SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Procomvax. This scientific discussion has been updated until 1 September 2004. For information on changes after this date please refer to module 8B.

1. Introduction

Haemophilus influenzae type b (Hib) has been an important cause of bacterial meningitis and other serious diseases in children. Infection with hepatitis B virus (HBV) is also a significant public health problem throughout the world that can lead to serious liver diseases such as cirrhosis and hepatocellular carcinoma. Both Hib and HBV infection can be prevented with vaccines, and a growing number of countries are recommending routine vaccination of infants against Hib and HBV. Routine use of these vaccines has been associated with a marked decline in the incidence of these diseases.

Much effort has been invested in combining as many vaccines as possible in a single multivalent dose, in an attempt to reduce the cost of vaccination and to increase the acceptability and affordability of vaccination. Achieving this final goal involves the choice of appropriate formulations in order to avoid potential interferences and to ensure compatibility and stability of the combined antigens. With Procomvax it will be possible to vaccinate infants against invasive Hib disease and HBV infection using only a total of three injections, whereas more than three injections would be needed if monovalent Hib and Hepatitis B vaccines were used (up to 6 injections, the exact number depending on the vaccine and the vaccination schedule used).

Procomvax is a combined Haemophilus influenzae type b and hepatitis B vaccine. The immunogenic components consist of polyribosylribitol phosphate (PRP) purified from Haemophilus influenzae and chemically coupled to the outer membrane protein complex (OMPC) of Neisseria meningitidis, and the hepatitis B surface antigen (HBsAg) of HBV, derived from cultures of a genetically recombinant strain of yeast, Saccharomyces cerevisiae. The immunogens in Procomvax are also the components of the licensed monovalent Hib vaccine PedavaxHIB and Pedvax HIB liquid [Haemophilus b conjugate vaccine (Meningococcal protein conjugate)] containing 15 and 7.5 µg PRP per dose and the licensed monovalent recombinant Hepatitis B vaccine (Recombivax HB, HB-VAX, H-B-VAX II, HB-VAX DNA, GEN H-B-VAX).

Procomvax is indicated for vaccination against invasive disease caused by Haemophilus influenzae type b and against infection caused by all known subtypes of hepatitis B virus in infants 6 weeks to 15 months of age.

The pharmaceutical form of this vaccine is a suspension for injection provided in glass vial.

2. Chemical, pharmaceutical and biological aspects

Composition

This combined vaccine contains the following active substances:

Haemophilus influenzae type b capsular polysaccharide (polyribosylribitol phosphate i.e. PRP) covalently linked to a carrier protein (the outer membrane protein complex (OMPC) of the B11 strain of Neiseria meningitidis group B) and hepatitis B surface antigen (HBsAg) derived from a recombinant strain of Saccharomyces cerevisiae

The active ingredients of Procomvax are 7.5 µg PRP, 125 µg OMPC and 5 µg HBsAg formulated with 225 µg aluminum as aluminum hydroxide in a 0.5 ml dose.

The dosage for Procomvax is based on the paediatric dosages for the marketed components of the vaccine, the new liquid PedvaxHIB formulation (PRP-OMPC) and H-B-VAX-II (Hepatitis B surface antigen; HBsAg). In Procomvax, the PRP-OMPC and HBsAg components are formulated with an aluminium hydroxide adjuvant to produce the bivalent vaccine. The active components are provided in a convenient,

pre-mixed, aluminium hydroxide-adsorbed, preservative-free, liquid formulation. It should be noted, that the formulation of Procomvax differs from the monovalent vaccines by the absence of a preservative.

A single dose of combined vaccine Procomvax is presented as a monodose in a glass vial (type I glass: nominal capacity 2/ml) with an grey butylstopper and a lacquered aluminium seal with a plastic cap. An overage of 0.2 – 0.25 ml is added (final volume 0.7/ml).

Method of preparation

Briefly, the formulation of Procomvax consists of the mixing/blending of the alum adsorbed PRP-OMPC and alum adsorbed HBsAg bulks together with the dilution medium.

The final bulk is mixed and stored for a maximum of 24 hours between 2-8°C until filling. The bulk is then mixed and re-circulated for 15 minutes to ensure that the suspension is homogenous prior to filling. The mixing is maintained during the filling operation.

The filled vials are stored in a cold room at 2-8°C. In accordance with the Ph. Eur., a sterility test is only performed on the final bulk immediately after mixing. The absence of a sterile filtration step during the formulation is justified by the fact that the two active ingredients as well as the diluent medium are aluminium adsorbed. Furthermore, adequate sterile filtration steps as well as sterility tests are carried out ensuring sterility of the active substances at the time of blending.

The validation plan of the process steps was considered to be satisfactory.

Control of starting materials

- **Alum adsorbed PRP-OMPC**

Haemophilus influenzae type b bulk production (PRP)

The Haemophilus influenzae type b strain was isolated from a patient in 1971, passaged several times and lyophilised. The pre-master, master and working seed lot of Haemophilus were prepared by culture on chocolate agar slants. The cultures obtained after suspension in Haemophilus inoculum medium and modified Gotschlich medium respectively, are pooled and dispensed into cryovials stored at a temperature of ≤ - 60°C. All the cultures are tested for purity, identity, viability immediately after preparation. The master and the working seed cultures are also tested at 1, 2, 4, 6, 8 and 10 years for identity and viability.

