig skin.

*Reverse Mutation* ----- (b)(4) -----  
----- (b)(4) ----- were treated with (b)(4) using the --- (b)(4) --- incorporation method at five dose levels (50 to 5000  $\mu$ g (b)(4)), in triplicate, both with and without the addition of a rat liver homogenate metabolising system. The vehicle (distilled water) ---- (b)(4) ----- produced counts of revertant colonies within the normal range. All positive reference compounds produced marked increases in the frequency of revertant colonies, with and without the metabolising system. - (b)(4) - caused no visible reduction in the growth of the bacterial lawn at any of the dose levels to any of the Salmonella strains tested, therefore, the test material was tested up to the maximum recommended dose of 5000  $\mu$ g/plate. (b)(4) was found to be non-mutagenic under all test conditions. Mouse lymphoma ---- (b)(4) ----- Assay (SPL ----- (b)(4) ----- +/- 3.7.2c mouse lymphoma cells (heterozygous at the thymidine kinase locus) were treated with (b)(4) at different dose levels (152.75, 305.5, 611 and 1222  $\mu$ g/ml) both with and without metabolic activation, together with vehicle (solvent) and positive control. (b)(4) did not induce any statistically significant dose-related increases in the mutant frequency either in the absence or in the presence of metabolic activation. All positive reference compounds produced marked increases in the mutant frequency, both with and without the metabolising system. (b)(4) was shown to be non-mutagenic to - (b)(4) --- cells under all conditions of the test.

## TOXICOLOGY SUMMARY

Pre-clinical toxicity studies have been conducted in order to identify and evaluate toxicity findings following intramuscular (IM) administration of the GSK Biologicals' Hib-MenCY-TT vaccine. The toxicological profile of the Hib-MenCY-TT vaccine has been studied in two GLP-compliant studies including:

- a local tolerance/single dose toxicity study in the ----- (b)(4) ----- rabbit,
- a repeated-dose toxicity study in the ----- (b)(4) ----- rabbit.

Each of these studies evaluated two formulations of the vaccine: (i) a formulation adsorbed on ----- (b)(4) ----- (Hib non-ads MenCY ads); and (ii) a non-adsorbed formulation (Hib non-ads MenCY non-ads). The latter formulation was selected for further evaluation of the candidate vaccine in the clinics. The content of each component administered i.m. to rabbits (10 micrograms each) in the toxicity studies was two to four times higher than in the Hib-MenCY-TT candidate vaccine (2.5/5/5 micrograms). Based on antigen content and bodyweight this clearly

represents an overdose compared to the antigen dose to be administered to infants. Furthermore, the number of injections given to rabbits (n = 5) in the repeated-dose study exceeded the intended number of injections to be given to infants (n = 4). In addition, and for informative and supportive purposes, data on the toxicity, mutagenicity and sensitization potential of -----(b)(4)-----, a byproduct of the reaction used to conjugate the purified polysaccharides of *Neisseria meningitidis* types C and Y to the tetanus toxoid carrier, have been summarized in section 8.1

## 9. DISCUSSION AND CONCLUSIONS

Acute toxicity, local tolerance and repeated dose studies have been performed with the HibMenCY-TT candidate vaccine. These toxicity studies have demonstrated that acute intramuscular injection of a full human dose produced no treatment-related changes in clinical signs or body weight in rabbits. Macroscopic changes related to treatment with was limited to a discolored area at the final (fifth) injection site in one male rabbits in the Hib non-ads MenCY ads/10-10-10 group sacrificed 3 days post last dose. Intramuscular injection of Hib non-ads MenCY non-ads/10-10-10 produced very slight to slight local inflammation observed microscopically in 2 male and 1 female rabbits. In the Hib non-ads MenCY ads/10-10-10 group, slight to moderate inflammation, consisting of a mixture of macrophages, monocytes and granulocytes, with dystrophic calcification or necrosis, was observed microscopically in 1 male and 2 females rabbits. Repeated intramuscular injection of the two Hib-MenCY-TT candidate vaccines (Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10) did not produce adverse systemic reactions compared with the concurrent control group. Treatment-related effects were limited to minor local inflammation reactions at the injection site which were slightly more pronounced in Hib non-ads MenCY ads/10-10-10. The toxicity and mutagenicity of (b)(4), a contaminant and by-product from the (b)(4) conjugation chemistry, was investigated following (b)(4) guidelines in several GLP-compliant tests. (b)(4) was found to be non-toxic at levels up to 10 mg/kg bodyweight given IM and up to 200 mg/kg bodyweight given orally. These non-toxic doses are high multiples of the (b)(4) residual dose (b)(4) measured at  $\leq 3\text{ ng}/5\mu\text{g}$  MenC and  $\leq 7\text{ ng}/5\mu\text{g}$  MenY, respectively, resulting in a maximal level of about 10 ng/dose of Hib-MenCYTT vaccine). (b)(4) was found to be a moderate skin sensitizer when applied to the guinea pig skin at high concentrations in the maximisation test. However, since residue doses in the final vaccine are much lower and local tolerance studies did not evidence any macroscopically observable sign of intolerance, the finding in the guinea pig is of no toxicological significance to the vaccine formulation. (b)(4) was found negative in the two *in vitro* genotoxicity studies. Another potential vaccine formulation contaminant is ---(b)(4)---, a solvent for (b)(4)-, used in the manufacturing of the Men-TT conjugates. ---(b)(4)--- is a well known volatile chemical and its toxicological properties have been reviewed. In Europe, the product is classified as a Class(b)(4) solvent following -(b)(4)-. "Note for guidance on impurities: residual solvents CPMP/ICH/283/95) - ICH Topic Q3 C". As demonstrated in m3.2.S.3.2- MenC-TT and m3.2.S.3.2- MenY-TT, the ---(b)(4)--- content was measured in MenC-TT and MenY-TT conjugate bulks and results in a maximal level of about 55 ng in the Hib-MenCY-TT (2.5/5/5  $\mu\text{gPS/dose}$ ) combined vaccine, similar to the residual level present in Menitorix™ (Hib-MenC) and well below the acceptable concentration limit specified for ---(b)(4)--- in CPMP/ICH/283/95. Therefore, it is of no toxicological concern. Potential contaminants resulting from the Hib-TT conjugation chemistry using direct (b)(4) coupling chemistry include -----(b)(4)----- for which release specifications have been issued. Levels of these compounds were found to be very low ((b)(4) level in the final vaccine is  $< 0.07\text{ ng/dose}$  and the maximum residual quantity of (b)(4) expected per vaccine dose is 9.6 ng) and they are of no toxicological concern.

