SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Hexavac. This scientific discussion has been updated until 1 October 2002. For information on changes after 1 October 2002 please refer to module 8B.

1. Introduction

Hexavac is a new formulated liquid formulation of a combination of existing vaccines, consisting of: diphtheria vaccine, tetanus vaccine, acellular pertussis vaccine, recombinant hepatitis B surface antigen vaccine, inactivated poliomyelitis vaccine and H*aemophilus influenzae* type b polysaccharide conjugated to tetanus toxoid vaccine. Administration of all these components in a single injection is a major step towards increased compliance with vaccination programs and control of infectious diseases, wide childhood vaccination coverage against six infectious diseases, as well as a better control of vaccination costs.

The diphtheria, tetanus and whole-cell pertussis (DTP) vaccine was the first combined vaccine containing pertussis and has been used for more than 50 years. DTP has been routinely administered mixed together with inactivated poliomyelitis virus vaccine (IPV) for more than 30 years in several countries. The reactogenicity profile of whole-cell pertussis vaccines led vaccine manufacturers to develop purified, well-characterised acellular pertussis vaccines (aP).

The administration of protein-conjugated Hib vaccines in infancy was shown to reduce dramatically the incidence of invasive Hib diseases and to diminish the oropharyngeal carriage of Hib. The same conjugated PRP-vaccine (Act-Hib) was also combined with DTaP or DTaP-IPV, and these new combination vaccines were recently licensed and are routinely used in a number of European countries.

The need for the control and prevention of hepatitis B (HB) infection has been recognised worldwide as a major public health goal. In 1992, the WHO assembly endorsed universal vaccination of infants against HB. Routine infant immunisation with HB vaccine is now recommended by various developed countries with low or intermediate endemicity, to effectively reduce the HB incidence by interrupting transmission before potential exposure. Economic evaluations have indicated that universal vaccination, even in low-endemicity countries, would be cost effective. Combination vaccines containing a HB component could accelerate integration of this vaccine into existing infant vaccination programmes even if in countries of high HB prevalence there is still need for a monovalent HB vaccine to be administered at birth. HB vaccine has already been successfully combined with DTP, DTaP or OMPC conjugated PRP (PRP-OMPC, Pedvax HIB) vaccines.

In recent years, diphtheria toxoid, tetanus toxoid, trivalent poliomyelitis concentrate, *Haemophilus influenzae* type b polysaccharide, and pertussis component (PTxd/FHA) have been licensed and marketed in several European countries (TETAVAX, D.T. VAX, TETRACT-Hib, PENTACT-Hib, TETRAVAC, PENTAVAC). The Merck recombinant hepatitis B vaccine has been licensed and marketed as a monovalent vaccine (HB VAX, RECOMBIVAX) or as a combined vaccine (PRIMAVAX, PROCOMVAX).

The pharmaceutical form of this vaccine is a suspension for injection by the intramuscular (IM) route. It contains aluminium as an adjuvant. The final product is preservative-free and presented in a pre-filled glass syringe.

This combined vaccine is indicated for active immunisation against diphtheria, tetanus, pertussis, hepatitis B caused by all known subtypes of viruses, poliomyelitis and invasive infections caused by *Haemophilus influenzae* type b.

- for primary vaccination in infants (from 2 to 12 months of age)

- for booster vaccination in toddlers (from 12 to 18 months of age)

provided the toddler has received a full primary vaccination course of each of the antigens contained in Hexavac regardless of whether they were administered as monovalent or combination vaccines produced by Aventis Pasteur MSD.

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

Hexavac is a new formulation of 6 antigens combined in a single suspension for injection. It contains as active ingredients:

Purified diphtheria toxoid, adsorbed		≥ 20 IU (30 Lf)
Purified tetanus toxoid, adsorbed		≥ 40 IU (10 Lf)
Purified pertussis toxoid, adsorbed		25 µg
Purified pertussis filamentous haemagglutinin adsorbed		25 µg
Recombinant hepatitis B surface antigen		5.0 µg
Inactivated poliomyelitis virus:		
	type 1	40 D units
	type 2	8 D units
	type 3	32 D units
Haemophilus influenzae polysaccharide type b conjugated to tetanus toxoid (24 µg)		12 µg

The antigen content for Hib is higher than in previous vaccines of the same manufacturer without affecting the ADR profile comparable to Hexavac. Similar amounts of antigen are used in other vaccines which have an ADR profile comparable to Hexavac.

The manufacturer has shown in clinical studies that the Hexavac acellular pertussis components PT and FHA confer sufficient efficacy comparable to the efficacy of other vaccines from the same manufacturer licensed already in Europe.

The product is contained in a 1 ml type 1 glass syringe with a stopper in contact with the suspension composed of a chlorobromobutyl elastomer. The syringe is supplied with or without a stainless steel needle.

Active substances

Diphtheria (PDT) and tetanus (PTT) toxoids are prepared from the respective toxins by formaldehyde detoxification followed by purification. The hepatitis B surface antigen (HBsAg) is produced by culture of a recombinant strain of *Saccharomices cerevisiae.* The poliomyelitis vaccine (IPV) is obtained from the propagation of polio viruses, types 1, 2 and 3, on Vero cells, purified and inactivated by formaldehyde. The acellular pertussis components (PT and FHA) are extracted from *Bordetella pertussis* cultures and separately purified. PT is detoxified with glutaraldehyde to create the toxoid (PTxd). The purified polysaccharide (polyribosyl ribitol phosphate [PRP]) of *Haemophilus influenzae* type b is conjugated to tetanus protein.

The addition of Haemophilus influenzae type b polysaccharide into the same container together with the other components and particularly the presence of aluminium hydroxide gave rise to the need to increase the content of PRP-T by 20% (ie 12 µg/dose). In fact, as stated by the manufacturer, it is important to obtain a low adsorption of PRP-T and a high adsorption of HBsAg.

Other ingredients

Hexavac also contains:

- Aluminium hydroxide (expressed as Al^{+++})	0.306 mg
- Buffer solution	up to 0.5 ml
disodium phosphate dihydrate,	

potassium dihydrogen phosphate,
sodium carbonate anhydrous,
sodium hydrogen carbonate,
trometamol, sucrose,
medium 199 Hanks without phenol red,
water for injection
(- 2.5 M sodium hydroxide or 10% acetic acid up to pH 6.80 to 7.20)
An aluminium content specification range 0.20-0.40 mg/dose was considered to be acceptable.

The selected formulation resulted in a partial adsorption for diphtheria toxoid and for tetanus toxoid in contrast to a complete adsorption of each of these antigens in other vaccines.

All tests on other ingredients used as starting materials, such as aluminium hydroxide, sodium chloride, sodium carbonate, etc. are performed according to the European Pharmacopoeia.

Product development and finished product

- Method of preparation

The preparation of the final bulk (blending), the filling into syringes and the packaging are carried out at Aventis Pasteur in Marcy l'Etoile (France). The finished product manufactured at Marcy l'Etoile is stored in a cold room at + 5° ± C 3°C.

The final bulk product (FBP) consists in blending all the 6 active ingredients with the excipient. The sequence of ingredient-excipient addition, the sterilization phases, and in process controls carried out during blending are detailed.

As Hexavac contains aluminium hydroxide gel, the FBP cannot be sterile filtered. The FBP manufacturing process is carried out aseptically according to the Eur. Ph. Requirement. All the components used for the FBP manufacture are sterile and are checked for sterility when produced.

All the packaging materials, syringes, needles, caps and stoppers are sterilised by steam in an autoclave. Filling is performed in a room with a controlled temperature (20°C), the Final Bulk Product is kept in a cold store (4°C) with stirring throughout the operation; after filling, the plunger stopper is inserted in each syringe. The syringes are then placed in a cold store.

The active ingredients are manufactured by Aventis Pasteur (in Marcy l'Etoile) except for the HBsAg component, which is prepared by Merck and Co (in the US). The manufacturing process has been presented in detail. Filling and secondary packaging operations with the relative in-process controls are also described.

All operations are stated to be carried out according to Good Manufacturing Practices (GMP).

- Production and control of starting materials

Diphtheria toxoid:

The preparation of PDT is based on a seed lot system. A highly toxigenic strain of *Corynebacterium diphtheriae* was used as primary seed lot (PSL). Several working seed lots (WSL) were obtained from this PSL in the form of lyophilised ampoules. At the end of the fermentation, the toxin-containing supernatant is clarified, concentrated, sterile filtered, detoxified in the presence of formalin, and purified by ammonium sulphate precipitation. This purified and concentrated toxoid preparation constitutes the bulk lot. The procedure and the relevant quality controls are described in the dossier. The antigenic purity for three batches of diphtheria toxoid is shown to meet the Eur. Ph. requirements.

Tetanus toxoid:

The preparation of PTT is based on seed lot system. The origin and the preparation of the primary seed lots and working seed are described in the dossier. A lyophilised strain of *Clostridium tetani* was used as the PSL. Several WSLs were obtained from this PSL in the form of lyophilised ampoules. Controls on primary and working seed lots fulfil the criteria of purity, identity and stability. The culture methods comply with the relevant Ph. Eur. monograph (452). At the end of the fermentation, the toxin is clarified, concentrated, detoxified in the presence of formalin and purified by ammonium sulphate (AS) precipitation.

Haemophilus influenzae type b polysaccharide conjugated to tetanus protein (PRP-T):

The polysaccharide (PRP) is extracted from cultures of *Haemophilus influenzae* type b, it is then purified and transformed into a derived polysaccharide PRP-AH by bonding with adipic dihydrazide acid (AH) after cyanogen bromide activation.

The tetanus protein is extracted and purified from *Clostridium tetani*, the same strain used for the preparation of the tetanus toxoid.
The derived polysaccharide PRP-AH is conjugated to the concentrated tetanus protein. After the conjugation process, the product is purified. The product obtained is referred to as *Haemophilus influenzae* type b conjugate concentrated bulk (PRP-T).

The production and purification steps, as well as all the routine control tests are the same as those carried out for the preparation of Act-Hib vaccine.

Pertussis components:

Hexavac contains two purified *Bordetella pertussis* antigens (PT and FHA). These antigens have been licensed and marketed in several countries in combination with other antigens.

The two pertussis components, PTxd (25µg) and FHA (25µg), are purified separately and adsorbed on aluminium hydroxide. PT is detoxified by glutaraldehyde.FHA is native.

The immunogenicity tests of pertussis antigens performed on independent final lots are satisfactory and show that the manufacturing processes are consistent and reproducible.
Hepatitis B surface antigen (HBsAg):

The yeast *Saccharomyces cerevisiae*, strain 2150-2-3, used as host for the recombinant HBsAg expression vector, was obtained from the collection of Leland Hartwell (University of Washington).

Cell bank system

A single cell clone was isolated following transformation of *Saccharomyces cerevisiae* strain 2150.2.3 with the plasmid PHBS56-GAP347/33 and used to prepare the master seed 13229-210.

Another lot of Master Seed was prepared, from the same Pre-Master Seed. DNA sequencing of the plus and minus strands of the plasmid from the newly prepared Master Seed was completed and the sequence was verified to be correct. Restriction analysis on the new Master Seed was performed and found to be satisfactory.

Production of the HBsAg active ingredient (bulk alum product)

The production site was Merck Manufacturing Division, Merck & Co. Inc., West Point, PA, USA. The fermentation process yields approximately 600-800 L of final culture.

The manufacturing process includes: fermentation, purification phase I (extraction of HBsAg, removal of detergent, aerosil purification), purification phase II (chromatography in Butyl-Agarose, Triton X 100 removal), removal of yeast proteins, denaturation (formaldehyde 3mM at 36°C for 60h), co-precipitation with aluminium hydroxide for the formulation of Bulk Alum Product.

The production takes place in a pilot unit and a general map including the different functional areas, the facilities and equipment together with cleaning and sterilisation procedures, the conditions of use and reuse of columns including loading conditions, yields, regeneration and storage between runs and life time of each column are described.

Validation of the process.

Five manufacturing lots of pure product were used for the biochemical and biophysical characterisation of the 24kDa protein (HBsAg). Analyses were performed on Purified Form III (PFIII) before and after filtration.