Fermentation: the culture is prepared after thawing and inoculating the working seed vial onto chocolate agar slants and is then transferred into liquid media. This inoculum is transferred to a fermentor containing 40 l of medium and incubated. The culture is then transferred to a 800 l fermentor containing approximately 550 L of the previous medium and fermented at a pH between 6.6 and 7.4 under stirring and aeration conditions. A phenol solution is added to inactivate the culture which is held at a temperature between +36°C and +42°C for at least one hour. The culture is cooled and transferred to a tank maintained at +2°C to +8°C. The culture is clarified and collected in a portable tank and held for up to 45 days at a temperature of +2°C and +8°C.

Chocolate agar also contains only category IV bovine material since December 1997 when the supplier implemented a change (replacement of the only category II risk material originally used in the chocolate agar by category IV bovine material). A certificate of origin is provided for Chocolate agar medium showing that bovine material used originates from U.S., New Zealand and Australia.

Purification: the culture broth is concentrated by ultrafiltration at a temperature below +12°C in order to reduce the level of low molecular weight impurities. Ethanol is added to precipitate protein and nucleic acid impurities at a temperature below +12°C. This step is repeated four times. At the fourth step, the product is precipitated, dried and stored at a temperature below -60°C.

Phospholipase D treatment is performed to separate the polysaccharides from the phospholipids. Protein, endotoxin and residual phospholipase D are removed by phenol treatment repeated four times. Lipopolysaccharides and residual phenol are removed using a polystyrene divinylbenzene resin. The PRP product is then precipitated using calcium chloride and ethanol.

All the steps of the fermentation and purification process are clearly described.

Deviations from Ph.Eur. requirements relating to the testing for protein, endotoxin and pyrogenicity are fully justified by the company.

Neisseria meningitidis bulk production (OMPC)

The Neisseria meningitidis group B (strain B11) was isolated from a patient in 1966 and passaged four times. In 1986, the master and the working seed lot system was prepared using Mueller-Hinton agar slants. In 1988, the master seed lot was re-qualified as a pre-master seed lot.

Culture and fermentation: after thawing, the working seed vial is inoculated onto Mueller-Hinton agar slants and incubated. The resulting colonies are resuspended using Gotschlich medium and added into larger flasks containing this medium, for incubation on a shaker until the optimum optical density is achieved at 37ºC. This inoculum is transferred to a fermentor containing 50 l of Neisseria meningitidis medium and incubated at 37ºC for up to 8 hours. After exposure of the culture in a 1000 L fermentor at 37ºC for at least 10 hours, the culture is inactivated by adding phenol. Inactivation is performed at 36-42ºC for up to 4 hours.

Mueller Hinton agar contains bovine material of category type IV and is therefore considered safe.

Purification: OMPC is isolated from the cell slurry by detergent extraction. Cell debris are removed first by a low speed centrifugation. The second and third centrifugation steps are carried out at a high speed level in order to pellet OMPC and to leave the soluble impurities in the effluent. The product is resuspended in TED buffer and filtered. Filtration concentrates the product to 4 L and a diafiltration with 20 L of sterile distilled water is performed. The purified OMPC bulk is stored at a temperature between +2°C and +8°C for up to 2 years.

Conjugation and adsorption of PRP and OMPC

The purified PRP and the OMPC are chemically derivatised in order to produce the reactive bridging chains for PRP and the terminal thiol for OMPC necessary for conjugation. The derivatised PRP bulk is stored at a temperature between +2°C and +8°C for up to 160 days. These groups react together to form the final PRP-OMPC conjugate which is concentrated and purified by diafiltration in order to eliminate impurities. The PRP-OMPC bulk is stored at a temperature between +2°C and +8°C for up to 1 year. The PRP-OMPC bulk is diluted and mixed with aluminium hydroxide diluent to form the bulk alum adsorbed liquid vaccine, which is stored at a temperature between +2°C and +8°C for up to 1 year. All reagents used are adequately sterile filtered.

Characterisation: characterisation testing of PRP-OMPC is performed by amino acid analysis of the conjugated product. The PRP/OMPC ratio and molecular sizing provide evidence of the conjugated PRP and free PRP.

Analytical validation: all the methods used for the in-process controls (IPCs) for the release of the active substance have been validated.

The company has shown that immunogenicity of PRP-OMPC in man does not clearly correlate with immunogenicity in mice. In addition, the company has identified some biochemical parameters which give more relevant immunogenicity information on the conjugation of PRP to OMPC. The omission of the mouse immunogenicity assay is scientifically justified and therefore this test may be omitted in agreement with the Ph.Eur. monograph.

Process validation: the process validation consists of a retrospective analysis of 35 production batches. Assay data are given for important intermediate products, as well as for the final products. Most of the 35 batches presented contained mercurothiolate as preservative, which has subsequently been withdrawn from the production of PRP-OMPC valence. Only few preservative-free batches have been produced so far. Hence no statistical analysis is proposed for these batches.

Results from a prospective validation study have demonstrated that the PRP-OMPC conjugation process removes residual reagents.

The active ingredient is defined as a Bulk Alum Product (BAP) which is a sterile suspension of the antigenic particles adsorbed onto alum in isotonic saline solution buffered with sodium phosphate.

Batch sizes and batch analysis: the batch sizes range from 9 to 100 l with a typical batch size of about 20 l. The data of six validation lots covering batch sizes from 7.4 - 29.4 L have been shown to meet

the criteria of passing all of the intermediate product release test specifications. Analysis certificates have been provided for eight production batches (PRP-OMPC conjugated alum adsorbed bulk). Controls were carried out at each step of the production process. All batches complied with the proposed specifications.