## 1. REPEAT-DOSE TOXICITY

### Toxicology Study Review:

**Title and study number:** Repeated-dose toxicity study with a Hib-MenCY candidate vaccine administered intramuscularly (five times) to male and female rabbits Study No. 4725/01 (males), 4725/02 (females)

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

Study initiation date: August 9, 2002

Study completion date: December 5, 2002

**Test article batch/lot:**

Hib non-ads MenCY non-ads/10-10-10 , batch ----(b)(4)-----

Hib non-ads MenCY ads/10-10-10, batch ----(b)(4)-----

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

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

**Number of animal per group and sex:** 10/dose/sex (5/dose/sex main study, 5/dose/sex recovery)

**Age:** 12 weeks

**Body weight range:** Day -7 : 2090-2405g, males, 2060-2373g, females

**Route and site of administration:** intramuscular injection, left hind leg 9calf) day 0, right hind leg (thigh) day 14, left hind limb (thigh) day 28, right hind limb (thigh) day 42, right hind limb (calf) day 56

**Volume of injection:** 0.5 mL

**Frequency of administration and study duration:** Animals (5/sex/group) received 5 doses on day 0, 14, 28, 42 and 56. Animals were necropsied on day 59 (main study animals) and on day 86 (recovery animals).

**Dose:** Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10 10µg PRT-TT, 10µg PST-TT and 10µg PSY-TT/0.5 ml

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose).

The study report shows that Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10 had expiration dates of 14 months and 12 months, respectively, at 2-10°C when stored in darkness as freeze-dried pellets containing 10µg PRT-TT, 10µg PST-TT and 10µg PSY-TT.

**Means of administration:** Intramuscular injection

**Report status:** Final

**Experimental design:**

**Males:**

Group	Treatment	Number of males (even nos.)	
		S1 (n=5)	S2 (n=5)
4725/01A	Saline	2-10	12-20
4725/01B	Hib non-ads MenCY non-ads/10-10-10	22-30	32-40
4725/01C	Hib non-ads MenCY ads/10-10-10	42-50	52-60

**Females:**

Group	Treatment	Number of females (odd nos.)	
		S1 (n=5)	S2 (n=5)
4725/02A	Saline	1-9	11-19
4725/02B	Hib non-ads MenCY non-ads/10-10-10	21-29	31-39
4725/02C	Hib non-ads MenCY ads/10-10-10	41-49	51-59

S1 = subgroup 1 was sacrificed on day 59 (i.e. 3 days post fifth inoculation)

S2 = subgroup 2 was sacrificed on day 86 (30 days post fifth inoculation)

From the submission provided by Glaxo SmithKline Biologicals

**Methods:**

The following parameters were evaluated:

clinical signs (once daily)

injection site approximately 3, 24 and 48 hours after dosing)

ophthalmoscopy: subgroup 2, predose, days 59, 84

body weights: -7, -4, 0, 3, 7, then weekly until day 84 and at sacrifice

food consumption: days -7, -4, 0, 3, 7, then weekly until day 84,

body temperature (rectal): day 0, 26; 4, 24 h post injection

haematology, coagulation and clinical chemistry: subgroup 2, days -4 males, -5 females, 1, 3, 52, 57, 59, 86

serology: days -4 males, -5 females, 59, 86 Samples were collected and analysed under nonGLP conditions from the abdominal aorta (at sacrifice) or ear artery

organ weights and histopathology on a selection of tissues.