Primary structure was characterised by N-terminal and C-terminal sequencing of the S protein. It was performed in order to verify the accuracy of the translation and to ensure that no chemical modifications occur during downstream processing. The sequence was verified to be correct. Aminoacid analysis was performed by prediction and by mass spectrometry.

Lipid analysis was performed by colorimetric assay, HPLC, enzymatic method and thin layer chromatography. Carbohydrate content was determined by HPAEC and DNA content by hybridisation.

Biophysical characterisation shows that there are no significant differences in the secondary structure of the HBsAg protein among the five PFIII lots, as determined by CD and FTIR spectroscopy.

The mean particle diameter, as determined from the TEM micrographs, is in the range of 18-22 nm. The apparent densities of the particles, measured by CsCl density gradient sedimentation, are nearly the same for all lots.

The validation of the process is considered to be satisfactory.

Validation of relevant methods

The validation included the Master Seed batch analysis and the Stock Seed batch analysis. Validation of the analytical methods; Replica Plating assay and Quantification of Less Than Full Length HBsAg polypeptides and control procedures have been provided. The other tests are described and are in compliance with Eur. Ph. monograph on hepatitis B vaccines (rDNA).

In vivo mouse potency and *in vitro* relative potency assays are applied for testing the Bulk Alum Product. The mouse potency assay was validated for the thiomersal-containing formulation of hepatitis B vaccine Bulk Alum Product. Although the hepatitis B bulk intermediate for Hexavac is thiomersal-free, no significant difference in the reaction system of the assay should occur because the vaccine is the direct sample for the animal body (mouse), whereas the direct sample for the EIA is the animal serum.

The potency is expressed as the ED_{50} (effective dose where 50% of the mice tested exhibit an immune response) which is determined based on the number of mice responders at different doses. The test has a specification of $ED_{50} \leq 1.5$ mcg. In order to be 95% confident that the true potency does not exceed this specification, an alert limit of $ED_{50} \leq 1.0$ mcg has been established.

The *in vitro* relative potency assay (IVRP) is used to measure the relative amount of HBsAg in alum-adsorbed samples through parallel comparison to an H-B-VAX-II reference standard. Reproducibility and linearity have been evaluated and reference standards were prepared calibrated for µg/ml antigen content (16.16 µg/ml).

The IVRP of the HBsAg Bulk Alum Product (BAP) of Hexavac batches used in the clinical trial program ranged between 0.95 and 1.41. The applicant has committed to implement a new IVRP

specification for the BAP which will be at least 1.0. Thus, in future Hexavac batches the IVRP of the HBsAg active ingredient will correspond to the clinical trial batches.

A linear relationship between the MP Assay and the IVRP Assay that supports the interchangeability of the two procedures has been established.

Inactivated polio vaccine

The three poliovirus strains are produced as monovalent pools and subsequently mixed: the production process is documented for both in detail.

Primary & Working Virus Seed Lots

The preparation of the vaccine is based on a «Seed Lot System». The viral strains originate from the Rijks Institute, NL, and are the well known wild strains Mahoney (type 1), MEF1 (type 2) and Saukett (type3) grown in primary cultures of *E. patas* kidney cells. The WVSLs in current use (three for type 1, two for type 2 and three for type 3) are all VERO passaged, from which the subsequent monovalent bulk lots are derived.

Monovalent Bulk Lots and their inactivation

Ampoules of VERO cells, taken from the WCB, are thawed and amplified.

The subsequent steps of the virus harvest are concentration by ultrafiltration and purification by passage in three chromatography columns.

After purification and filtration, inactivation by formalin is started. At the end of the process the suspension is filtered and kept at 5°C ± 3°C up to the end of the due controls.

Concentrated trivalent bulk

The concentrated trivalent is manufactured under laminar flow.

The filtered concentrated trivalent is stored at 5°C ±3°C before blending with the other antigens of Hexavac.

Impurities

Impurities routinely determined are: DNA, protein, residual bovine serum albumin, antibiotics and DEAE beads. Validation studies have shown that the purification process is capable of removing these impurities to an acceptable level.

- Control tests on intermediate product

The quality tests are carried out on the intermediate product «Final Bulk Product». The release specifications are reported from data specific to five pilot FBP and five industrial batches.

Diphtheria toxoid

The requirement to set the LFL to ≥20 IU/dose is based on data of clinical batches and takes into account the high variability of the potency testing.

Tetanus toxoid

The potency test has been performed according to the Ph. Eur. for the monovalent vaccine. As reference, a monovalent product calibrated with the WHO reference (Reference toxoid ETIM-2, 300 IU/ml) was used. The limit ≥ 40 IU/ml selected is that of the Eur. Ph. .

The specific toxicity test is carried out according to the Ph. Eur. technique on 5 guinea pigs each of which receive an injection of 5 human doses (2.5 ml).

Non adsorbed PRP-T

The release specification of "≥ 8 µg PRP/dose" lower limit for the final product is acceptable.

However, it should be mentioned that the amount of tetanus toxoid-conjugated PRP is not covered by this specification.

HbsAg

Quality Control Tests and specifications are in accordance with European Pharmacopeia requirements.

Inactivated polio vaccine

The immunogenicity test in chicken (potency in vivo) has been performed in a different way from the specific Ph.Eur. monograph. However, the assay has been validated through statistical analysis of the data.

The results from five consecutive pilot sized batches have been provided. In all cases consistency in the results of IPV potency *in vivo* is achieved.

- Control tests on the finished product

For practical reasons, many control tests are carried out on the Final Bulk Product so that only the identification of the different active ingredients and sterility are performed on the finished product. The vaccine is filled in single-dose syringes

Specifications and control tests for the finished product are reported in the following table:

Tests	Ph. Eur.	Release specifications
Appearance	/	Whitish and cloudy suspension
Determination of extractable volume	2.9.17	≥ 0.5 ml for each of the 5 syringes tested
Aluminum content	2.5.13	0.20 – 0.40 mg/dose
Endotoxin content	2.6.14	For information
Bacterial and fungal sterility	2.6.1	Conform to Eur. Ph.
Pyrogen test (1/100)	2.6.8	Conform to Eur. Ph
Diphtheria identification	2.7.1	Positive
Tetanus identification	2.7.1	Positive
Haemophilus identification	2.7.1	Positive
Pertussis identification		
- FHA	2.7.1	Positive
- Pertussis toxoid	2.7.1	Positive
HBsAg identification	2.7.1	Positive
Polio virus identification (D Antigen)	/	Positive

The finished product shows good consistency in 5 production lots examined.

- Viral safety

HbsAg

No biological products, from human or animal origin are used in the production / purification process of recombinant HBsAg.

Inactivated polio vaccine

The WVSLs have been properly checked. The inactivation process of the polioviruses is validated and appears to be safe. The PCBs and the WCBs have been appropriately checked as described according to WHO and Ph Eur Requirements. For each new production, a sample of the cell cultures deriving from the WCB undergoes the appropriate controls.

- Animal-derived materials

The controls comply with the «CPMP BSE – Guideline CPMP/BWP/877/96«.

Stability of the Product

- Stability of the active substances/intermediate product

Diphtheria and tetanus toxoids

The stability of the two toxoids was studied at the following stages:

Crude diphtheria toxoid; At +5°C ± 3°C, there was no change for periods of 8 to 33 weeks. The study conducted for 6 months at 5°C ± 3°C demonstrates the stability of the product.

Crude tetanus toxoid; The study conducted for 20 weeks at +5°C ± 3°C demonstrates the stability of the product.

Purified diphtheria and tetanus toxoids; the mixture of the purified tetanus and diphtheria toxoids can be stored for 36 months at + 5°C ± 3°C.

Pertussis components

The stability studies have been carried out on Hexavac vaccine in final containers intended for licensing, in final bulk product, in native and adsorbed purified antigens.

Native purified PT: 48 months at 5°C

Native purified FHA: 48 months at 5°C

Adsorbed purified PT: 48 months at 5°C

Adsorbed purified FHA: 48 months at 5°C

Haemophilus polysaccharide;

PRP-T (concentrated bulk): Based on the data provided a 36 months period of stability (-35°C) is acceptable for the PRP-T.

PRP: Based on the data provided a 36 months period of stability (-20°C) is acceptable for the PRP.

PRP-AH: Based on the data provided a 24 months period of stability (-35°C) is acceptable for the PRP-AH.

Tetanus protein:

A 12 month stability period has been accepted.

Concentrated tetanus protein :

Based on the data provided a 24 months period of stability (+5°C ± 3°C) is considered to be acceptable for the concentrated tetanus protein.

HbsAg

Yeast cell paste; may be stored at 70°C for up to two years.

Clarified aerosil product; may be stored at 2-8°C for up to two weeks.

Sterile filtered product ; may be stored for up to one year at 2-8°C.

Final aqueous product; may be stored for up to one month at 2-8°C.

Bulk alum product; may be stored at 2-8°C for up to three years.

The applicant commits to only use HBsAg active ingredient with a cumulative holding period not exceeding 3 years (not considering the 2 years storage period at –70°C of the washed yeast cell paste). In the exceptional case bulk alum product (BAP) older than 24 months (a holding period of 36 months is approved for the monovalent Hepatitis B vaccine produced by the applicant) is used as an active ingredient in Hexavac, the applicant intends to set up a specific stability testing program to provide supportive data justifying the use of BAP older than 24 months.

Inactivated polio vaccine

Monovalent product

The shelf-life of 36 months is considered to be acceptable.

Concentrated trivalent product

30 months at 5°C ± 3°C.

- Stability of FBP

Based on the data provided the shelf life 6 months (4-8 °C) for the final bulk product is considered to be acceptable.

Ongoing study, currently 9 months at 5°C ± 3°C.

- Stability of finished product

The approved shelf-life for the finished product is 24 months stored at +5 °C ± 3 °C.

3. Part III: Toxico-pharmacological aspects

Pharmacodynamics

- *In vivo* studies

Immunogenicity (or potency) in mice

Immunogenicity (capacity for producing a specific immune response) or potency (capacity for triggering an immune protection to a challenge) has been quantified for each antigen on the various Hexavac lots produced. For each valence, the response was evaluated against a standard reference and compared with the response obtained from monovalent vaccine lots or from other marketed combination vaccines.

With respect to diphtheria, tetanus, polio and *Haemophilus* type b valences, the results obtained from 7 Hexavac lots were compiled and compared with the results obtained under the same conditions from 10 lots of various marketed vaccine preparations containing one or more of these valences. For each of the 4 valences (polio valence being analysed for 3 types : 1, 2 and 3) all the Hexavac lots passed the Pharmacopoeia tests and the statistical comparison with the results from the other vaccines revealed no significant decrease in response in any of the cases studied.

With respect to the pertussis valence, Hexavac contains two acellular antigens (PT+ FHA = acP). The antigenicity of acP was evaluated in the mouse by comparing, the antibody response to PT and FHA obtained in Hexavac, with that obtained in parallel under the same experimental conditions with the corresponding acP lots injected alone. The results obtained for the 6 Hexavac lots compared with those for the 6 corresponding acP lots showed a comparable response for the FHA and PT components.

The absence of interference from other valences on the antigenicity of the hepatitis B surface antigen (HBsAg) was evaluated through a lot by lot comparison in the mouse, as described above for the acP valence and the antibody response obtained with Hexavac was compared with the corresponding HBs response in the mouse administered alone and under the same experimental conditions. A study of 8 lots of Hexavac and corresponding HBsAg lots concluded that the other valences in Hexavac did not interfere with HBsAg immunogenicity.

- *Immunogenicity in rhesus monkeys*

The antibody response to the *Haemophilus* type b valences (PRP-T), Hepatitis b (HBs), Diphtheria (D), Tetanus (T) and pertussis (PT and FHA) obtained with Hexavac was compared with that obtained with 3 other monovalent or combined vaccines: ACT Hib (PRP-T) – Recombivax (Hbs) –DTaP-IPV combined vaccine.