- **Alum adsorbed recombinant Hepatitis B surface antigen (HBsAg)**

Development genetics: the biosynthesis of the HBsAg antigen utilizes a recombinant plasmid expressed in the host cell Saccharomyces cerevisiae. The plasmid codes for a 24 kDa membrane protein called the S protein.

Cell bank system: the pre-master, master and working seed lot were prepared according to the same procedure consisting of re-suspension in a medium containing 17% glycerol. The cultures obtained were aliquoted, frozen and stored at a temperature of ≤ -60°C. A new lot of Master Seed has recently been prepared, for which extensive analyses, including a full-scale fermentation showed satisfactory results.

Culture and fermentation: the working seed is expanded to sufficient cell suspension volume for inoculation in a production fermentor (800 or 1000/l). At the optimum optical density, the culture is transferred to the production fermentor containing the YEHD medium (containing dextrose, yeast and soya peptone). The entire fermentor is harvested through a 0.65 micron pore size filtration membrane. The cellular suspension obtained after concentration can be stored at -20°C for up to one year.

HBsAg is extracted with Triton X-100 after the mechanical disruption of cells in the presence of a protease inhibitor.

Purification: the process purification is defined as two phases of purification, followed by several steps of chemical treatment leading to the aluminium adsorbed active ingredient Bulk Alum Product (BAP).

A diafiltration is carried out in order to eliminate residual thiocyanate as well as other impurities and a sterile filtration and an appropriate dilution (or concentration) are then performed. The product is stored into glass bottles at a temperature between +2°C and +8°C for up to one year.

Formaldehyde treatment; this treatment is carried out at +36°C ± 2°C for at least 60 hours and results in the Final Aqueous Product (FAP).

Formulation: an alum solution is added to the FAP. The pH increase caused by the addition of NaOH causes the precipitation of aluminium hydroxide and antigen adsorption. After a 2-hour incubation, the bulk alum adsorbed product is tested for completeness of adsorption. After at least
5 settle/decant/resuspension cycles, the active substance is obtained at a concentration of 40 µg/ml. It can be stored for 2 years at a temperature between +2°C and +8°C. The process is well described.

Characterisation: characterisation has been carried out on five manufacturing batches of HBsAg at two different steps, using physicochemical, biological and immunological techniques.

Specifications and routine tests: on the basis of the experience gained with this product, it can be concluded that the active substance is released for further processing after satisfactory control tests (IPCs and tests are performed on the BAP). These tests comply with the requirements of the Ph. Eur. monograph and provide assurance of the quality and consistency from batch to batch.

Process validation: the active ingredient BAP is well characterised. A sufficient number of batches have been analysed in detail and show the consistency of the production process.

Based on this experience, the company proposed to tighten the in-process control for:

- proteins for filtered sterile solution
- aluminium for the bulk adsorbed product
- formaldehyde
- endotoxins

Impurities: potential impurities such as yeast protein, thiocyanate, triton X-100 and formaldehyde arising during the production process are routinely tested for. On the basis of batch analysis results, other impurities such yeast DNA, carbohydrates, lipids and proteins, are not routinely tested.

Batch analysis: results are provided for 8 batches, 3 containing mercurothiolate as preservative and 5 preservative-free. Satisfactory answers have been given to the authorities with regard to the present fiducial limits for IVRP test for future batches and with regard to the batch sizes of the consistency lots.

- **Other Ingredients**

Information on each constituent of the dilution medium and the Ph. Eur specifications for routine control tests has been provided. The composition of the dilution medium is used for both monovalent bulks.

- **Packaging materials**

The packaging materials are quality controlled. The vaccine is contained in glass vials (Type I glass) enclosed by rubber stoppers, aluminium seals and plastic caps.

Control of the finished product

Specifications and routine tests: the proposed routine tests to be performed are: sterility, general safety, pyrogenicity, completeness of adsorption, PRP/HBsAg identity, in vitro relative potency (IVRP for HBsAg), PRP content, pH determination, aluminium content, sodium chloride content and filling volume.

Evaluation of data to set a specification for the hepatitis B IVRP test is currently in process.

Improvements were made during the assessment concerning specifications for the IVRP test, pH and aluminium content. The deletion of the mouse immunogenicity assay for the Hib component of the vaccine was fully justified by the company.

Determination of aluminium by atomic emission spectrometry, pyrogenicity, testing according to 21 CFR, part 610.13 method and LPS testing at the bulk conjugate are not in full compliance with the Ph.Eur. requirements, but have been fully justified by the company.

Validation data have been provided for the non Ph. Eur. methods. Batch analysis data have been provided for 8 batches, 3 containing thiomersal as preservative and 5 preservative-free.

Stability

Extensive stability data have been provided for three lots of each of the two aluminum hydroxide-adsorbed bulks used to manufacture Procomvax, as well as three lots of the finished product vaccine.

- **Active substances**

The data demonstrate that the Preservative-Free Liquid Alum Adsorbed PRP-OMPC bulks and the HBsAg bulks remain stable when stored at 2-8° C for 24 months, supporting a 24 month dating period.

All of the assays used are described and have been validated. Mouse potency data for the HBsAg after storage of the BAP for 24 months have been presented for 3 lots.