Terminal necropsy

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with a w were weighed. Tissues with an h were examined for histopathology

Organ/Tissue	Collected (y)	Not collected (X)
Adrenal glands	wh	
Aorta	h	
Bone (sternum & femur)	y	
Bone marrow (sternum & femur)		X



Brain (cerebrum, cerebellum, medulla and pons)	wh	
Cervix	y	
Colon	y	
Duodenum	y	
Epididymides	wh	
Esophagus	y	
Eyes (optic nerve)	h	
Fallopian tubes (oviduct)		X
Gall bladder		X
Gross lesions (if any)	h	
Harderian gland (if applicable)		X
Heart	wh	
Ileum	y	
Jejunum	y	
Kidneys	wh	
Lacrimal glands		X
Larynx		X
Liver	wh	
Lung (main-stem; bronchi)	wh	
Lymph nodes (cervical)		X
Lymph nodes (mandibular)	y	
Lymph nodes (mesenteric)	y	
Mammary glands	y	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	wh	
Pancreas	h	
Peyer's patch (if applicable)		X
Pituitary gland	wh	
Prostate	wh	
Rectum	y	
Salivary glands (mandibular)	y	
Sciatic nerve	h	
Skeletal muscle	h	
Skin	y	
Spinal cord (cervical, lumbar, thoracic)	h	
Spleen	wh	
Stomach	h	
Testes	wh	
Thymus	wh	
Thyroid (w/ parathyroid glands)	wh	
Tongue		X
Ureters		X
Uterus (w/ cervix)	wh	
Urinary bladder	h	

Vagina	y	
Zymbal's gland (if applicable)		X

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

**Results:**

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

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ ))	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		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, fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	A/G ratio: day 3 group 3m, 1.7x Day 52, group 2m, 1.5x Group 2 f, 0.72x Day 53, group 2m, 1.26x CK day 3, group 2 m, 0.25x; group 3m, 0.44x Day 82 Group 2, 3 f: 0.65x Triglycerides: group 2, 3 m, 1.36x	Albumin (A) Globulin (G, calculated) or Total cholesterol Cholinesterase Total protein Creatine kinase

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ )	NOT OF NOTE
RED BLOOD CELLS	Reticulocytes day 53, group 2, 3 m, 0.85x	Hematocrit (Hct) Hemoglobin conc. (Hb) Mean corp. Hb. (MCH) Mean corp. Volume (MCV) Total erythrocyte count (RBC)
WHITE BLOOD CELLS	Basophils: day 55, group 3 m, 1.3x	lymphocyte count Macrophage Total leukocytes (WBC) Large unstained cells (LUC)
CLOTTING POTENTIAL	Fibrinogen: day 47: group 3 f, 1.19x, day 53 group 2, 3 f, 1.3x, day 55, group 2,3 f: 1.17x	Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume
OTHERS		Bone marrow cytology

**Table of Hematology Results****Systemic toxicity:**

## Clinical signs

No distinct clinical signs related to the intramuscular treatment of the animals were observed during the study period. Incidentally, due to a punctured subcutaneous blood vessel upon injection a haematoma at the site of injection was observed in a few animals of both the control group and the vaccine group. The other individual clinical signs occasionally observed were considered not related to treatment.

## Ophthalmoscopy

Ophthalmoscopic examination of subgroup 2 animals did not reveal any treatment related abnormalities of the eyes examined. Occasionally, (multi)focal corneal opacities or focal lens opacities were observed in control and vaccine formulation animals (see appendix 1). These findings are considered normal background findings for rabbits of this strain and age.

## Body temperature

The body temperatures recorded around the first and fifth inoculation did not show significant differences between the vaccine formulation groups and the saline control. The 24-hour recording after the first inoculation showed a slightly but statistically significant different body temperature

of the "Rib-MenCY na" females when compared to the pre-dose recording, but the difference with the pre-dose temperature was minor (39.6 pre-dose to 39.5 °C post-dose) and the toxicological significance of this finding was considered negligible.

#### Body weights and food intake

No statistically significant differences in body weights were observed in both vaccine formulation groups when compared to saline control group during the study period. Apart from a slightly lower mean food intake (-3%,  $P < 0.05$ ) in the "Rib-MenCY a" males on day 28, and in the absence of effects on group mean body weight, no statistically significant differences in food intake were observed in both vaccine formulation groups when compared to saline control group during the study period.

#### Haematology

The red blood cell (RBC) and white blood cell (WBC) variables of all post inoculation sampling dates were statistically evaluated against the values of the saline control group by means of covariance analysis using the pre-dose values as co-parameter.

##### Red blood cell variables

The pre-dose values only showed a statistically significantly low haemoglobin (Hb) concentration of the "Rib-MenCY a" males. Individual and group mean values were noted by the sponsor to be within the historical background range. In males, no statistically significant differences were observed in the RBC variables post-inoculation. Compared to the saline control group, the following statistically significant changes were observed in the females: Thrombocytes. A lower thrombocytes count was observed in the "Rib-MenCY a" females on day 1 after the fifth inoculation. Compared to the previous pre-dose sampling the value was hardly different. Therefore, no toxicological significance was attached to this transient finding, no longer present on day 3 post fifth injection.