In a vaccine scheme consisting of 3 intramuscular injections (0-4-8 weeks) carried out on 2 groups of 8 animals each (one group receiving Hexavac and the other 3 vaccines simultaneously at separate sites), blood samples were taken to evaluate the antibody response to the five valences mentioned.

On week 10, the peak antibody responses obtained for the hepatitis B, diphtheria and pertussis toxoid valences with the three separate vaccines were statistically significantly higher than that of the hexavalent combination thus showing evidence for interference in the peak response to HB, D and pertussis toxoid when the responses to Hexavac were compared with the control group. These apparent differences in potency are unlikely to translate into clinically meaningful differences since (a) the response to each component of the vaccine is > 100-fold higher than the pre-immune titers, and (b) the difference in responses to Hexavac and the control arm became indistinguishable over time. By study week 48-51, there was no significant difference in the antibody response to any component of the vaccine between monkeys injected with Hexavac vs. separate site injection of Hib + HB + DTaP-IPV.

Due to a shortage of sera, serology was not performed to detect antibodies against poliomyelitis antigens.

Boostering was not carried out within this monkey study.

- General and safety pharmacology programme

Potential secondary pharmacodynamic effects were investigated by the daily observation of the animals during the repeated dose study. No abnormal reactions were observed. Considering the background information from the marketed vaccine with the same antigens, these animal data and the lack of animals models specifically designed to assess secondary pharmacodynamic effects of vaccine, no additional investigations were thought to be necessary to assess secondary pharmacodynamic effects.

Pharmacokinetics

The mechanisms of action triggering humoral and cellular immunogenicity are complex and essentially qualitative. The duration of the vaccine effect far outlasts the presence of antigenic fractions even if adsorbed, and depends upon the immunocompetent cells of the receiver, not on antigen persistence. An exact knowledge of the distribution and degradation of the antigens in the composition of Hexavac was not considered to be useful since the listed antigens are well known and widely used in vaccinology.

Toxicology

- ***Single dose toxicity:***

Hexavac safety was evaluated in the mouse by subcutaneous route to mimic the human route of exposure (injected dose: 40 human dose/kg) and in the rat by intraperitoneal route to obtain a rapid bio-availability (injected dose: 12.5 human dose/kg). Both studies were performed according to Good Laboratory Practices. The doses tested are relatively high considering that one human dose for a 6 kg infant is equivalent to 0.17 human dose/kg of human body weight.

No signs of toxicity were noted in both species through clinical observation and gross necropsy examination. The local reactions observed in the mouse at the subcutaneous injection site were as expected for an adjuvanted vaccine.

The lack of a single dose toxicity study in rodents by intramuscular route is compensated by the rabbit local tolerance study performed by intramuscular route. The intraperitoneal route in the rat was used as a "worst case scenario".

- ***Repeat dose toxicity:***

The absence of toxicity was evaluated after repeated administrations in the rat according to a treatment schedule mimicking a human vaccination scheme (i.e. 3 injections at monthly intervals).
The fourth dose performed six months to one year after the third dose in the human vaccination scheme was not included in this toxicology study design. However, it is considered that the second and the third injections were sufficient to boost the immune response and to reveal potential adverse reactions.

The subcutaneous route was considered equivalent in terms of bioavailability to the intramuscular clinical route and a dose level of 5 human doses per kg was tested (human dose level: 0.17 human dose/kg of human body weight). A control group only received «aluminium» adjuvant, while a reference group received, in an identical manner, a pentavalent adjuvanted vaccine lot which is already marketed (differs from Hexavac by the presence of cellular (whole cell) *pertussis* valence and the absence of HBs valence).

All clinical observations followed by gross and histopathologic examinations and blood assays (haematological and biochemical) at completion of treatment revealed changes attributed to the vaccine properties of the reference and tested vaccines. None of these findings had a toxicological significance.

Based on the design of this repeated dose toxicology study, no immuno-toxicological findings such as autoimmune reactions were observed. It is noted that this animal study revealed no kidney or vascular lesions justifying further examinations or indicating potential immune complex deposition.

No hypersensitivity reactions were noted after the second or the third administrations. However the protocol used can be considered to be unable to detect this kind of reaction.

Each dose may contain residual but undetectable traces of antibiotic, namely neomycin, streptomycin and polymyxin B which in principle could be responsible for hypersensitivity reactions. However contact sensitisation rather than anaphylaxis have been reported for these antibiotics.

- ***Mutagenicity and carcinogenicity:***

Bearing in mind the nature of the Hexavac active constituents, namely the various antigens, mutagenicity and carcinogenicity were not carried out.

All manufacturing residual products and excipients are well known and no other contaminants with potential mutagenicity are present in the formulation.

- ***Reproduction Toxicity:***

No macroscopic or microscopic lesions were observed on testes and ovaries examined in the vaccine treated animals from the repeated dose toxicology study suggesting that there was no effect of Hexavac on reproductive organs and thus no impairment of fertility.

This vaccine is only intended for paediatric use, therefore the risk of embryo/fœtal toxicity is not considered to be relevant.

- ***Local Tolerance:***

A preclinical local tolerance study of the Hexavac preparation was carried out in the rabbit using the intramuscular administration which is the one used for humans. The study consisted of administration of one human dose of vaccine to a group of 8 animals and, at a different site, one equivalent dose of aluminium adjuvant, with subsequent sacrifice of groups of two animals 2, 4, 7 and 14 days after injection for macroscopic and histopathologic examination of the injection sites.
The results showed the local minor and reversible inflammatory reactions generally found with adjuvanted vaccines.

- ***Other toxicity studies***

Abnormal toxicity testing was performed for the first pilot and industrial lots, showing no signs of toxicity. According to Ph.Eur. requirements the test was not maintained as a routine batch release test.
Specific toxicity tests for tetanus, diphtheria and pertussis are applied on the final bulk product on a routine basis (batch release). All batches passed these tests.

Irreversibility testing of tetanus, diphtheria and pertussis toxoids are routinely performed on bulks of diphtheria, tetanus and pertussis toxoids. In addition, irreversibility tests were carried out on 5 expired batches of Hexavac (after 36 months), after exposure to elevated temperatures. No diphtheria, tetanus and pertussis toxins have been detected.

Five batches of Hexavac vaccines have been tested satisfactorily for pyrogens according to the third edition of the Ph. Eur. (2.6.8.).

- ***Environmental risk assessment***

This hexavalent vaccine contains no Genetically Modified Organisms (GMOs) that would warrant an environmental risk assessment as defined in Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms (98/C 139/01). In addition, no attenuated live micro-organisms are used in this vaccine; therefore the risk associated with reverse mutations to wild type micro-organisms is not applicable.

Vaccination is performed in a medical environment, which reduces the risk of potential misuse of this vaccine. There are no ingredients, which could be responsible of an ecological concern.

4. Part IV: Clinical aspects

In the nine clinical trials initiated by the applicant (one Phase I, one Phase II study and 7 Phase III studies), 3,905 infants received the investigational vaccine Hexavac as a three-dose primary series and 4,467 children were boosted with Hexavac, regardless of the vaccine used for primary immunisation. Two different vaccination schedules currently used in European countries for primary immunisation were investigated. Infants were vaccinated at 2, 3, 4 or 2, 4, 6 months of age. The 3-5-12 month immunisation regimen relevant for European Nordic countries and Italy is currently under investigation in Sweden. Six of the nine clinical trials were performed in France. All nine were conducted according to current European requirements and all conformed to Good Clinical Practice.
The development plan of Hexavac included the following studies:

<u>Phase I/ II: Choice of formulation</u>:

- Study 1: A phase I study investigating the safety profile of a single injection of two different formulations of hexavalent vaccine (single or dual chamber) in healthy 14 – 18 month children, previously immunised with against diphtheria, tetanus, pertussis and poliomyelitis in the first year of life

- Study 2: A multicentre phase II study which assessed the immunogenicity and safety profile of two formulations (single and dual chamber) administered in infants at 2, 3 and 4 months of age followed by a booster dose given between 12 and 14 months of age

Phase III primary series studies

- Study 3: A pivotal study which compared immunogenicity and safety of Hexavac to that of PENTAVAC and H-B-VAX II vaccines administered at two separate sites in infants at 2, 4 and 6 months of age.

- Study 4: Two different vaccination schedules at 2, 3 and 4 months of age and 2, 4, and 6 months of age were investigated.

- Study 5: A consistency lot study of immunogenicity induced by three consistency lots of Hexavac were studied

- Study 6: A large scale safety study was undertaken assessing the safety profile of Hexavac administered as a three-dose primary series at 2-4-6 months of age

Phase III booster vaccination studies

- Studies 2, 3, 4 and 6: Four studies were conducted in children who received primary series with Hexavac to assess the immunogenicity and safety of a fourth dose of Hexavac given during the second year of life

- Study 7: One study assessed safety and immunogenicity of a booster dose of Hexavac in children primed with PENTAVAC plus H-B-VAX II

- Studies 8 and 9: Two studies evaluated the booster response of Hexavac in children primed with PENTACOQ and with 0 to 3 doses of a licensed HB vaccine.

Clinical pharmacology

- *Pharmacodynamics*

Phase I

Study 1

The pilot study was conducted in France in 1995 to assess the safety and reactogenicity profile of two presentations (single or dual chamber) of the hexavalent vaccine, administered as a single booster dose to 58 children between 14 and 18 months of age. The children had been previously immunised in the first year of life with PENTACOQ, a vaccine widely used in France. Half of the subjects (n=30) received the liquid hexavalent combined vaccine Hexavac (0.5 ml volume) and the others (n=28) were given the dual-chamber hexavalent vaccine (1 ml total volume).
At this stage that both formulations were well tolerated and suitable for further clinical development.
Phase II

Study 2, Choice of formulation

A randomised, multicentre, open study was conducted in French infants in 1995-1996 to assess the immunogenicity and safety of two presentations (single or dual-chamber) of hexavalent vaccine to

select one for further clinical development. The vaccines were administered as a primary series to 312 infants at 2, 3, 4 months of whom 294 infants also received a booster injection at 12-14 months of age.

The data from this Phase II trial showed that for both formulations, the performance of the hexavalent vaccine, in terms of achieving predefined reference seroconversion or seroprotection rates, was not inferior to that expected, except for the anti-PRP seroprotection rate (≥ 0.15 µg/ml) achieved after administration of the Hexavac formulation. Thus, apart from the anti-PRP response obtained after administration of the Hexavac presentation, the two hexavalent formulations elicited adequate immune responses to all components.

The immune responses to PT, FHA, D, T and poliovirus components were of the same magnitude for both formulations, with the exception of anti-HBs and anti-PRP antibody responses. Anti-HBs antibodies GMT values and seroprotection rates were lower in the dual-chamber recipients.

One month after the booster dose, 96.9 to 100% of children achieved protective levels of antibodies to HBs, PRP, D, T, poliovirus components, and a substantial increase in GMT values was shown for all components. PT and FHA antibody responses were similar for both formulations and comparable to those previously reported. After the booster dose, the GMT values for anti-HBs antibodies were higher in the Hexavac group than the dual-chamber group (1458 vs 530 mIU/ml), but the seroprotection rates were of the same magnitude for the two formulations (> 97%).

The frequency of local and systemic reactions appeared to be somewhat lower after administration of the liquid presentation and this formulation was obviously much easier to use therefore, it was decided to adopt the liquid Hexavac vaccine for further clinical development.

Phase III

Study 5: Immunogenicity of three consistency lots

A randomised and double-blind study, designed to assess the equivalency of immunogenicity of 3 consistency vaccine lots (S 3127 - S 3128 - S 3170) of Hexavac, was conducted in 1,028 Chilean children who received the vaccine at 2, 4, 6 months of age. An equivalence testing approach was used and the two sided 90 % confidence intervals of the difference of the response rates between each pair of vaccine lots and for each antigen were calculated with the predefined maximum acceptable difference δ of 10 %.

The secondary objectives were to compare the seroprotection and seroconversion rates to each antigen and for each vaccine lot with an historical reference (derived from immunogenicity results observed with PENTAVAC and H-B-VAX II in the same population). Lots S3127 and S3128 were also used in study 4 and study 3 respectively.