- **Finished product**

Three lots of the finished product Procomvax were assessed for stability at 2-8º C over a 24 month period with an additional 12 months planned in the study. No significant changes were observed for any of the parameters tested on the three lots over a 24 month period. The data provided support a 24-month dating period for the finished product vaccine. Data on the 36 month time point were

prepared at the time of the initial submission and the company intended to file a variation for the extension of the shelf life. This data has later been supplied. Due to the deletion of the mouse immunogenicity test for Hib, the date of filling of final containers is used for calculation of the period of validity.

Accelerated stability studies were carried out for six months at 20-25º C and 36-38º C. No changes were observed in any of the parameters tested for 6 months at 20-25º C. Notable decreases in PRP polysaccharide levels as assayed by EIA could be observed for all three lots at 36-38º C. Due to the high variability of the EIA the company has implemented a new HPLC assay to assess the PRP content of Procomvax. The new assay is more reliable and variability is about 5% RSD in contrast to the EIA that shows variability of 20% relative standard deviation (RSD).

3. Toxico-pharmacological aspects

Pharmacodynamics

The company to progress to the clinical studies of the combined vaccine regarded the preclinical data gained with the mono components as sufficient. However the combination Hib-Hep B as a new vaccine is addressed with some deficiency, especially in the preclinical part, with respect to the 'Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines' (CPMP/465/95). However, this Note for Guidance was not in force at the time of preparation and submission of the dossier. Nevertheless the company has provided the relevant clinical data for the combination product. From the current point of view this procedure may be criticised, but information and data presented form a sufficient basis to allow approval of Part III of the dossier.

- **Immunogenicity**

Anti-PRP

Preclinical studies have been conducted in animals to demonstrate the equivalence of antibodies raised against the Hib component of the newly formulated combination vaccine to those induced by the monovalent PedvaxHIB formulation (P). These include infant Rhesus and African Green monkey immunogenicity studies and immunogenic potency determined in BALB/c mice by estimation of the minimum dose required to induce an antibody response in 50% of immunized mice (ED_{50}).

Infant Rhesus Monkeys: Since the primary target population for initiating the immunization series for the Hib conjugate vaccine is 2-month-old infants, the immunogenicity of the Hib component of the vaccines was tested in 2- to 3-month old infant Rhesus monkeys.

Infant Rhesus monkeys were injected intramuscularly on days 0 and 28 with doses of either 1 or 0.01 µg of polysaccharide as PRP-OMPC conjugate or a monovalent PedvaxHIB. The monkeys were bled prior to each injection and on day 42.

Doses of either 1 or 0.01 µg of polysaccharide as PRP-OMPC conjugate both induce high-titer anti-PRP antibody responses in 2- to 3-month-old infant Rhesus monkeys. However 1µg was selected for further evaluation, as it is closer to the dosage level used in human infants.

The results demonstrate that a 1 µg dose of polysaccharide as PRP-OMPC conjugate in Procomvax (PR), is as immunogenic as a 1 µg dose of the monovalent PedvaxHIB vaccine (P). Both induce high antibody titers in infant Rhesus monkeys. The immune response was found to increase with the number of injections. The geometric mean antibody titers (GMT's) at day 42 were: 39 – 404 for thiomersal-free PRP-OMPC conjugate in combined vaccine, 79 - 164 thiomersal-containing PRP-OMPC conjugate in combined vaccine and 30-159 for the monovalent vaccine.

Anti-HBsAg

African Green Monkeys: Infant African green monkeys were chosen to test the combination vaccine, since the target population for the combination vaccine is children less than 2 years old.

Infant African green monkeys were injected intramuscularly on days 0, 28 and 96 with 0.5 ml of inoculum containing a full human dose of either Procomvax or a monovalent Recombivax HB (R) control. The same dosage, i.e. 5 µg, of Recombivax HB and Procomvax (from thiomersal-containing

lots) was used. The monkeys were bled prior to each injection and on days 42 and 110. The sera were evaluated for anti-HBs antibody by using a commercial RIA kit.

Evaluation of the antibody titers obtained by immunization with Recombivax HB and the combination vaccine Procomvax show that both vaccines induce a strong immune response in the infant African Green monkey model. However the titers achieved by Procomvax vaccination are only one third compared to Recombivax HB. The company comments that anti HBsAg titers observed in African green monkeys should not be extrapolated to humans. In addition the GMT increase observed after the second and third dose are comparable for both Recombivax HB and the combined vaccine. A statistically significant lower GMT for the PR group than that for the P+R group has also been observed in the pivotal clinical study study 002. However the reduction in titer is of questionable clinical significance as anti-HBs titers =10mIU/ml are regarded as fully protective.

No analysis has been performed with respect to the class of antibodies formed and the duration of the immune response. However the company notes that the isotype response in African green monkeys is not predictive of the response in humans and consequently the study was focussed on determining antibody responses, which is generally accepted as a surrogate marker for protective immunity.

Inbred Mice: Studies of immunogenic potency were carried out in 6 to 8 week-old female BALB/c mice. Groups of 6 to 8 mice were injected intraperitoneally with 0.5 ml of vaccine containing 0.5 µg, 0.1 µg, 0.02 µg or 0.004 µg PRP-OMPC in aluminium diluent on days 0 and 14. The mice were bled on day 21. ED_{50} values were calculated by determining the number of mice that responded with greater than or equal to 1 µg anti-PRP antibody/ml for each dosage level.

The results show that the dosage range of the combination vaccine required to induce = 1 µg anti-PRP/ml in at least 50% of the mice is comparable to the dosage range of the monovalent liquid PedvaxHIB formulation.