Fibrinogen. A higher fibrinogen concentration was observed in both vaccine formulation groups on day 1 after the fifth inoculation. The value was still increased (although not statistically significant) on day 3 and normalized by day 30. Fibrinogen is a marker of an inflammatory process accompanying the induction of the immune response. This appears to be a transient finding.

Mean corpuscular haemoglobin concentration (MCHC). A slightly lower MCHC (-2 to -3% of controls) was observed in both vaccine formulation groups on day 30 after the first inoculation. Compared to the previous samplings (also in controls) the MCHC value was hardly different. In the absence of effects on HB concentration on RBC count, may not be significant.

No other statistically significant differences were observed in the RBC variables of the females.

##### White blood cell variables

A slightly higher percentage of basophils was noted in the "Hib-Men CY a " males on day 3 after the fifth inoculation (Table 6.13). This incidence was already present, although not reaching statistical significance, on day 4 prior to the fifth injection. However, absolute basophil counts were never different from controls. A small increase in relative number of eosinophils was measured in the "Hib-MenCY na" females on day 1 after the first inoculation (Tables 6.16 and 6.23), although absolute mean counts were rather similar among groups, and therefore considered to be of no biological significance. Because of its isolated and transient nature and the minor effects observed, no toxicological significance was attached to these findings.

#### Clinical chemistry

The clinical chemistry values of all post inoculation sampling dates were statistically

evaluated against the values of the saline control group by means of covariance analysis using the pre-dose values as co-parameter. Compared to the saline control group, the following statistically significant changes were observed:

Aspartate aminotransferase (ASAT). In "Hib-MenCY a" males, a high mean ASAT activity was observed on day 1 after the fifth inoculation .

Chloride. In "Hib-MenCY a" males, a higher mean chloride concentration was observed 4 days prior to the fifth inoculation and in "Hib-MenCY na" females on day 1 after the first inoculation . Because of its isolated and transient nature, no toxicological significance was attached to these findings.

Sodium. In the "Hib-MenCY na" males, a slightly higher mean sodium concentration was observed on day 30 after the fifth inoculation and in "Hib-MenCY a" females on day 1 after the first inoculation. Compared to the previous samplings, the calcium concentrations had hardly changed and the individual values were within the pre-dose range. Therefore, no toxicological significance was attached to this finding.

Albumin/Globulin ratio (A/G ratio). In the "Hib-MenCY na" females, a lower mean A/G ratio was observed 4 days prior to and on day 1 after the fifth inoculation. The individual values were within the pre-dose range.

Creatinine Kinase (CK). In both female vaccine formulation groups, a lower mean creatinine kinase activity was observed on day 30 after the fifth inoculation . Compared to the pre-dose values (Table 7.32) the CK activity was slightly changed and the statistical significance was due to higher pre-dose values of these groups. Incidentally in saline controls and in the vaccine formulation groups (predominantly males), the blood creatine kinase activity, a marker of muscle damage also known as creatine phosphokinase (CPK), showed only incidentally high individual increases mainly on day 3 after the first and fifth inoculation. The other clinical chemistry parameters determined did not reveal distinct differences.

#### Body temperature

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

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

#### Organ weights

##### *Subgroup 1 (sacrificed 3 days after the fifth inoculation)*

In males and females, no statistically significant differences in organ weights were observed.

##### *Subgroup 3 (sacrificed 30 days after the fifth inoculation)*

Compared to the saline controls, a statistically significantly lower mean absolute adrenals weight and a higher absolute and relative right popliteal lymph node weight of the "Hib-MenCY na" males were observed. Because the relative adrenal weight was not statistically significantly different, no toxicological significance was attached to this finding. Moreover, histopathology of the adrenals and of the popliteal lymph nodes did not reveal distinct abnormalities. Because the right popliteal lymph node is draining the most recent injected site (right calf muscle), the change in its weight is most probably due to the immunization. In females, no statistically significant differences in organ weights were observed.

#### Pathology -gross

##### *Subgroup 1 (table 9; appendix 9)*

At necropsy a white area was observed at the inoculation site of the right calf muscle of one "Hib-MenCY a" male (C44). In addition, haemorrhages and red areas were found in inoculated as well

as untreated skeletal muscles and in the skin of several animals including controls. They were considered to be related to trauma during restraint as this batch of rabbits showed quite some stress against fixation during blood sampling. No other gross changes were remarkable.

#### *Subgroup 2*

At necropsy no gross changes were observed that could be related to the inoculation of the vaccine formulations. Haemorrhages and red appearance/spots were observed in inoculated as well as untreated skeletal muscles and other organs of several animals including controls. They were considered to be related to trauma during restraint. All other gross changes did not appear to be remarkable.