The three consistency lots of Hexavac were shown to be statistically equivalent in terms of seroprotection or seroconversion rates and in terms of GMTs achieved one month after the third dose, for all antigenic components. The absolute value of the bounds of the 90% CI of the observed differences in seroconversion or seroprotection rates between the vaccine lots was less than 2% for anti-D, T, and poliovirus type 1, 2, 3, antibodies, less than 4% for anti-PRP and HBs antibodies, and less than 7% for anti-PT and FHA antibodies. Homogeneity of the three vaccine lots was assessed by the rejection of the null hypothesis of a 2-fold ratio of GMTs between any two lots. The ratios of GMT values between any two lots, ranged between 0.85 for anti-FHA antibodies and 1.39 for anti-HBs antibodies. For all vaccine components, the three lots of the vaccine induced an antibody response that met the pre-defined requirements.

Clinical efficacy

Main studies (phase III, therapeutic confirmatory trials)

Seven Phase III studies were conducted between 1996 and 1998 involving 3,748 infants who received Hexavac as a three-dose primary series between the age of two and six months (studies 3 to 6), and 4,286 toddlers given a booster dose of Hexavac during the second year of life (studies 3, 4 and 6 to 9). Among the 3,905 infants who received at least one dose of Hexavac, 3,788 (97%) received the complete three-dose primary regimen. Of these, 1,993 infants received the complete 3-dose primary series and had a blood sampling one month after the third dose. Of these 1,993 infants, 1,654 were included in the PP analysis of immunogenicity of the primary series.

Description of the studies

Study 3: Pivotal immunogenicity study of Hexavac and of PENTAVAC (pentavalent DTaP-IPV-Hib) and H-B-VAX-II (recombinant monovalent hepatitis B) administered concurrently at two different sites

The potential interference of the hexavalent liquid combination on the immunogenicity of the different antigen components, i.e. the PENTAVAC, and H-B-VAX II vaccine was evaluated in a multicentre, randomised study conducted in France. A total of 848 infants received at least one of the three doses given at 2, 4 and 6 months of age of either the investigational vaccine or PENTAVAC and H-B-VAX II given concurrently intramuscularly but at two different sites (in the two thighs).

For PRP and HBs components, according to the statistical hypotheses, the observed seroconversion rate of Hexavac was non-inferior to that of PENTAVAC and H-B-VAX II; 93.7% of children achieved a seroprotective response at the threshold of 0.15 µg/ml. However, the 95% CI of the seroprotection rates did not include zero, suggesting the vaccine performances might not be similar.

The immune responses to all other components in terms of seroconversion or seroprotection rates and GMTs were similar between the two groups. For PT, a 19-26% reduction in GMT value was observed depending on the titration method (CHO or EIA). In contrast, for FHA, GMT values were higher with the investigational vaccine (19% increase). No effect was observed on the percentage of children achieving a four-fold rise in titres of antibodies to PT and FHA. Furthermore, the immune response to both pertussis antigenic components was within the range reported after primary vaccination with DTaP in the Senegal efficacy trial and with PENTAVAC in different clinical studies.

Study 4: Comparison of two immunisation schedules: 2, 3, 4 vs 2, 4, 6 months

A multicentre, open, randomised comparative study with two groups was conducted in France in order to assess the safety and immunogenicity of Hexavac given at two different immunisation schedules, 2, 4, 6 months and 2, 3, 4 months of age, respectively. The objective of this study was to ascertain whether the investigational combined vaccine (DTaP-IPV-PRP~T-HBs) is more immunogenic when administered at 2, 4, 6 months of age than at 2, 3, 4 months of age. Five hundred and sixteen healthy infants were enrolled in the trial, 258 infants per group.

Primary objective was the comparison of the percentage of children showing a PRP antibody titre ≥ 1.0 µg/ml one month after the third dose vaccinated at 2, 4, 6 months or 2, 3, 4 months of age. As secondary objectives the immunogenicity of each antigen against referenced percentages one month after the primary series was studied. A reference seroconversion/ seroprotection rate for each vaccine component was derived from the database developed from the current tetravalent and monovalent vaccines. For each component it was decided to assess whether the seroconversion/ seroprotection percentage was smaller than the expected reference rate.

The percentage of infants with a anti-PRP antibody titre ≥ 1 µg/ml one months post-dose 3 was significant higher with the 2-4-6 month immunisation schedule than with the 2-3-4 schedule (62.5 % vs. 73.5 %, p= 0.0165).

When compared with the expected seroconversion/ seroprotection rates, defined according to the historical references for serum titres correlated with seroprotection, all the vaccine antigens except for PRP satisfied the pre-established requirements, based on the predefined δ, in both groups and

according to the per protocol analysis. For the ITT-analysis, only the group with the accelerated vaccination schedule failed to comply with the predefined criteria.

The seroconversion or seroprotection rates to all other components were within a clinically acceptable range for the two immunisation regimens and comparable to those seen with the historical control.

One month after primary immunisation the GMT values for antibodies to all antigens with the exception of those for pertussis antibodies measured by EIA, appeared to be higher when Hexavac was given using the extended immunisation regimen. However, these differences are clinically not relevant. Taking into account the pre-booster titres both vaccination schedules are comparable.
Pre- and post-booster immunogenicity results:

The pre-booster GMTs for PRP antibodies were 0.623 μg/ml (95 % CI; 0.498-0.781) and 0.416 μg/mL (95 5 CI; 0.342-0.506) in infants primed at 2, 3 and 4 months (Group 1) and 2, 4, 6 months (Group 2) of age respectively. Protective levels of PRP antibody (≥ 0.15 μg/ml) persisted in 77.5 % of children in group 2 and 85.2 % in Group 1. Nineteen children in group 1 (2, 3, 4 month) and 14 in group 2 (2, 4, 6 month) included in the per-protocol population for the primary and the booster vaccinations did not achieve an anti-PRP response ≥ 0.15 μg/ml, after the primary series. Fourteen/ 17 children in group 1 and 11/12 in group 2 had antibody titres ≥ 1 μg/ml after the booster dose. These titres are assumed with long-term protection.

GMTs for HBs antibodies were 51.4 mIU/ml and 36.7 mIU/ml in infants primed at 2, 3, 4 and 2, 4, 6 months of age respectively. The percentage of children still seroprotected at the 10 mIU/ml threshold was 85.5 % in Group 1 and 72.8 % in group 2. The post-booster response was high with 94.9 % (accelerated regimen) and 97.7 % (extended regimen) seroprotected. The GMTs were 1465 mIU/ml and 1089 mIU/ml respectively.

A total of 15 children (11 in group 1 and 4 in group 2) did not achieve seroprotective titres to HBs after booster. Six (5 in group 1 and 1 in group 2) had a high pre-immunisation antibody titre corresponding to maternal antibodies ranging from 455 to 48691 mIU/ml, and four had a weaker antibody titre pre immunisation ranging from 16 to 76 mIU/mL.

The antibody persistence for D, T, FHA and PT (EIA and CHO assayed) was similar in both groups. In contrast the persistence of antibodies to polio 3 virus types was higher in group 2 than in group 2. The antibody titres ≥ 5 (17dil) were measured in at least 88.2 % of children. Nevertheless, the booster immune response was high to these components and similar in both groups.

Study 6: Large scale safety study of Hexavac given as a primary series and as a booster dose during the second year of life

This single group, open, multicentre study was conducted in Germany between 1996 and 1998 involving 1, 783 infants who were to receive three i.m. doses Hexavac at 2, 4 and 6 months of age. 1,735 infants effectively primed, received a booster dose of the vaccine at 12 to 14 months of age. A summary of the safety data obtained during the trial program is provided separately.

As a secondary objective, in a subset of 118 infants, immunogenicity analysis of the PRP response was performed. After the primary series, 95.5 % of infants of whom immunogenicity data were available were seroprotected at the 0.15 μg/ml level and 69.6 5 at the 1.0 μg/ml level. One month after the booster, all children were seroprotected at the 0.15 μg/ml level and 95.4 % had titres ≥ 1 μg/ml.

Study 7: Analysis of immune responses to a booster dose of Hexavac in children primed with DTaP-IPV/Hib combination vaccine (PENTAVAC) and Hepatitis B vaccine

The immunogenicity of a booster dose of Hexavac was assessed in 129 14-18-month old children primed with PENTAVAC and HB-VaxII given concurrently as a primary series at 2, 3 and 4 months of age, in an open one-arm descriptive study conducted in Turkey.

One month after the booster dose, a strong immune response was elicited for all components of the vaccine indicating a high secondary response. All children achieved seroprotective titres to HBs, PRP (≥1 µg/ml), diphtheria and tetanus (≥0.1IU/ml) and to the three types of poliovirus. Antibody GMT values increased by at least 5-fold from pre-booster levels for poliovirus antigens to 161-fold for PRP antigens. The anamnestic response to the pertussis components was strong and titres increased at least 18-fold from pre- to post-booster levels.

These data indicate that Hexavac, administered as a booster dose in children already primed in infancy with PENTAVAC and HB-VAXII given concurrently in two separate sites is able to elicit a strong secondary immune response to all vaccine antigens.

Study 9: Analysis of immune responses to a booster dose of Hexavac in children primed with whole-cell pertussis DTP-IPV/Hib combination vaccine (PENTACOQ) and Hepatitis B vaccine.

All 307 children enrolled in this study to receive a booster dose of Hexavac received PENTACOQ as a three-dose primary regimen; one-third of infants had received two doses of HB vaccine, and two-thirds had received three doses. The booster dose of Hexavac was administered at the mean age of 16 months [range 14-21 months].

Antibodies to HBs and PRP persisted at higher levels (93.8% to 95.5% for HBs and PRP respectively) up to the time of the booster dose in those children primed with PENTACOQ compared with children primed with Hexavac.

After primary immunisation with PENTACOQ and HB vaccine, high antibody levels to D, T poliovirus components were still present at the time of the booster dose, with concentrations being of the same order of magnitude as those observed after priming with Hexavac.

In contrast, the level of persistent antibodies to pertussis antigens appeared higher in children primed with Hexavac than in those primed with whole-cell PENTACOQ.

A strong anamnestic response was observed for all components of Hexavac in children primed with PENTACOQ and HB vaccine, with seroprotection rates of the same order of magnitude as those achieved after a fourth dose of Hexavac. However except for pertussis and poliovirus antibody responses, GMT values appeared higher after a booster of Hexavac when the children were primed with PENTACOQ, this being most marked for anti-PRP and anti-HBs antibodies.

One month after the booster, GMT values for anti-HBs antibodies appeared lower in children who had received two priming doses of HB vaccine compared with those who had received three doses, although no impact on seroprotection rates was observed. These findings show that a booster dose of Hexavac elicits an anamnestic immune response to all vaccine components in children who had been primed with a complete three-dose primary series of a whole-cell pertussis pentavalent combined vaccine and two or three doses of HB vaccine.

Primary endpoints/assays

All serological analyses were carried out in the same laboratory at Aventis Pasteur.

Diphtheria toxoid (IgG) antibody titres were assayed by EIA with a LLOQ of 0.0006 IU/ml. Anti-D response was also assessed by neutralisation test on Vero cell culture in comparison to the WHO equine antitoxin standard. Results are reported in IU/ml as the highest reciprocal dilution of the test serum that allowed cell metabolism in the presence of four times the minimum cytopathic dose of diphtheria toxin.

PRP antibody titres were measured by RIA in comparison to the FDA human reference (test derived from Farr's method) with a LLOQ of 0.07 µg/ml.

Anti-HBs titres were assayed by RIA (AUSAB-ABBOTT kit) in comparison to the WHO standard with a LLOQ of 2 mIU/ml.

FHA (IgG) antibody titres were measured by EIA with a LLOQ of 2 EU/ml (EU: EIA Unit).

PT (IgG) antibody titres were measured by measured by EIA with a LLOQ of 2 EU/ml.