Pharmacokinetics

Pharmacokinetic studies have only been performed with the Hib component of the combination vaccine Procomvax. The company has justified this.

Toxicology

- **Safety Testing**

General Safety testing is conducted on each lot of Procomvax. This testing is conducted in two animal species, guinea pigs and mice. The results complied with the requirements.

- **Acute toxicity (for the HepB component)**

Recombivax HB was evaluated for acute toxicity by the oral, intraperitoneal, and subcutaneous routes in mice and rats. The resulting responses were not significantly different from the placebo (aluminium diluent) and the LD_{50} observed was > 50 mL/kg.

- **Rabbit Pyrogen Testing**

PR was tested for pyrogenicity by injecting New Zealand White rabbits intravenously with 1.0 ml of diluted test vaccine (containing at least 0.025 µg PRP/ml) per kg of rabbit body weight. A second pyrogenicity test also was conducted in which 1.0 ml of aluminium-adsorbed vaccine containing 7.5 µg of PRP-OMPC was administered intramuscularly (IM) to each rabbit, and temperatures were monitored for five hours. PR with or without thiomersal was found not to give an increase of more than 0.6ºC in temperature. All lots of Procomvax were found to pass the pyrogen tests. It is confirmed that pyrogenicity tests were performed according to the U.S. CFR.

- **Repeated dose toxicity studies**

The lack of repeated dose toxicity studies is accepted now in view of the arguments and data presented. Firstly, Procomvax represents a combination of two well-known components, which have been proven to be safe and well tolerated in clinical use, without addition of any new component. Secondly, the potential for toxicity was addressed in clinical studies, based on the positive results of the few preclinical tests performed.

- **Mutagenicity studies**

Mutagenicity studies were not performed in accordance to relevant guidelines.

- **Carcinogenicity studies**

Studies were not performed in laboratory animals for PR

- **Local tolerance toxicity study**

The company refers to the information available for the mono components. Since no new components were included within the combination, the evaluation of safety in clinical study is regarded as justified and sufficient.

Overview of part IV of the dossier: clinical aspects
Clinical data of Procomvax (PR) are summarised in the following table:

Study	Vaccination Period	Study Sites	Number Enrolled/ Receiving PR	Study Design
General safety in adults				
001	8/90-9/90	US	21/21	open study, PR administered at Day 0 and one month later
Safety and immunogenicity in infants				
002 Pivotal	3/92-10/93	US	882/661	randomized, open, infants given PR from 1 of 3 lots or concurrent injections of monovalent vaccines (P+R), at 2, 4, 12-15 months Concurrent administration of other pediatric vacines permitted
004	6/93-4/95	US	126/126	open, infants given PR with coadministration DTwP/IPV or DTwP+OPV, MMR at 15 months
005	12/93-12/95	US	208/69	randomized, open, infants given PR, P+R, P?R with co-administration of DTwP+OPV and MMR
009	1/96 ongoing	US	254/254	randomized, open, children 12-15 months of age given a dose of PR concurrently or 6 weeks prior to MMR and Varicella vaccine
411-295	1/96 ongoing	US	67/67	control arm of a randomized of open study, infants given PR+IPV and DTaP at 2, 4, 6 months.
Expanded surveillance for serious adverse events				
013 014 017	1/93-2/96 12/92-4/96 3/95-ongoing	US	721/721 214/214 60/60	randomised, double blind (or open) study, infants given PR concomitantly with an investigational pneumococcal conjugate vaccine (with DTwP, OPV)
U93-3663-01 U93-3663-02	5/93-5/95 5/93-5/94	US	294/294 527/527	randomised, double blind (or open) study, infants given PR concomitantly with investigational DTwP/IPV vaccine.

The submitted dossier included only the complete reports of four of these studies (001-002-004-005). Summaries of protocol 009 and 411-295 have been submitted; complete final reports are awaited.

- **Phase I studies**

Protocol 001 was a general safety study involving 21 adults. This was an open-label study designed to evaluate the safety of PR in healthy adults prior to initiating studies in healthy infant population. The combined vaccine was administered at day 0 and one month later.

- **Phase III studies**

Pivotal study (002) was an open, randomized study of safety and immunogenicity comparing three batches (CW927, CW928, CW929) of combined PR vaccine with the two monovalent vaccines (P+R) administered concurrently at different sites.

Two schedules 2-4-15 months and 2-4-12 months were assessed. At each study site, infants were initially assigned to the 2-4-15 month schedule until approximately half of the total targeted enrollment was achieved. All infants enrolled after that were assigned to the 2-4-12 month schedule. However, the scheduling of a third dose of vaccine was not randomised.

One of the three consistency lots of PR was not available when the study started; it was put into use only when subjects were assigned to vaccination at 2-4-12 months. At that point, allocation to treatment was based on the second randomisation schedule.

Concurrent administration of other standard pediatric vaccines with PR or P+R according to standard immunization practices (DTwP, OPV, MMR) was permitted but not required. In these cases, they were administered at a separate injection site.

The vaccines were administered by intramuscular injection in the thigh. Antibodies to PRP and HBsAg were measured in blood samples taken at 2, 4, 6, 12 or 15 and 13 or 16 months. By amendment, the protocol allowed for the collection of blood samples at approximately 7 months of age from a non-random volunteer subset of subjects who had received PR or P+R concurrently DTwP and OPV to ascertain antibody responses.