### **Histopathology**

#### *Subgroup 1, sacrificed day 59,3 days post fifth inoculation*

Treatment-related inflammation was observed at the injection site of a few "HibMenCYna" animals and of several "Rib-MenCY a" animals, and was denoted 'mixed inflammatory-cell infiltration'. It was characterized by a mixture of granulocytes and a few small (possibly lymphocytes) and several larger (probably macrophages) mononuclear inflammatory cells. The inflammation was most often very slight to slight, although in one "Rib-MenCY a" male (C44) it occupied a considerable part of the sectioned muscle tissue and exhibited central necrosis denoted as 'moderate'. It is questionable whether the very slight mixed cell inflammation is related indeed to inoculation with one of the vaccine formulations, because very slight mixed cell inflammation was also observed in the untreated triceps of a female saline control, at day 30 after the fifth inoculation (see below). In addition, very slight mononuclear cell infiltrate was observed at the injection site of "Rib-MenCY a" females. Because it was observed also at the injection site of saline controls, and in the untreated muscle (triceps), it was considered to be background pathology, and thus unrelated to the treatment. The same applies to very slight fiber degeneration, which also occurred in the untreated triceps, including that of a saline control female. Several macrophage aggregates were observed in the draining popliteal and inguinal lymph nodes, including in those of saline control animals. Therefore, they were considered to be unrelated to the vaccine formulations. All other histopathological changes were considered to be unremarkable, with the exception of a statistically significantly increased incidence in slight, focal alveolitis in the lungs of "Hib-MenCY na" males. The increase was considered to be of no toxicological significance because the incidence of alveolitis is rather variable, and there was no increase in severity.

#### *Subgroup 2, sacrificed day 86,30 days post fifth inoculation*

Treatment-related mixed-cell inflammation was observed in the right calf muscle of only one "Rib-MenCY na" male and female (slight inflammation) and one "HibMenCY a" male (moderate inflammation). The inflammation was not necrotizing. Very slight mononuclear cell inflammation was observed in the right calf muscle of a few animals. These very slight inflammations were considered to be unrelated to the inoculation of the vaccine formulations, because they were also observed in one saline control female and in the untreated triceps. In addition, very slight mixed cell inflammation was observed in the triceps of one female saline control. Several macrophage aggregates were observed in the draining popliteal and inguinal lymph nodes, including in those of saline controls. Therefore, they were considered to be unrelated to the vaccine formulations. All other histopathological changes occurred in one or a few animals or they were distributed about equally amongst the groups, with the exception of basophilic tubules in the kidneys. The variability in incidence of this common histopathological change is considered to be normal, within historical control data and, therefore, no toxicological significance was attached to the absence of this change in the "HibMenCYna" females.

**Organ Weights at Terminal Sacrifice:**

SEX		MALES				FEMALES			
GROUPS		1 (CONTR OL)	2	3	4	1 (CONTR OL)	2	3	4
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
BODY WEIGHT (gram) <sup>a</sup>		2986.9	2840.8	2945.3	2863.0	3050.5	3023.2	3068.8	3062.8
BRAIN									
Absolute Weight <sup>a</sup>	gram	10.32	10.14	10.27	9.98	10.84	9.83	10.88	10.59
Per Body Weight <sup>a</sup>	%	0.3458	0.3569	0.3492	0.3489	0.3559	0.3254	0.3551	0.3452
ADRENALS									
Absolute Weight <sup>a</sup>	gram	0.3313	0.3904	0.3390	0.4013	0.2744	0.2771	0.3619	0.3114
Per Body Weight <sup>a</sup>	%	0.0111	0.0137	0.0115	0.0140	0.0090	0.0092	0.0118	0.0102
Per Brain Weight <sup>a</sup>	%	3.2153	3.8526	3.3177	4.0198	2.5585	2.8281	3.3312	2.9579
LUNGS									
Absolute Weight <sup>a</sup>	gram	13.07	12.84	12.60	13.60	12.60	13.11	11.82	12.66
Per Body Weight <sup>a</sup>	%	0.4358	0.4533	0.4272	0.4738	0.4144	0.4347	0.3852	0.4135
Per Brain Weight <sup>a</sup>	%	126.16	126.57	123.39	136.50	116.17	132.83	108.79	119.96
HEART									
Absolute Weight <sup>a</sup>	gram	5.68	6.13	5.41	5.62	5.94	6.07	6.02	5.31
Per Body Weight <sup>a</sup>	%	0.1902	0.2156	0.1837	0.1961	0.1950	0.2010	0.1967	0.1734
Per Brain Weight <sup>a</sup>	%	54.99	60.43	52.89	56.34	54.77	61.78	55.22	50.29*
KIDNEYS									
Absolute Weight <sup>a</sup>	gram	17.30	18.70	17.32	15.81	15.61	16.28	16.53	17.08
Per Body Weight <sup>a</sup>	%	0.5781	0.6582	0.5892	0.5519	0.5119	0.5395	0.5372	0.5561
Per Brain Weight <sup>a</sup>	%	167.27	184.22	168.28	158.49	144.09	165.01	152.45	160.81
LIVER									
Absolute Weight <sup>a</sup>	gram	77.17	75.75	77.17	70.91	62.06	63.76	68.43	69.16
Per Body Weight <sup>a</sup>	%	2.58	2.6612	2.62	2.4773	2.0361	2.1105	2.2274	2.2500
Per Brain Weight <sup>a</sup>	%	746.26	745.98	752.00	710.44	571.80	649.47	630.55	650.95