PT functional antibody titres were also determined by a toxin neutralisation test on Chinese Hamster Ovary (CHO) cell culture with a LLOQ of 2 (1/dil). Titres were expressed as the highest reciprocal dilution of the test serum resulting in 100 % inhibition of the clustering effect of the toxin. In addition the antibody response to PT (EIA, CHO) and FHA (EIA), the percentage of infants with titre ≥ 4 and ≥ 32 EU/mL were calculated.

Anti-polio virus 1, 2, 3 antibody titres were measured by microneutralisation on Human Epidermoid Carcinoma Cell line (Hep-2) culture according to the WHO standardised procedure (WHO/EPI/GEN 93.9) with a LLOQ of 5 (1/dil). Results were expressed as the highest reciprocal dilution of the test serum that inHibited the cytopathic effect of the challenge virus.

Seroconversion and seroprotection percentages are defined as follows:

anti-PRP response:	% ≥ 0.15 µg/ml
anti-HBs response:	% ≥ 10 mIU/ml
anti-diphtheria response:	% ≥ 0.01 IU/ml
anti-tetanus response:	% ≥ 0.01 IU/ml
anti-PT and anti-FHA response:	% with a four-fold rise in titre
anti-polio virus type 1, 2 and 3:	≥ 5 (1/dil)

Statistical analysis

Statistical methodology for the immunogenicity evaluation of Hexavac was based on the hypothesis that the observed antibody response to each antigen, one month after completion of the primary series, would not be inferior to predefined reference seroconversion or seroprotection rates by an appropriate pre-established maximum acceptable difference δ (Tab. 1). Reference values and acceptable differences were based on previous experience or literature data regarding combination vaccines. The same approach to assess non-inferiority of Hexavac immunogenicity was used when a control group was included in the study design, i.e. PENTAVAC plus H-B-VAX II in study 3.

Tab 1: Criteria of judgement of clinical and statistical assumptions (studies 2 – 5)

Study code/Study no	**A3R03/no 2**		**A3R08/no 3**		**A3R13/no 4**		**A3R09/no 5**	
CRITERION FOR SEROCON-VERSION/ SERO-PROTECTION (%) PER ANTIGEN	**Pref***	**δ****	**Pref**	**δ**	**Pref**	**δ**	**Pref**	**δ**
HBs ≥ 10 Miu/mL	90%	14%	90%	15%	92%	10%	92%	10% (15%)[#]
PRP ≥ 0.15 µg/mL	98%	9%	98%	10%	98%	9%	99%	10%
PRP ≥ 1.0 µg/mL	-	-	-	-	65%	at least 15%	-	-
D ≥ 0.01 IU/mL	99%	9%	99%	10%	99%	9%	99%	10%
T ≥ 0.01 IU/mL	99%	9%	99%	10%	99%	9%	99%	10%

PT ≥ 4-fold rise	90%	14%	90%	15%	92%	10%	92%	10% (15%)[#]
FHA ≥ 4-fold rise	90%	14%	90%	15%	92%	10%	92%	10% (15%)[#]
Polio 1, 2, 3 ≥ 5	99%	9%	99%	10%	99%	9%	99%	10%

Pref: Reference response rate.

*** δ: Pre-defined acceptable difference*

[#]: For secondary criteria

Reference values for seroconversion or seroprotection rates as well as for acceptable differences δ, were established based experience available at that time with monovalent vaccines or previously developed combination vaccines but had to be adjusted following the experiences with the first studies.

The primary immunogenicity analysis was performed on the Per Protocol (PP) population (n=1,654), i.e. excluding infants with at least one major protocol deviation. Most of the reported major protocol deviations concerned infants who were outside the interval range allowed between injections or between the last dose of vaccine and the subsequent blood sampling. For this reason, the percentage of infants excluded from the PP analysis of immunogenicity was rather high (approximately 17% of infants who completed the primary series and who had a blood sample collected one month after the third dose). Results from the Intent to Treat (ITT) population (n=1,993) were consistent with those of the PP population.

Antibody titres were summarised using geometric mean titres (GMTs), calculated by taking the anti-log of the mean of the log transformed titres, with their 95% CI.

Efficacy results

Overall analysis of the immune response

- HBs antigen (Studies 2 – 5)

Except in the Phase II study (study 2), in which a lower seroprotection rate was observed (91.6%), the percentages of infants with seroprotective titres (≥ 10 mIU/ml) of anti-HBs antibodies were high and consistent (over 95%) in all Phase III studies whatever the immunisation schedule used (2, 3, 4 month and 2, 4, 6 month) The lower bounds of 95% CI for seroprotection were above 91% in all Phase III studies. When all the Phase II and Phase III studies are considered together, 96% [95% CI 94.9-96.9] of the 1,634 children included in the PP analysis had seroprotective titres of HBs antibodies (≥ 10 mIU/ml) after completion of the three-dose primary regimen, whatever the immunisation schedule used. The seroprotection rate achieved after primary immunisation with Hexavac (91.6%-97.1%) is within the range of what has been reported for licensed HB vaccines given, as a primary series in infants (87.7% - 100%).

Anti-HBs antibody GMT values were found to be somewhat higher with the extended schedule than with the accelerated schedule (study 4) after the primary immunisation, as may be expected due to the longer time interval between the second and third doses of vaccine

GMTs for HBs after Hexavac were lower than that observed after three doses of H-B-Vax-II given alone (literature data) or together at two different sites with PENTAVAC (study 3). On the other hand, seroprotection rate and GMT values for HBs antibodies after completion of the primary series with Hexavac appeared comparable or higher to those obtained after two 5 µg doses of HB vaccine combined with PRP-OMP vaccine, PROCOMVAX, given at 2 and 4 months of age (primary immunisation) with a seroconversion rate of 92.1 % and a GMT of 114 mIU/ml (Pivotal trial). In order to investigate further the immune response to Hexavac, the company has committed to perform antibody persistence studies with Hexavac and also to provide data on anti-HBsAg antibody kinetics over long-term follow-up. These studies have been shown to be adequately designed to provide further information on the persistence of the anti-HBsAg-antibodies. The studies will also provide data on the need for a booster and with 3 time points of blood sampling some information on the antibody kinetics.

- PRP antigen (Studies 2,3,4,5)

One month after the third dose of Hexavac given as a primary series, the seroprotection rate to PRP (≥ 0.15 µg/ml) varied from 92.7 to 98.3% when all Phase III studies were considered (studies 3-6). In each Phase III study the lower bound of the 95% CI for the seroprotection rate was above 88%. When all Phase II and Phase III studies are considered together, 95.4% [95% CI 94.3-96.3] of the 1,646 children included in the per protocol (PP) analysis had titres of anti-PRP antibodies (≥ 0.15 µg/ml) after completion of the primary series. The percentage of vaccinees with antibody titres ≥ 0.15 µg/ml was comparable in French infants vaccinated according to either the accelerated (2, 3, 4 months) or the extended schedule (2, 4, 6 months) (studies 2, 3, 4). Anti- PRP titres ≥ 0.15 µg/ml calculated in the PP analysis was achieved by 93.1% [95% CI 91.2-94.8] of 801 French children vaccinated at 2, 3, 4 or 2, 4, 6 months of age. Except for the Phase II study (study 2) anti-PRP immune response, in terms of seroprotection rate (≥ 0.15 µg/ml) met the predefined requirements of non inferiority to PENTAVAC™, as established for the pivotal study (study 3), or when a reference seroprotection rate of 98% with a maximum acceptable difference of 10% were used as predefined clinically relevant criteria.

In contrast, GMT values of PRP antibodies, as well as the seroprotection rate ≥ 1 µg/ml, varied according to the schedule and to the population studied (from 1.42 to 4.37 µg/mL and from 62.0 to 85.1%, respectively) with the highest seroprotection rate ≥ 1 µg/ml and GMTs in the Chilean infants.

The 2, 3, 4 month schedule induced lower anti-PRP GMT values and lower seroprotection rates (≥ 1 µg) than the 2, 4, 6 month regimen as demonstrated in study 4.

The percentage of infants who achieved the threshold of at least 1 µg/ml for anti-PRP antibodies was lower after immunisation with Hexavac (between 62% and 73.5% in the European populations) than observed after vaccination with the PRP-T, Act-Hib monovalent vaccine. A decrease of GMT values and seroprotection rates (≥ 1 µg/ml) has been reported with other licensed combination vaccines containing a Hib component, when compared with data obtained following separate administration of the same vaccines. On the other hand, the seroprotection rate obtained with Hexavac was in the range with those reported for the monovalent PRP-D vaccine that has been shown to be effective in protecting against invasive Hib disease during efficacy trials conducted in Finland. In addition, the anti-PRP response of infants after three doses of Hexavac observed in the studies was within the same range (91.7%-98.3%) as that seen in native American Navajos, which was associated with a dramatic reduction in the incidence of invasive disease in this population. These results of the booster dose demonstrate that three doses of Hexavac given within the first six months of life can adequately prime infants and can stimulate their immunological memory.

The GMT values were approximately twice as high in the Chilean population (study 5) than in the French population although the same lots of Hexavac were administered and the same 2, 4, 6 month immunisation schedule used. A higher immune response in the Chilean population compared to the European population has also been previously reported in other clinical studies (Hopenbrouwers K Safety and immunogenicity of Haemophilus influenzae type b-tetanus toxoid conjugate (PRP-T) and diphtheria-tetanus-pertussis (DTP) combination vaccine in infants in a dual-chamber syringe to infants in Belgium and Chile. Vaccine 1998, 9/10, 921).

- Pertussis components

When all Phase III studies are taken into consideration, the percentage of infants achieving a four-fold rise in antibody titres to PT or FHA, as measured by EIA after primary immunisation with Hexavac, was remarkably consistent throughout the clinical studies, ranging from 89.5% to 93.0% for the response to PT (EIA), and from 90.5% to 93.5% for the response to FHA (EIA), whatever the population or the vaccination schedule considered. Of 1,561 children included in the PP analysis of Phase II and Phase III studies, 91.7% [95% CI 90.3-93.1] showed a four-fold rise in antibody titres to

PT (measured by EIA) after the three-dose regimen. A four-fold rise in antibody titres to FHA was observed in 91.1% [95% CI 89.6-92.5] of 1,539 children included in the PP analysis.

For these two vaccine components, there was a slight tendency for higher GMTs in the Chilean infants (study 5) compared to French infants (studies 2, 3, 4).

Titres and seroconversion rates for PT-neutralising antibodies (% ≥ 32 and % of four-fold rise) measured in a CHO cell assay were somewhat lower than those for anti-PT antibodies measured by EIA and, more importantly, were lower when Hexavac was given at 2, 3 and 4 months of age compared with the extended schedule. The impact of the accelerated immunisation regimen on the PT neutralising antibody response and its lack of effect on anti-PT antibodies measured by EIA has previously been reported in Senegalese DTaP recipients.

It should be noted that, regardless of the immunisation schedule or population, GMT values, as well as the percentages of infants who developed a four-fold rise in PT (EIA or CHO assays) or FHA antibodies, which is considered as a sign of seroconversion, were strictly within the same range as those observed in infants primed with PENTAVAC or TETRAVAC (DTaP IPV) vaccines. These data were confirmed in study 3 when seroconversion rates to PT and FHA achieved after immunisation with Hexavac were compared with those obtained with PENTAVAC plus H-B-VAX II administration. The same held true for the proportion of infants who achieved a titre of anti-PT antibodies ≥ 32 measured by a CHO assay (69.6%-90.7).

- Diphtheria, Tetanus and Poliovirus type 1, 2, 3 components

When all Phase III studies are considered together, one month following completion of the primary series, all infants studied achieved diphtheria antibody titres ≥ 0.01 IU/ml (as measured by EIA), regardless of the immunisation schedule or population concerned. Of the 1,609 included in the PP analysis, all but one infant, in study 2, i.e. 99.9% [95% CI 99.7-100] had seroprotective titres ≥ 0.01 IU/ml, as measured by EIA. This child who did not attain the seroprotective threshold after primary series had received Hexavac at 2, 3 and 4 months of age. This same child achieved the seroprotective level (≥ 0.1 IU/ml), as measured by the seroneutralisation assay, after receiving the booster dose.