The original analysis tested for difference as failure to reject the null hypothesis and was intended to lead to a conclusion of similarity. A re-analysis of the immunogenicity data was requested by the **US** FDA: analysis was performed consistent with the objective of evaluating equivalence among the PR lots and between the PR lots and P+R (difference of 10 percentage points in the rates of subjects with anti-PRP titer > 1µg/ml and anti-HBs titer = 10 mIU/ml). A per-protocol approach was used. A total of 882 subjects were enrolled.

Protocol 004 was an open-label, multicenter study designed to evaluate the safety and immunogenicity of routine paediatric vaccines given concomitantly with PR. A total of 126 infants were enrolled. Infants were given PR at 2, 4, and 15 months with coadministration of DTPw+OPV at 2 and 4 months of age (or DTPw/IPV at 2 months of age) and DTPa+OPV+MMR at 15 months of age. Blood samples were taken at 6 months of age and 1 month after the third injection. The null hypothesis being that the immunological response rates to PR given concomitantly with other routine paediatric vaccines is more than 10 percentage points lower than the pre-specified expected responses.

Protocol 005 was a randomized open-label multicenter study. A total of 208 subjects were enrolled. Infants given PR, P+R, or P followed by R a month later, at 2, 4, and 15 months of age with co-administration of DTPw+OPV at 2 and 4 months of age and MMR at 15 months of age. Blood samples were taken at 2, 4, 6, 7, and 17 months of age. The objective of the study was to estimate anti-PRP and anti-HB levels at 6 months of age and the levels of antibodies to diphtheria toxin, tetanus toxin, pertussis and poliovirus at 7 months of age. No formal statistical comparisons were performed.

Antibody to (Assay Methods)	Protocol 002	Protocol 004	Protocol 005
PRP (RIA)	% > 1.0 µg 2 months post injection 2 (P2)	% > 1.0 µg 2 months post injection 2 (P2) expected response 75%	% > 1.0 µg 2 months post injection 2 (P2) expected response 70%
HBsAg (AUSAB RIA)	% ≥ 10mIU/ml 1 month post injection 3 (P3)	% ≥ 10mIU/ml 2 months post injection 2 (P2) expected response 90%	% ≥ 10mIU/ml 2 months post injection 2 (P2) expected response 86%
Poliovirus types 1, 2, 3 (inhibition test)	% neutralising antibody ≥ 4 3 months post injection 2 (P2)	% neutralising antibody ≥ 4 3 months post injection 2 (P2) expected response 90%	% neutralising antibody ≥ 4 3 months post injection 2 (P2) expected response 90%
Diphtheria (vero cells) Tetanus (EIA) Pertussis (microagglutination EIA)	% ≥ to fourfold rise relative to baseline 3 months post rejection 2 (P2)	% ≥ to fourfold rise relative to baseline 1 month post rejection 3 (P3) expected response 90%	% ≥ to fourfold rise relative to baseline 1 month post rejection 3 (P3) expected response 90%
Measles (EIA) Mumps (EIA) Rubella (EIA)	seroconversion in initally seronegative subjects	seroconversion in initally seronegative subjects expected response 90%	

Immunogenicity

Antibody levels of >1.0 µg/ml anti-PRP titer and ≥10 mIU/ml of anti-HBs titer were designated as the primary endpoints for assessing immunogenicity. In the original protocol, Geometric Mean Titers (GMT) was defined as the primary outcome variable. In the final data analysis plan, GMT was designed as a secondary outcome variable because it was felt that the development of certain antibody levels were more relevant clinically, than GMTs.

- **Anti-PRP response**

Two months after the second dose of vaccine, at the primary time point of 6 months of age, the serum anti-PRP response was considered to be satisfactory, within the range of anti-PRP responses obtained in immunogenicity studies of monovalent vaccine (53% to 100%).

However in the pivotal study 002 the responses in the PR and P+R treatment groups are considered as slightly low and are not considered to be equivalent. Moreover, data on anti-PRP responses (% achieving > 0.15 µg/ml and > 1 µg/ml) against different lots demonstrated a lack of consistency that required clarification. During the oral explanation on 15 December 1998, the company clarified the variability observed between lots.

Eight to 11 months after the second dose of vaccine, before administration of the third dose, the percentages of subjects developing anti-PRP > 1.0 µg/ml were found to be very low - lower than those usually reported, whatever the schedule of vaccination.

Although precise immunological correlates of protection against invasive Hib disease have not been established, antibody estimates such as = 0.15 µg/ml (assumed to give short-term protection) and =1 µg/ml (assumed to correlate with long-term protection) have been widely used in all studies with modern conjugated Hib vaccines. Before the third injection, the responses of children in the PR and

P+R treatment group were not considered to be equivalent for both % > 0.15 µg/ml and the % > 1.0 µg/ml.

Therefore in order to address the question of adequate protection of infants between the second and the third booster dose, the company provided additional information. In several studies, PR was shown to induce similar levels of anti-PRP titers and GMTs when compared to P containing 7.5 µg liquid PRP-OMP (Monovalent PedvaxHIB containing either 7.5 µg liquid or 15 µg lyophilized PRP-OMP are equivalent as far as immunogenicity is concerned). Prior to the third dose (at 12-15 months of age), 77.0 % of the infants given PR had > 0.15 µg/ml and 22.1 % had > 1.0 µg/ml of anti-PRP.