SEX		MALES				FEMALES			
GROUPS		1 (CONTR OL)	2	3	4	1 (CONTR OL)	2	3	4
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
SPLEEN									
Absolute Weight <sup>a</sup>	gram	0.9873	0.8398	0.8677	0.9967	1.0972	0.9111	1.0820	1.2605
Per Body Weight <sup>a</sup>	%	0.0329	0.0295	0.0294	0.0348	0.0359	0.0302	0.0355	0.0412
Per Brain Weight <sup>a</sup>	%	9.5349	8.2903	8.5347	9.9920	10.2497	9.2630	9.8937	12.0359
TESTES									
Absolute Weight <sup>a</sup>	gram	5.25	4.26	4.31	4.44				
Per Body Weight <sup>a</sup>	%	0.1757	0.1501	0.1463	0.1544				
Per Brain Weight <sup>a</sup>	%	50.78	41.96	42.01	44.60				
THYMUS									
Absolute Weight <sup>a</sup>	gram	3.54	2.14	3.81	2.46	3.61	4.38	3.72	3.61
Per Body Weight <sup>a</sup>	%	0.1188	0.0758	0.1297	0.0857	0.1182	0.1448	0.1219	0.1181*
Per Brain Weight <sup>a</sup>	%	34.34	21.22	37.65	24.78	33.08	44.59	34.14	34.40
OVARIES									
Absolute Weight <sup>a</sup>	gram					0.2094	0.2277	0.3064	0.2101
Per Body Weight <sup>a</sup>	%					0.0069	0.0075	0.0099	0.0068
Per Brain Weight <sup>a</sup>	%					1.9559	2.3039	2.8412	1.9714

Table of organ weight and their normalization (terminal sacrifice). Absolute weights are expressed as mean (grams). \*different from controls at  $P \leq 0.05$



**Organ Weights at Recovery Sacrifice:**

SEX		MALES				FEMALES			
GROUPS		1 (CONTR OL)	2	3	4	1 (CONTR OL)	2	3	4
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
BODY WEIGHT (gram) <sup>a</sup>		2771.8	2865.4	2776.6	2968.8	3020.7	3010.1	3011.3	2990.7
BRAIN									
Absolute Weight <sup>a</sup>	gram	10.1824	9.5682	10.2122	9.9343	10.2157	10.0414	10.2449	10.211 3
Per Body Weight <sup>a</sup>	%	0.3688	0.3339	0.3685	0.3346	0.3385	0.3333	0.3402	0.3415
ADRENALS									
Absolute Weight <sup>a</sup>	gram	0.3719	0.3829	0.3478	0.2694	0.2857	0.3081	0.3418	0.3586
Per Body Weight <sup>a</sup>	%	0.0135	0.0133	0.0126	0.0091	0.0094	0.0103	0.0113	0.0120
Per Brain Weight <sup>a</sup>	%	3.7069	3.9908	3.4377	2.7333	2.7781	3.0913	3.3254	3.5105
LUNGS									
Absolute Weight <sup>a</sup>	gram	12.0385	10.8050	12.0534	11.9526	10.5969	12.1838	11.8491	11.530 4
Per Body Weight <sup>a</sup>	%	0.4362	0.3774	0.4370	0.4031	0.3506	0.4052	0.3934	0.3858
Per Brain Weight <sup>a</sup>	%	118.06	113.01	117.72	121.23	103.50	122.00	115.91	112.91
HEART									
Absolute Weight <sup>a</sup>	gram	5.6566	5.5164	5.4460	5.8728	5.9826	6.4005	5.5759	5.8535
Per Body Weight <sup>a</sup>	%	0.2042	0.1927	0.1967	0.1981	0.1972	0.2130	0.1852	0.1957
Per Brain Weight <sup>a</sup>	%	55.78	57.70	53.55	59.33	59.31	64.18	54.54	57.33
KIDNEYS									
Absolute Weight <sup>a</sup>	gram	15.7913	16.0116	15.1955	16.8623	16.4495	16.1138	15.7185	16.321 2
Per Body Weight <sup>a</sup>	%	0.5695	0.5584	0.5479	0.5677	0.5457	0.5359	0.5223	0.5454
Per Brain Weight <sup>a</sup>	%	156.25	167.22	149.31	170.10	161.19	161.28	153.36	159.85
LIVER									
Absolute Weight <sup>a</sup>	gram	65.1244	67.0278	62.4089	70.6091	63.6721	64.6329	70.0602	74.399 8
Per Body Weight <sup>a</sup>	%	2.3524	2.3370	2.2356	2.3794	2.1091	2.1534	2.3263	2.4880
Per Brain Weight <sup>a</sup>	%	646.27	699.94	611.09	711.50	622.84	650.27	682.27	728.61