Among the 1,095 infants included in the PP analysis and for whom sera were assessed by both the Vero cell and the EIA assays, only 4 children did not achieve the seroprotective titre (≥ 0.01 IU/ml) for diphtheria neutralising antitoxins after completion of the primary series.

For all studies, the overall evaluation of the immune response to diphtheria toxin in terms of GMTs and seroprotection rates was within the same range as that observed with PENTAVAC and TETRAVAC.
One month after completion of the primary series, all 1,630 infants included in the PP analysis achieved seroprotective titres for tetanus antitoxins at the 0.01 IU/ml level, and 99.8% [95% CI 99.5-100] of children achieved the 0.1 IU/ml level. The 3 children who did not reach the 0.1 IU/ml threshold were in the group that had received Hexavac at 2, 3 and 4 months of age.

Similarly, the neutralising antibody response to poliomyelitis virus types 1, 2 or 3 was not affected by the combination of the pentavalent vaccine with HB vaccine in the same syringe. The percentages of infants with detectable antibody levels ≥ 5 (expressed as the reciprocal dilution) and GMT values after immunisation with Hexavac were comparable to results obtained after administration of PENTAVAC and H-B-VAX II at different sites (study 3) or to historical clinical data obtained with PENTAVAC or TETRAVAC.

Among the 1,531 infants who completed the primary series and who were included in the PP analysis, one month after the third dose of the primary series, all infants but one showed detectable antibodies to poliovirus type 1, 2 or 3, and at least 94.7%, 91.0% and 96.1% of children achieved a neutralising antibody titre ≥ 50 to poliovirus types 1, 2, 3, respectively.

As was seen for the anti-PRP and anti-HBs antibody responses, a much higher serological response to D, T and poliovirus components was observed in Chilean infants compared with a European infant population, when GMTs and seroprotection rates at the higher threshold (≥ 0.1 IU/ml for D and T, and ≥ 50 for poliovirus) were considered.

The accelerated immunisation schedule (2, 3, 4 months) induced lower GMTs for antibodies to D, T and poliovirus than the extended one, although no major impact on seroprotection rates was observed.

Persistence of antibodies and anamnestic response after a booster dose of Hexavac

Three studies evaluated the persistence of antibodies up to the time when a booster dose was given at 12 to 18 months of age, in a total of 1,008 French children who had been primed with the combined Hexavac (studies 2, 3 and 4). A subset of 110 children were followed in Germany (study 6) for anti-PRP antibody persistence as well as anti-PRP anamnestic responses.

In addition, the immune response following a booster injection of Hexavac was assessed in two other populations: in 129 children primed with PENTAVAC and H-B-VAX II (study 7) and in 307 children primed with PENTACOQ and a hepatitis B vaccine currently used in France (study 9).

- *Persistence of antibodies up to the fourth vaccination*

Following primary immunisation with Hexavac, serum concentrations of antibodies to HBs decreased over the 8 to 13 months before booster vaccination. Geometric mean titres ranged from 35.3 to 62.3 mIU/mL, and 72.8 to 85.7% of children were still seroprotected (≥ 10 mIU/ml) at the time of booster, regardless of the immunisation regimen of the primary series. Levels of anti-HBs antibodies, in terms of GMT values and seroprotection rates persisting up to booster were within the range of what has been reported at 12 to 15 months of age after administration of two doses of the combined HB-Hib PROCOMVAX vaccine given at 2 and 4 months of age.

Among children who had received primary immunisation with Hexavac at 2, 4 and 6 months of age, GMT values and the proportion with seroprotective levels of HBs antibodies was lower 9-12 months after primary immunisation (i.e. booster given after 15 months of age) than those seen 6-9 months after priming (i.e. booster given at 12 months of age). In contrast, when the primary immunisation regimen had been 2, 3 and 4 months, persistence of antibodies appeared to be similar from 8 to 13 months after priming (studies 2, 4). However, the overall antibody persistence to HBs antigen appeared of similar magnitude between 12 and 18 months, whatever the immunisation regimen of the primary series.

In terms of GMT values and seroprotection rates, levels of anti-HBs persisting up to booster were lower after primary immunisation with Hexavac than when children were primed with PENTAVAC and H-B-VAX II given separately.

Seroprotective titres of PRP antibody (≥ 0.15 µg/ml) were maintained up until the time of booster dose (12 to 18 months of age) in 73.6 % (study 2) and 85.2% (vaccination at 2,3, 4 months of age in study 4), of children primed with Hexavac at 2, 3 and 4, or 2, 4 and 6 months of age respectively. Persistence of antibodies to PRP did not appear to be affected by the immunisation schedule used to prime the children. When primary immunisation was given according to a 2, 4, 6 month schedule, antibodies to PRP persisted at higher levels, in terms of GMT values and seroprotection rates, when children were primed with PENTAVAC and H-B-VAX II than when Hexavac was used (study 3). Nevertheless, GMT values and seroprotection rates (PRP ≥ 1 µg/ml) at the time of booster, 11 to 13 months after immunisation with Hexavac at 2, 3 and 4 months of age, were of the same magnitude as those observed after priming with PENTAVAC and H-B-VAX II (study 4). When all the Phase II and Phase III studies are considered together, 78.6% [95% CI 75.6-81.4] of the 794 children included in the PP analysis still had seroprotective titres of anti-PRP antibodies (≥0.15 µg/ml). When children were primed with two doses of HB-Hib, PROCOMVAX vaccine or with Pedvax-Hib (PRP-OMP conjugate) vaccine administered at 2 and 4 months of age, levels of anti-PRP antibodies persisting at 12 to 15 months of age were similar to those observed when Hexavac was used for primary immunisation (Fig.

2). The Pedvax-Hib and also PRP-D vaccines were shown to be effective in protecting against invasive Hib disease in double blind efficacy trials.

The serum concentrations of antibodies to PT and FHA, as measured by EIA, decreased over the 8 to 13 months following completion of the primary immunisation series. Mean antibody titres measured 8 to 13 months after the primary series with Hexavac ranged from 8.50 to 15.3 EU/ml for anti-PT antibodies and from 28.9 to 32.2 EU/ml for anti-FHA antibodies. Despite the decrease in antibody titres over time, levels measured at the time of booster injection were nevertheless approximately 4- to 6-fold higher than pre-immunisation levels, indicating persistent anti-PT and anti-FHA antibodies in these children. Levels of antibodies to pertussis antigens that persisted up to the time of booster injection were within the range of those observed after primary immunisation with PENTAVAC.

The persistence of antibodies to Tetanus was satisfactory. All children were still seroprotected against T (titres ≥ 0.01 IU/ml). Pre- and post-booster antibody response did not differ between the two immunisation schedules (2-3-4 month or 2-4-6 months) investigated. GMTs before the 4th injection ranged in studies 2 to 4 between 0.279 – 0.447 IU/ml. After the booster the GMTs increased with a post- to pre- ratio between studies of 12.9 – 33.0 demonstrating a strong booster response.

For Diphtheria between 89.3 % and 95.8 % of the children primed with Hexavac were still seroprotected (≥ 0.01 IU/mL, Vero cell assay) and GMTs ranged in the studies between 0.049 and 0.060 IU/ml. The booster dose resulted in a strong immune response of GMTs with a Geometric Mean of post- to pre-titre ratio between 31.0 and 60.1 (studies 2-4). One month after the booster between 99.1 % and 100 % of children in the studies 2, 3 and 4 were seroprotected. Pre- and postbooster titres of Hexavac were comparable with PENTAVAC + H-B-VAX II.

For poliovirus types 1, 2 and 3 up to 8 to 13 months after primary immunisation with Hexavac was satisfactory, with more 87% of children (study 2 polio type 2) still had detectable neutralising antibodies to poliovirus types 1, 2 and 3 at the time of booster.

Although the 2, 4, 6 month primary immunisation schedule appeared to induce a higher immune response one month after the third dose than the 2, 3, 4 month schedule, antibody titres were similar at the time of booster, regardless of the immunisation regimen used.

- *Anamnestic response after a booster dose of Hexavac*

A strong anamnestic response to a booster injection of Hexavac was obtained for all components, regardless of the immunisation regimen used during the primary series.

The anamnestic response to HBs antigen was marked, for both primary immunisation regimens, as indicated by the 15.6- to 29.6- fold increase in geometric mean titres after the booster immunisation (Tab. 10). GMT values for anti-HBs antibodies one month after the booster appeared lower than those observed in children primed with simultaneous but separate administration of PENTAVAC and H-B-VAX II and boosted with the same two vaccines given separately (study 3). GMT values as well as seroprotection rates obtained after booster appeared lower than those observed after immunisation with HB vaccine given as a three-dose regimen at 0, 1, 6 months or as a four-dose regimen given at 0, 1, 2, 12 months in infants.

Nevertheless, the overall seroprotection rates among the 802 children who received four doses of Hexavac (irrespective of the immunisation schedule) and who were included in the PP analysis, were 96.8% [95% CI 95.3-97.9] at the 10 mIU/ml level.

Of the 26 children who had not achieved seroprotective titres of anti-HBs antibodies (> 10 mIU/ml) after four doses of Hexavac, 14 had high titres before the first dose and 12 were seronegative before immunisation. Of these 26 children, 23 assayed for anti-HBs antibodies before booster immunisation already had titres below 10 mIU/ml and did not respond to the booster dose.

One month after the administration of the booster dose of Hexavac, of the 807 children included in the PP analysis, all children but one had anti-PRP titres greater than 0.15 µg/ml and 97.4% [95% CI 96.0-98.4] of children achieved titres greater than 1 µg/ml (Tab. 11). The immune response to PRP in terms of GMTs were 16.7 (14.4-19.4) and 23 (20.4-26) respectively, and the seroprotection rates appeared to be similar after four doses of Hexavac as after four doses of separately administered PENTAVAC and H-B-VAX II (study 3). The priming effect provided by the primary course of different combined DTaP-Hib vaccines has been previously demonstrated by the induction of a strong anamnestic response to PRP antigen after administration of a booster dose of plain unconjugated PRP at 12 to 14 months of age. GMT values for anti-PRP antibodies following a booster dose of plain PRP in children primed with PMC DTaP-Hib vaccine appeared to be similar to those observed in Finnish children primed with PRP-T (Act-Hib) vaccine alone. The priming effect for PRP afforded by Hexavac was strongly supported by the high GMT values (13.7 to 45.7 µg/ml) and the marked 29.3- to 73.3- fold increase in mean antibody titres following the booster dose administration.

An anamnestic response to pertussis antigens was evidenced by the high GMT values as well as the 3.1- to 11.4-fold increase of mean antibody titres observed after the booster immunisation.

Of the 785 children who received a booster dose of Hexavac and who were included in the PP analysis, 96.4% [95% CI 94.9-97.6] had pertussis toxin neutralising antibody titres greater than 32. Mean titres of antibodies as measured by EIA were higher than those induced after primary immunisation and ranged from 74.6 to 99.9 EU/ml for anti-PT and from 149 to 205 EU/ml for anti-FHA antibodies. As was also seen after primary immunisation, GMT values for anti-PT antibodies measured by EIA after booster with Hexavac appeared somewhat lower than those induced by booster with PENTAVAC. In contrast, a difference in response to Hexavac and PENTAVAC boosters was not apparent in terms of the percentage of children with a four-fold rise of pre-booster antibody titres to PT (study 3). The clinical relevance of the minor quantitative differences in GMT values after Hexavac and PENTAVAC booster remains to be established. The overall antibody responses to pertussis antigens after a booster dose of Hexavac was of the same order of magnitude as those observed previously for PENTAVAC.

After booster, all children achieved seroprotective titres of tetanus antibodies greater than 0.1 IU/ml.

Concerning diphtheria antitoxins, all children had titres above the seroprotective threshold of 0.01 IU/mL after booster, and 97.5% to 100% had titres greater than 0.1 IU/ml.

Of 802 children included in the PP analysis, 98.9% [95% CI 97.9-99.5] had titres greater than 0.1 IU/ml for diphtheria antibodies measured by EIA compared to 34% [95% CI 30.5-37.7] before the booster dose. This strong anamnestic response was confirmed by the assessment of neutralising diphtheria antitoxins, as measured in the Vero cell assay. Of 718 children included in the PP analysis, all but two children achieved diphtheria neutralising antibody titres greater than 0.01 IU/mL and 98.7% [95% CI 97.6-99.4] of children had titres greater than 0.1 IU/ml after the booster dose.