The company also refers to a former study in the Navajo population. In this study, anti-PRP responses elicited by 2 doses of lyophilized PedvaxHIB given at 2 and 4 months of age were similar to those of protocol 002. The efficacy for protection in the Navajo study was estimated at 93 % (for follow-up through 18 months of age) and 100 % (for follow-up through 15 months of age).

Nevertheless considering the low levels of anti-PRP titers before the third injection and the fact that the primary immunisation schedule comprises only 2 doses, an injection of a third dose at 12/15 months of age is considered to be absolutely mandatory; a statement indicating that all three doses must be administered to complete the vaccination regimen has been incorporated into the SPC.

- **Anti-HBs response**

In the pivotal study 002, the percentage of infants with anti-HBs ≥ 10 mIU/ml before the third dose were lower-following administration of the combined vaccine PR (78% - 80%) than that observed with the monovalent anti-hepatitis B vaccine (95% - 97%). One month after the third dose of PR vaccine, more than 97% of subjects in each of lots of schedule groups developed ≥ 10 mIU/ml of anti HBs.

However, the comparison of GMTs of PR to P+R following the third injection showed a statistically significant difference (p=0.011), lower for the PR group than for P+R group. However, the reduction in titer (4468 mIU/ml as compared to 6944 mIU/ml) is of questionable clinical significance as anti-HBs titers =10 mIU/ml are regarded as fully protective.

- **Concomitant use with other vaccines**

Antibody responses to poliovirus types 1-3

In study 004, 95% to 100% developed antibodies to all polio types following the third dose which was considered to be satisfactory. Discrepancies between centres and vaccines administered have been observed. Among the 23 non-responders from the largest study site, serologically followed up after the third dose, some remained poor responders with low antibody titers. More detailed information has been provided by the company and data are available for 22 of 23 non-responders after a 3rd dose of OPV. Of the 22 non-responders 19 seroconverted after an additional dose of OPV, however,
3 failed to develop specific antibodies (1 to polio type 1 and 2 to polio type 3). It is not clear why the response to polio vaccine in protocol 004 differed qualitatively by Centre. Nevertheless it seems rather unlikely that PR impairs the immune response to polio antigens.

Antibody responses to DTP

In studies 002, 004 and 005, 90% to 100% of the children receiving PR concomitantly DTwP, displayed a ≥ 4-fold rise in antibody to diphtheria toxin and 100% displayed a ≥ 4-fold rise in antibody to tetanus toxin. There is good evidence that PR can be co-administered with DTwP.

The initial data on co-administration of PR with DTaP for the primary series was too small
(18 subjects) to allow any definite conclusions. However additional data (51 subjects) on the efficacy evaluation of the primary vaccination series of an acellular pertussis vaccine based upon defined serological correlates of protection was presented by the company during the oral explanation on
15 December 1998. A statement indicating the limited data has been incorporated into the SPC.

Antibody responses to measles, mumps and rubella (MMR)

In studies 002, 004 and 005, among the children given MMR concomitantly with PR and P+R, 94 to 100% of subjects developed antibodies to measles virus, 97.3 to 100% developed antibodies to mumps virus and 91.3 to 100% developed antibodies to rubella virus. Additional data (GMT and seroconversion rates compiled from studies 002, 004, 005 and 009) have been provided by the company showing that the PR can be administered concurrently with MMR.

- **Interchangeability**

Although there is indirect evidence from published data on the interaction of Hib vaccine, direct evidence for interchangeability of PR (Procomvax) with other single Hib conjugate vaccines in a priming series is presently unavailable. The SPC has been amended accordingly.

Safety

Body temperature, injection site and systemic adverse events in studies 001, 002, 004 and 005 were recorded for at least 6 days after each injection.

In addition, subjects vaccinated in all 11 studies were monitored for serious adverse events.

- **Local reactions**

Of the subjects enrolled in study protocol 001, 95% (20/21) reported injection site reactions after the first dose (one subject withdrew from the study) and 85% after the second dose.

In study protocol 002, no significant differences were found for injection-site reaction between PR and P + R.

Local reactions in study protocol 004 were reported with a higher frequency following administration of DTwP/IPV (dual-chambered syringe) +PR than after the administration of DTwP+OPV+PR. These differences may reflect the dose volumes given at 2 months of age, since DTwP/IPV is a 1.0 ml injection and DTwP+OPV is only a 0.5 ml injection.

- **Systemic reactions**

Of the subjects enrolled in study protocol 001, systemic adverse reactions were reported in 14% of the subjects (following the first dose): none were serious.

In study 002, the frequency of unusual high-pitched crying (UHPC) following administration of PR as compared to P+R, was significantly higher after the second injection (p=0.016). Regarding other systemic adverse events, irritability was significantly more frequent in the PR treatment group at any injection (p=0.045). Cases of rash were more frequently reported following administration of PR than following administration of P+R.

In a post hoc investigation of the data, the odds-ratios were calculated to compare the frequency of unusual high-pitched crying in subjects receiving DTwP+PR versus those receiving PR alone. The odds ratio was 1.72 (95% CI 0.77-3.88) post dose 1 and 1.63 (95% CI 0.57-4.66) post dose 2. This suggests that unusual crying is more likely with concurrent DTwP. However, the lower bound of the 95% CI is below 1.0 (no association) and therefore it cannot be firmly concluded that DTwP is associated with a higher incidence of these adverse events.

The company provided review of studies with P and R in infants who received these vaccines without concurrent DTwP. The frequency of unusual high-pitched crying was 0.8%-1.7% following administration of 15 µg dose of the licensed lyophilised Hib formulation (P) and this event was not reported in two previous studies of R (2.5 µg).