SEX		MALES				FEMALES			
GROUPS		1 (CONTR OL)	2	3	4	1 (CONTR OL)	2	3	4
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
SPLEEN									
Absolute Weight <sup>a</sup>	gram	0.8703	0.7686	0.7523	0.7885	1.1760	1.2549	1.0536	0.9056
Per Body Weight <sup>a</sup>	%	0.0311	0.0268	0.0269	0.0266	0.0390	0.0416	0.0350	0.0303
Per Brain Weight <sup>a</sup>	%	8.4490	8.0203	7.4408	7.9326	11.4630	12.4405	10.2778	8.8693
TESTES									
Absolute Weight <sup>a</sup>	gram	4.9025	4.6282	4.2358	4.1665				
Per Body Weight <sup>a</sup>	%	0.1778	0.1617	0.1541	0.1405				
Per Brain Weight <sup>a</sup>	%	48.52	48.41	41.77	41.88				
THYMUS									
Absolute Weight <sup>a</sup>	gram	2.5575	2.2639	2.8608	2.9391	3.6916	3.8712	3.9776	3.2888
Per Body Weight <sup>a</sup>	%	0.0917	0.0792	0.1016	0.0990	0.1219	0.1290	0.1318	0.1101
Per Brain Weight <sup>a</sup>	%	25.21	23.69	27.97	29.83	36.22	38.98	38.52	32.20
OVARIES									
Absolute Weight <sup>a</sup>	gram					0.2307	0.2543	0.2662	0.2533
Per Body Weight <sup>a</sup>	%					0.0076	0.0085	0.0089	0.0085
Per Brain Weight <sup>a</sup>	%					2.2407	2.5455	2.5863	2.4796

Table of organ weight and their normalization (recovery sacrifice). Absolute weights are expressed as mean (grams). \*different from controls at  $P \leq 0.05$

There was a 19% decrease in heart/brain weight ratios in group 4 females with no macroscopic or microscopic pathology associated with this decrease. Group 4 females also showed statistically significant decreases in mean thymus weight (17%), thymus/body weight ratio (18%) and thymus/brain weight ratio (23%), when compared to their respective control (group 2). These decreases were not considered test article related. No changes in organ weights or ratios were reported in the recovery groups.

#### Gross Pathology:

#### Terminal sacrifice

Group	Findings
1M	Hematoma at injection site 3/10
2M	Hematoma at injection site 1/10
3M	Hematoma at injection site 1/10
1F	Hematoma at injection site 4/10
2F	Hematoma at injection site 2/10
3F	Hematoma at injection site 2/10

(NF = no findings); \* (number of animals with the observation/total number of animals in the group).

### Microscopic finding

#### (terminal sacrifice)

Groups	Findings
1M	Slight-subcutaneous/muscle foci of granular amorphous material; minimal-myofiber degeneration
2M	Minimal to slight-subcutaneous/muscle hemorrhage; minimal-mixed inflammatory infiltrate, lung alveolitis
3M	Minimal-subcutaneous mixed inflammatory infiltrate; minimal to slight-subcutaneous/muscle hemorrhage; minimal to slight-mixed inflammatory infiltrate, lung alveolitis
1F	Minimal-subcutaneous/muscle hemorrhage; minimal-subcutaneous/muscle foci of granular amorphous material; minimal-mixed inflammatory infiltrate; lung alveolitis;
2F	Minimal-subcutaneous/muscle hemorrhage; minimal to slight-histiocytic aggregates; minimal to slight-mixed inflammatory infiltrate
3F	Slight to moderate-subcutaneous mixed inflammatory infiltrate; minimal to slight-subcutaneous/muscle hemorrhage; minimal -mixed inflammatory infiltrate, minimal-myofiber degeneration; lung alveolitis

#### (recovery sacrifice)

Groups	Findings
1M	Minimal epithelium erosion; slight-histiocytic aggregates
2M	Minimal-subcutaneous/muscle foci of granular amorphous material; minimal to slight-histiocytic aggregates; slight- mixed inflammatory infiltrate
3M	Minimal-subcutaneous/muscle hemorrhage; minimal-mixed inflammatory infiltrate
1F	Minimal-subcutaneous/muscle hemorrhage; slight-histiocytic aggregates; minimal myofiber degeneration
2F	NF
3F	Slight-histiocytic aggregates; minimal-mixed inflammatory infiltrate

(NF = no findings)

**Local toxicity:**

All animals appeared within normal limits in the dermal irritation assessment of the injection sites. One animal (animal number 2004) only reported with moderate erythema on day 57.

Macroscopically, discoloration at the injection site 1 and/or 2 was reported in all, except male control, treated groups at terminal sacrifice. Firmness at injection site 1 was reported in male groups 3 and 4. Discolored cervical and mediastinal lymph nodes and enlarged mediastinal lymph node were reported in one female of group 1. Discolored thymus and edematous injection site 1 were reported in females group 3. At recovery sacrifice discolored injection site 1 were reported in males groups 1, 3, and 4 and females group 1.

Microscopically the injection site, in the single dose-treated animals at the terminal sacrifice, showed subcutaneous/muscle hemorrhage in male groups 2 and 4 and in female groups 1, 3, and 4. Muscle histiocytic aggregates were reported in female group 2. Subcutaneous/muscle foci of granular amorphous material were seen in female groups 3 and 4. At recovery sacrifice, muscle histiocytic aggregates were reported in male group 4 and in female groups 2 and 4.