All 768 children included in the PP analysis had detectable poliovirus type 1, 2, 3 neutralising antibodies after the booster dose, and 99.6% [95% CI 98.9-99.9] of children achieved titres above 50 (1/dil).

These data considered together confirm the ability of Hexavac to prime infants effectively, regardless of the three-dose primary immunisation regimen used, and to induce a good anamnestic response after a booster dose given between 12 and 18 months of age.

Vaccine failures

Four »vaccine failures» were clinical episodes of pertussis infections. No cases of Diphtheria, Tetanus, Poliomyelitis, Hepatitis B, or *Haemophilus influenzae* type b infections were identified. Two of them occurred after the first dose of Hexavac (break-through in partially immunised infants). Among the two cases which occurred after a complete primary series, one was after vaccination with Pentavac and H-B-Vax II and one after vaccination with Hexavac.

Supportive studies

The efficacy of the PMC DTaP vaccine was compared to DTP in a randomised double-blind controlled trial involving 4181 children in Senegal. Participants were vaccinated at a 2, 4 and 6 months schedule. The study was designed to estimate the relative efficacy against pertussis disease comparing DTaP to DTP the absolute vaccine efficacy from observational studies (a household contact study and a cohort study). The relative risk of DTaP was 1.5 (95% CI 1.23-1.93). For more severe forms of pertussis the relative risk was estimated to be higher demonstrating better efficacy of the whole cell pertussis vaccine used. In the nested case contact study both vaccines were highly efficacious with an absolute efficacy of the DTaP vaccine of 85% [95% CI 66-93] against typical pertussis as defined by the World Health Organisation (WHO). PT-neutralising antibody titres, as well as anti-PT and FHA antibodies, induced by the trivalent DTaP in Senegalese children were within the same range as those obtained in various populations and to those obtained following administration of more complex DTaP-IPV or DTaP-IPV/Hib combination vaccines. The limitations of household contact studies are well known. The case contact and the cohort study are small studies, especially the groups of unvaccinated subjects were limited resulting in wide confidence intervals. However, in the context of the relative efficacy study both studies are considered to support the efficacy of the two component acP vaccine.

One open, one-arm study (A3R17398) is conducted in Sweden and has started in March 1999. The aim of the study is to assess the immunogenicity in 180 infants vaccinated at 3, 5 and 12 months of age. A historical comparison with PENTAVAC. will be performed. The final study report is targeted for 4Q2000.

Two other studies have been launched during the review process in order to study the possibility of administering other vaccines concurrently, but at separate sites. In one study the concurrent administration of a hepatitis A vaccine (VAQTA paediatric) will be studied in 600 infants at 6 and 12 months of age. In the second study the immune response and safety of simultaneous administration of Hexavac and MMR-II vaccines in 600 toddlers between 12 ands 15 months of age will be assessed.

A Post Marketing survey was started in 1998 in Sweden in order to assess the occurrence of Hypertonic Hyperresponsive Episodes (HHE) following administration of two doses of Pentavac in infants at 3 and 5 months of age.

Clinical safety

Safety and reactogenicity data were recorded during three successive periods after each injection: immediate reactions occurring within 15 minutes after injection (except study 9, where immediate reactions were recorded within 30 minutes after injection); solicited and unsolicited local and systemic adverse events occurring after the 15 minutes following injection up to and including the 3rd day after injection; and adverse events occurring from the 4th day until the 30th day after each injection requiring a medical contact. Immediate reactions were recorded at the investigational site and were analysed independently of solicited local and systemic reactions reported during the study.

All serious adverse events (AE) were reported during the entire study period covering the period from one month after the primary vaccination until one month after the booster dose. The adverse event relationship with vaccination was assessed and rated by the investigator. Since the scale for relationship was changed during the course of the development, the analysis focused on the absence or presence of a relationship with vaccination rather than on the degree of relatedness (doubtful, possible, probable, or definite).

Parameters measured were redness, induration and oedema = 2 cm, rectal temperature = 38 ° C, unusual drowsiness, irritability and/ or unusual crying, inconsolable crying for more than 3 hours, vomiting and diarrhoea, insomnia, anorexia and other local and systemic adverse events.

Patient exposure

- Primary immunisation

During the course of the clinical development of Hexavac, 3,905 infants received at least one dose of this vaccine, administered as a primary immunisation series, in France (Studies 2, 3 and 4), Chile (Study 5) and Germany (Study 6). Among the 3,905 infants who received the first dose of Hexavac, 3,831 received two doses, and 3,788 had a complete three-dose primary series. Complete safety and reactogenicity assessments are available for 3897 infants post-dose 1, 3826 infants post-dose 2 and 3784 infants post-dose 3. Overall, a total of 11,525 doses of Hexavac have been administered in primary immunisation: 3,246 to French infants (Studies 2, 3 and 4), 5,278 to German infants (Study 6) and 3,001 to Chilean infants (Study 5). Of the 11,525 doses administered, 11,507 (99.8%) have completed safety and reactogenicity assessment.

- Booster immunisation

In order to establish the safety profile of the booster immunisation with Hexavac, 4,437 children received a dose of Hexavac during the second year of life. Among these children, 2,697 had been primed with Hexavac (Studies 2, 3, 4 and 6), and thus were given a fourth dose of Hexavac; 129 had been primed with PENTAVAC (Study 7), and 1,611 with PENTACOQ (Studies 8 and 9). A similar overall analysis of the safety and reactogenicity profile to that conducted after the primary series is presented for the booster vaccination with Hexavac.

Local and systemic reactions

- Local reactions

Primary immunisation:

During the three days after immunisation, excluding the 15-minute period immediately following injection, of the 11,507 doses administered to French, German and Chilean infants, 10.0 to 36.1% were associated with at least one local reaction, 2.44 to 17.4% with local redness ≥ 2 cm, and 6.97 to 23.8% with induration and/or swelling ≥ 2 cm, depending on the infant population studied, and considering all doses in the primary immunisation series.
Within three days of vaccination (excluding the 15-minute period immediately following injection), 19.9% to 22.8% of French infants (Tab. 18) experienced at least one local reaction, depending on the dose considered. Redness ≥ 2 cm occurred in 11.8% to 14.0% of infants and induration and/or oedema ≥ 2 cm in 14.8% to 16.1%, depending on the dose. No increase in the rate of local reactions with successive injections were observed. In Germany, rates of local reactions were lower than those reported in France: 10.0 to 10.6% of German infants experienced at least one local reaction depending on the dose of the primary series considered, with less than 8% presenting redness ≥ 2 cm or an induration and/or swelling ≥ 2 cm. These seeming differences between France and Germany might be attributed to differences in the injection techniques, reporting procedures, trial populations and experience of investigators.

Among the total of 11,507 doses administered for primary immunisation, 1.39 % (95 % CI 1.18 % – 1.62 %) were associated with a local redness ≥ 5 cm, and 0.87 % (95 % CI, 0.79 – 1.16 %) were associated with local induration ≥ 5 cm.

Local reactions after Hexavac. Primary series

Study No.	No. of doses	Local reactions ≥ 5 cm		Local reactions (5-7 cm)		Local reactions ≥ 7 cm	
		Redness %	Induration %	Redness %	Induration %	Redness %	Induration %
All studies	11507	1.39 (1.18-1.62)	0.96 (0.79-1.16)	1.27 (1.07-1.49)	0.87 (0.71-1.06)	0.12 (0.07-0.20)	0.10 (0.05-0.17)

When Hexavac was compared directly with PENTAVAC and H-B-VAX II, administered simultaneously but at two different sites (study 3), the rates of local and systemic reactions reported

within three days following the primary vaccination were comparable between the two groups with the exception of redness. A higher incidence of redness appeared to occur after Hexavac compared to PENTAVAC and H-B-VAX II.

A slight trend towards a higher local reactogenicity was seen after Hexavac compared to Pentavac. However, due to the relatively small sample size no clear conclusion can be drawn. Small differences may not be detectable because the trial was not powered to detect small differences. The applicant commits to perform a large post-marketing safety and reactogenicity study of Hexavac.

Booster immunisation:

Within the three days of booster immunisation with Hexavac, of the 2,688 doses administered to French and German toddlers and analysed, 16.7 to 28.9% were associated with at least one local reaction, 13.4 to 24.1% with local redness ≥ 2 cm, and 12 to 21.5% with induration and/or swelling ≥ 2 cm.

As in the primary immunisation series, local reactions were more frequently reported in French infants than in German children. However the rates of local reactions ≥ 5 cm after a fourth dose of Hexavac appeared to fall within the same range in the French and German populations of study children.

As expected, local and systemic adverse event rates were somewhat higher after the booster immunisation, compared with rates observed after the primary series. Thus the proportion of children with at least one local reaction increased from 21.2 during the primary series to 28.9% after booster in France (studies 2-4) and from 10.3 to 16.7% in Germany (study 6).

Local and systemic adverse event rates observed after booster did not differ markedly with the type of vaccine given as a primary series (Hexavac or PENTACOQ or PENTAVAC).
The rates of local reactions ≥ 5 cm observed in toddlers who received Hexavac as a booster immunisation after having been primed with PENTACOQ appeared to be of the same magnitude as that observed in Hexavac-primed children.

However the rates of local reactions ≥ 5 cm reported for Turkish toddlers primed with PENTAVAC and H-B-VAX II who subsequently received a booster dose of Hexavac ranged from 19.8% for redness to 21.4% for induration and/or swelling.

A higher frequency of the local reactions ≥ 5 cm was reported in the Turkish study group for unknown reasons. Differences in injection techniques cannot be excluded. Twenty percent of the children received the vaccine in the arm instead of the thigh, in direct violation of the protocol. It could not be verified retrospectively whether immunisations were given strictly intramuscularly. Such a frequency of local reactions (≥ 5cm) was not observed in other studies conducted in France or Germany using the same lot of Hexavac (studies 3, 4, 6), and under the same conditions of administration, at least per protocol.

Of the 126 Turkish children who were assessed for safety, 13 (10.3%) presented with an induration and/or swelling > 7 cm after booster with Hexavac (all associated with a large swelling of the limb) and 20 children (15.9%) experienced a large swelling of the vaccinated limb. These large local reactions generally began the day of injection or the day after. These reactions were considered as benign, and resolved over a period of a few days without any sequelae, and did not fulfil the criteria of SAEs.

Similar swelling of the entire limb has been already described with other DTaP vaccines given as a booster immunisation within the second year of life in Swedish, Japanese or German children . Large swelling was also observed in a few cases in the other clinical studies during the primary series with Hexavac as has also been described after administration of DTP-IPV or DTP-IPV-Hib vaccines as a three-dose primary immunisation series, which suggests that this phenomenon is not restricted to a booster dose. The mechanism causing this benign swelling has not been elucidated. The SPC has been amended to include swelling of the entire limb under the section Undesirable Effects.

- Systemic reactions

Primary immunisation:

Rates of solicited systemic adverse events reported by the parents, such as irritability and/or unusual crying and drowsiness, also depended on the population studied. Compared with French or German infant populations among which irritability and /or unusual crying appeared to decrease in frequency with dose, irritability occurred more frequently in Chilean infants and increased from 17.7% to 40.7% with successive doses. The high rate of irritability in Chilean infants might be explained by an association with respiratory disorders, which were reported at a high frequency in the study. It is of note that this study was performed in the winter, at a time when a bronchiolitis epidemic was reported and when the third dose was given.

The rate of mild fever [38°C-38.9°C] tended to be lowest after the first dose, varying from 7.3% to 16.2% in France, from 8.8 to 17.7% in Germany and from 11.3 to 23.9% in Chile.