The rate of unusual high-pitched crying reported in a large, historical study following DTwP vaccination is 0.1% which is much lower than the rate observed in the studies provided in this dossier. According to the company reporting of "unusual high-pitched crying" may be an artefact because this event was prompted for on diary cards without a precise definition (parents might have confused unusual prolonged crying with high-pitched screaming).

In study 004, as there was no control group (DTwP – PR) the analysis of the results is difficult. Systemic reactions, particularly unusual high-pitched crying, were reported with an exceptionally high frequency (with a higher incidence in the treatment group DTwP/IPV).

As with the two previous study protocols 002 and 004, systemic reactions, particularly unusual high-pitched crying, were reported with an exceptional high frequency infants enrolled in study protocol 005.

- **Serious adverse events**

Thirty nine events which met one or more of the defining criteria of a serious adverse event (SAE), were reported within 14 days in a total of 3353 subjects in the groups PR, P+R, P followed by R one month later. Of the 39 SAEs the numbers observed in each group were:

- 33/2993 (1.1%) receiving PR
- 6/290 (2.1%) receiving P+R
- none of the 70 subjects receiving P followed by R one month later

None were considered by the investigator to be possibly, probably or definitely related to the study vaccines. A re-evaluation and a detailed report of the 39 cases with serious adverse events was submitted by the company. After analysis of the data, a causal relationship seems rather unlikely between administration of PR and these serious adverse events.

- **Deaths**

Detailed reports on the 4 deaths have been submitted. An association between these deaths and preceding vaccinations (including PR/ Procomvax or P+R) seems unlikely and the deaths have been attributed to Sudden Infant Death Syndrome (SIDS).

Consistency of lots

Data from study 002 on anti-PRP responses (percentage achieving > 0.15 µg/ml and > 1 µg/ml) against different PR lots demonstrated a lack of consistency that required clarification.

During the oral explanation on 15 December 1998, the company clarified satisfactorily the variability observed between lots.

Post-marketing experience
Data collected from a large post-marketing database show that PR is generally well-tolerated and no condition consistent with, or suspicious for, unusual or high-pitched crying is documented. However it is noted that adverse events such as crying may not be spontaneously reported by physicians.

Two post-marketing safety studies of PR are ongoing; a trial evaluating the concomitant use of PR with DTaP and IPV (Protocol 011) and a field trial to evaluate medical events following vaccination (Protocol 012). The design of these trials do not address the question of UHPC as they do not compare the 2 groups PR and P+R.

Further information provided by the company
The company discussed the following outstanding issues during the oral presentation on 15 December 1998.

1. efficacy of PRP-OMPC; necessity of the third dose
2. interaction with other paediatric vaccines (DTaP)
3. interchangeability with other Hib vaccines
4. additional data demonstrating batch consistency
5. safety; unusual high-pitched crying (UHPC)

During the oral presentation on 15 December 1998, the company provided satisfactory explanations to all except the safety issue. The SPC was amended with respect to the first three issues relating to the efficacy, interaction, interchangeability.

Regarding the safety issue,it was discussed and agreed by the CPMP that further written explanation was required; in particular, the potential significance of "UHPC" in relation to brain damage and neurological sequelae.

Following the submission of the additional written explanation by the company which included opinions from four independent experts on the clinical and scientific meaning of "UHPC", it was concluded that there are no scientific data pointing to an association between "UHPC" occurring after administration of inactivated vaccines such as PR, and brain damage.

A higher frequency of "UHPC" and irritability with PR compared to P+R has been observed, the difference being statistically significant only at some time points. Thus in the case of Procomvax, there has been considerable difficulty in concluding whether the reactogenicity profile of PR is similar to the reactogenicity profile of P+R. However the higher reactogenicity of the combined vaccines compared with the mono components has been a general problem in the past and is not peculiar to Procomvax alone. Furthermore it was noted that the severity of "UHPC" was rated as mild to moderate by parents and no drop-outs were recorded due to this systemic side effect

In order to determine whether the differences observed between the 2 groups are an artefact resulting from the method of data collection and multiple comparisons, the company committed to perform a post-authorisation clinical safety study to compare the reactogenicity profile of Procomvax versus concomitant administration of P + R in infants vaccinated at 3 and 5 months of age.

This study will be a controlled study, with the assessment of the frequency of "UHPC" as primary criterion and the power of the statistical analysis will assume that the previous observed difference is not reproducible.

4. Overall conclusions and benefit/risk assessment

Although there has been some difficulty in concluding whether the reactogenicity profile of the combined vaccine Procomvax is similar to that of the monovalent components P and R, there have also been some trends such as the mild to moderate severity of "UHPC" and the lack of drop-outs due to this effect, which allow the reactogenicity profile for this combined vaccine, to be considered acceptable.
Considering all the information submitted by the company throughout the procedure, and also the pharmaceutical, biological and clinical follow-up measures undertaken by the company, the risk-benefit appeared favourable for the following indication approved by the CPMP:

'Procomvax is indicated for vaccination against invasive disease caused by Haemophilus influenzae type b and against infection caused by all known subtypes of hepatitis B virus in infants 6 weeks to 15 months of age.'

The CPMP granted a positive opinion for Procomvax, monodose suspension for injection in a vial.

At the time of the 5-year renewal, the CPMP considered that the benefit/risk profile of Procomvax continued to be favourable and therefore recommended the renewal of the Marketing Authorisation.