At the terminal sacrifice of the multiple dose-treated animals at injection site 1, minimal and/or slight-subcutaneous/muscle foci of granular amorphous material were reported in male groups 1, 3, and 4 and in female groups 1, 2, and 3. Minimal-myofiber degeneration was seen in males and females groups 1 and 4. Minimal, minimal to slight, or marked-subcutaneous/muscle hemorrhage was reported in male groups 2, 3, and 4 and female groups 1, 2, 3, and 4. Minimal, minimal to slight, or minimal to moderate-mixed inflammatory infiltrate were reported in male groups 2, 3, and 4 and female groups 1, 2, 3, and 4. Moderate and slight-subcutaneous edema was reported in male group 4 and female group 3, respectively. Surface-inflammatory cells/debris and slight-acanthosis were reported in male group 4. Minimal to slight or slight-histiocytic aggregates was reported in male group 4 and female groups 1, 2, and 4. Minimal epithelium erosion was seen in female group 3.

At the recovery sacrifice of the multiple dose-treated animals at injection site 1, minimal epithelium erosion and slight-histiocytic aggregates were reported in male group 1.

**Serology:**

Blood samples were taken 4 or 5 days before the first injection and at necropsy: 3 days after the fifth injection (subgroup 1) and 30 days after the fifth inoculation (subgroup 2). Presence of antibodies against the MenC polysaccharide was measured at these time-points in individual sera. Administration of Rib-MenCY vaccines induced anti-PSC antibodies (seroconversion) in 95% (38 of 40) of vaccine recipient animals after five vaccine dose administrations.

*Seroconversion rate obtained in ELISA anti-PSC for male and female rabbits at the different time-points*

Formulation		Day -4 or -5	D3 post V (day 59)	D30 post V (day 86)
<b>Group A :</b> <i>saline</i>	Males	0/10	0/5	0/5
	Females	0/10	0/5	0/4*
<b>Group B :</b> <i>Hib non-adj MenCY non-adj</i>	Males	0/10	4/5	4/5
	Females	0/10	5/5	5/5
<b>Group C :</b> <i>Hib non-adj MenCY adj</i>	Males	0/10	5/5	5/5
	Females	0/10	5/5	5/5

\* One invalid result

from sponsor submitted material

(Test article related effects are listed in the table below)

Test article related effects	Effects considered incidental*
↓LDH M&F, ↑AST M&F, ↓creatinine kinase, ↑ cholesterol F G3 only, ↑ neutrophil F G3 and ↓ M G4, ↓ eosinophils, ↓ basophils, ↓ monocytes M G3 & G4 Discoloration at the injection site1 and/or 2	↑↓ALT, ↑ ALP

#### Assessment:

Repeated intramuscular treatment (five inoculations) of rabbits with two Hib-MenCY candidate vaccine formulations (Hib non-ads MenCY non-ads/10-10-10 and Hib non-ads MenCY ads/10-10-10) was associated with minor findings when compared to a saline control group. No treatment-related, mortality, nor distinct changes in clinical signs, ophthalmoscopy, body temperature, body weight and food intake were observed. Evaluation of the hematology and clinical chemistry parameters did not appear to show any clearly treatment related effects. The few slightly affected parameters observed were all considered of minor, if any, toxicological significance. The changes were transient and in most cases incidental. Moreover, they were mostly within the pre-dose ranges. Blood creatine kinase activities showed only a few increases possibly related to damage due to the injected muscle. Evaluation of the relative and absolute organ weights at necropsy on days 3 and 30 after the fifth inoculation only revealed a higher absolute and relative right popliteal lymph node weight of the "Hib-MenCY na" males. Histopathology of the popliteal lymph nodes did not reveal distinct treatment-related abnormalities and because the right popliteal lymph node is draining the most recent injected site (right calf muscle), the change in its weight is most probably due to the immunization. The toxicological significance of this finding is, therefore, considered negligible. Macroscopic observations at necropsy on days 3 and 30 after the fifth inoculation did not reveal treatment-related changes, apart from an incidental discolored area at the site of (the final) injection. Microscopic examination revealed only one inoculation-related finding, i.e. mixed inflammatory cell infiltrate at the injection site, which was scored 'slight' in one "HibMenCY na" male and one "Hib-MenCY a" male and female, and 'moderate' (with necrosis) in one "Hib-MenCY a" male, at day 3 after the fifth inoculation. The inflammation was still present and to a comparable extent at 30 days after the fifth inoculation, although necrosis was no longer present. Overall, the intramuscular inoculation with the two Hib-MenCY candidate vaccine formulations did not result in systemic adverse reactions, when compared to the saline control group. The effects were

limited to a minor local reaction at the site of injection which tended to be only slightly more pronounced with the Hib non-ads MenCY ads/l 0-1 0-1 0 formulation. Because of the already slight nature of the inflammatory reaction, locally in the muscle, a recovery process was not distinctly noted at the end of the 30-day observation period. Immunology performed in this study verified that an active dose was administered.

**GLP study deviations or amendments:** No deviations to methods were reported for this study.

Concurrence: Martin D. Green