Fever above 39°C was reported infrequently, with a proportion ranging from 0.34-0.37% at the first dose to 2.02-2.42% at the third dose of the primary series given to German and French infants. In Chilean infants, a higher rate of fever [39°-39.9°C] was reported, from 0.68% at the first dose to 4.09% at the third dose, with a trend towards an increased rate with successive doses of vaccine. This trend was observed for both mild and high fever (≥ 40°C). High fever ≥ 40° C occurred in 0.03 % of doses after the first dose, 0.13 % after the second dose and 0.16 % after the third dose.

Inconsolable crying, corresponding to crying lasting for more than three hours, but not high-pitched or abnormal crying, was infrequently reported in the three populations (between 0 and 0.34% irrespective of the dose of the primary series and the population considered). The subjective nature of the reporting of this event should be emphasised, and it is of note that more than half of the children who were noted to present with inconsolable crying, were also reported to present with irritability and unusual crying < three hours.

Concerning the immunisation schedule, the data show that when Hexavac is administered to French infants as a three-dose primary series, the rates of local and systemic reactions within three days following vaccination appeared to be similar regardless of the dosing schedule used (2, 3, 4 or 2, 4, 6 months). As mentioned above the reactogenicity profile appeared to vary somewhat depending on the study population.

Booster immunisation:

The rate of fever (≥ 38°C) in French children increased from 7.7. to 17.6% during primary immunisation to 30.3% after booster immunisation, and from 9.2 to 19.9% after primary immunisation to 28.5% after booster in German children.

Rates of solicited systemic adverse events following administration of a booster dose of Hexavac, did not differ markedly between the two study populations. Fever between 38° and 38.9°C was observed in 23.9 to 26.4% of children, with 3.5 to 3.7% having fever ≥ 39°C. Less than 1% of children experienced a fever ≥ 40°C, and 0.12 to 0.30% presented with inconsolable crying persisting for more than three hours.

Adverse events and serious adverse event/deaths

- Adverse events

Primary immunisation:

Of the 11,525 doses of Hexavac administered to 3,905 infants as a primary series, only one case of HHE (within three days after vaccination) (0.025%) was reported. This event was reported as being probably related to vaccination.

High fever of at least 40.0°C was observed in 12 infants (0.3%). All episodes of fever ≥ 40.0°C except one occurred after the second or third injection.

Half of the episodes did not require any medical contact. Five episodes of fever were isolated, with no associated infectious symptoms. One infant presented with a fever at 40.1°C the day of the third dose that was associated with a large local reaction ≥ 7 cm. All episodes of fever ≥ 40.0°C, except two, were considered by the investigators to be related to vaccination.

Two cases of febrile convulsions were reported within seven days after vaccination in Chile (study 5), one occurring seven days after the second dose, and one five days after the third injection. The former case was associated with bronchopneumonia and declared as a SAE. Both cases were associated with gastrointestinal symptoms and were considered by the investigators to have no relation to vaccination.

One infant (study 6) experienced a bilateral swelling of the limbs on the day of the first injection. This event lasted two days, did not lead to a medical visit and resolved without any sequelae.

Inconsolable crying, a solicited event reported on the parental diary card, was noted for 18 infants (0.46%) among a total of 11,525 doses administered (0.15% per dose). This event consisted of crying that persisted for more than three hours, in spite of efforts to calm the child by feeding or nursing.

Most infants who experienced inconsolable crying presented this event on the day of the injection, or the day after. Half of them were seen by a physician. All of the cases of inconsolable crying except one were considered to be related to vaccination by the investigator. One infant (study 6) who presented with a high-pitched crying on the day of injection that lasted for four hours was recorded as an SAE by the investigator. No neurological abnormalities were found and the child recovered spontaneously.

Among the 18 children with inconsolable crying, 12 did not have any associated symptoms, while three experienced at the same time a large local reaction (≥ 7 cm). There were no sequelae associated with these adverse events.

Seventeen large local reactions > 7 cm were observed in 16 infants who received primary immunisation with Hexavac, i.e. 0.40% of infants and 0.14% of doses administered. These reactions consisted of redness or induration and/or swelling, and did not fulfil the criteria of a SAE. Seven of the 16 children presented with both redness and induration/swelling at the same time, 6 had only redness, and 3 had only induration and/or swelling. All large local reactions occurred on the day of injection and 14 of them resolved within one or two days. Five of the reactions required a medical contact.

One child (study 6) presented with a local reaction > 7 cm after both the first and the second dose. It is noteworthy that this child went on to receive a third dose as well as a booster dose of Hexavac, without experiencing such a reaction.

Booster immunisation:

Following administration of a booster dose of Hexavac to children who received either Hexavac or PENTACOQ as a primary series, there were 298 reports of unsolicited adverse events occurring within the three days after vaccination.

A total of 165 events were reported for 150 (5.5%) of the 2,688 children who were primed with Hexavac; 133 events occurred in 123 (7.6%) of the 1,608 children who were primed with PENTACOQ. Unsolicited local reactions consisted mainly of injection site pain (25 out of 39 adverse events reported). Unsolicited local reaction rates did not appear to be affected by the vaccine used during the primary series. The SPC has been amended to include ‘injection site pain’ under the section Undesirable Effects.

In children primed with PENTACOQ and who received Hexavac as a booster dose, two-thirds of the other unsolicited events reported did not require any medical attention (42 out of 133); slightly more than half (58%) (n=78) were considered by the investigators to have some relation to vaccination, accounting for 4.8% of the doses administered. These events were similar to those reported after administration of four doses of Hexavac, and consisted mainly of respiratory or skin disorders, which are common events in childhood.

After booster immunisation with Hexavac, regardless of the vaccine administered as a primary series, only a few adverse events occurring within the three days after vaccination could be considered as clinically relevant

	Primary series with the Hexavac	**Primary series with the PENTACOQ+HB-VAX II**	**Primary series with the PENTAVAC+HB-VAX II**
Study code/Study no	**A3R03-08-12-13/ nos 2, 3, 6, 4**	**A3R14+20 /nos 8,9**	**A3R16/no 7**
Number of vaccinated children	**2,697**	**1,611**	**129**
CLINICALLY RELEVANT ADVERSE EVENTS WITHIN THE 3 DAYS OF VACCINATION			
HHE	**0**	**0**	**0**
Fever ≥ 40°C	**20**	**5**	**0**
Convulsions*	**0**	**1**	**0**
Inconsolable crying > 3 h	**5**	**4**	**1**
Local reactions > 7 cm	**9**	**5**	**13**

- ***within 7 days of vaccination*** HHE= Hypotonic hyporesponsiveness

- Serious Adverse Events (SAE)/deaths

Primary immunisation:

During the course of primary immunisation with Hexavac in France, Germany and Chile involving 3,905 infants (Studies 2, 3, 4, 5, 6), 247 SAEs were reported. More than half of the SAEs occurred in Chile (n=140), with 107 reported in France and Germany.

Of the 247 SAEs reported, 5 were considered to be related to vaccination by the investigators.

One infant (study 6) presented with a high-pitched crying, that occurred 2.5 hours after the first injection and lasted for four hours. The infant was withdrawn from the study. There were no sequelae associated with this adverse event that was considered to be probably related to vaccination by the investigator.

One infant (study 5) in Chile experienced an hypotonic-hyporesponsiveness episode (HHE) the day after the first dose of Hexavac, which lasted for two days and was considered to be probably related to vaccination by the investigator. This SAE did not meet the definition for HHE recently proposed in a workshop sponsored by the FDA and the US Public Health Service, because the expected skin decoloration was not observed. There were no sequelae associated with this adverse event.

In addition, a case of intussusception (Study 4) occurring the day after the second dose, a case of pyelonephritis due to *Escherichia coli* infection (study 4) occurring five days after the third dose and a

case of febrile influenza-like syndrome associated with gastroenteritis (study 6) occurring two days after the third dose were reported. The three latter cases were rated probably, possibly and doubtfully related to vaccination by the investigators, respectively. Two cases occurred in the context of infectious disease.

There have been no reports of neurological disorders after Hepatitis B vaccination in infants and toddlers below 24 months of age.

Booster immunisation:

During the period between the end of the primary series (one month post-dose 3) and the administration of a booster dose with Hexavac, 119 SAEs were reported. None was considered to have any relationship with vaccination.
During the month following the booster immunisation with Hexavac administered to 4,437 children (studies 2-4,6-9) 33 SAEs were reported; three were considered by the investigators to be related to vaccination, and were reported as such to the Health Agencies. The 30 SAEs considered to have no relation to vaccination by the investigators consisted of common diseases of childhood, i.e. mainly gastrointestinal or respiratory diseases (n=18), and also three cases of febrile convulsions.

The 3 SAEs considered to be related to vaccination by the investigators were all cases of febrile convulsions; two cases occurred 8 and 10 days after the vaccination, respectively, both in the context of infectious rhinopharyngitis (subjects 145A07 and 709 studies 4, 6).

- Deaths

Primary immunisation:

Among the 3,905 infants involved in the primary immunisation series with Hexavac, seven deaths occurred which were attributed to a sudden infant death syndrome, SIDS. Four cases occurred in Chile (in 1,028 infants), two out of 1,094 infants in France, and one out of 1,783 infants in Germany. All the SIDS cases were considered to be unrelated to vaccination either by the investigator himself or, for one infant (subject 57V02 study 3), by the study co-ordinator. The SIDS cases were not associated with any one batch of vaccine.

Each SIDS case was reviewed extensively by experts. It is noteworthy that the age at death was under six months in all cases, that four children out of five for whom the position at the time of death was known were found in a prone position (including the two French cases of SIDS), and that four infants had a personal history of disease just prior to the death. The diagnosis of SIDS was questioned for two cases, one that occurred in Germany and one in Chile. Three of the four cases of SIDS in Chile occurred during the winter, a period reported to be associated with an increased incidence of SIDS. For both experts, the most important risk factor to be considered was the age at death. Four deaths occurred after the first dose, before the fourth month of age, one in France and three in Chile, and three after the second dose, and thus before the sixth month of age. The peak incidence of SIDS has been reported to be at 12 weeks of age between 2 and 4 months of age (with 90% of deaths occurring before the age of 6 months).

One death occurred in a child (study 6) 151 days after the third dose of the primary series with Hexavac, which was attributed to a pneumococcal septicemia.

Post marketing data

Since the Marketing Authorisation was granted, new safety data have been received which led to changes in the product information. These concerned the following adverse reactions, all reported very rarely: convulsions, encephalitis/ encephalopathy, Guilain Barré syndrome, neuritis, allergic reactions and angioedema; thrombocytopenia and purpura; abdominal pain, meteorism and nausea; dyspnoea.

Overall conclusions, benefit/risk assessment and recommendation

Benefit/risk assessment

Combined vaccines are considered to contribute significantly to the simplification and harmonisation of vaccination schedules, to increase vaccine coverage and for straight-forward introduction of new vaccines.
Hexavac belongs to the new generation of hexavalent vaccines and is based on the combination of a previously known pentavalent vaccine and recombinant hepatitis B component. Safety and efficacy of these vaccines has been demonstrated for many years.

However, Hexavac consists of a new formulation: all vaccine components including the Hib component are presented in a liquid formulation. A defined buffer system was selected during the pharmaceutical development, that maintains the Hib component in an unadsorbed status and the D and T components in a partially adsorbed status. Since these changes compared to the established vaccines were considered to be important, close attention was paid to the quality aspects of Hexavac.

During the dossier evaluation process the following quality issues were identified and discussed intensively. In particular:

- Cumulative holding periods of vaccine intermediates
- Impact of aged intermediates on the quality of the active substances
- Control of the quality of the vaccines from the time of batch release to the end of shelf life

Following an in-depth discussion, the company was able to provide a clear concept of how to monitor the consistent quality of Hexavac. The measures undertaken by the applicant include:

- Reduction of holding periods of vaccine intermediates
- Setting up extended stability study programs
- Reduction of the shelf life to 24 months

The CPMP, based on the recommendation given by the BWP, concluded that the quality of Hexavac is acceptable.

Concerning the clinical issues, the differences in the new vaccine formulation compared to the formulation for the established vaccines, were not considered to be clinically significant.

The diminished antibody levels against Hib at the time of the booster dose was discussed in depth during the Ad hoc Expert Group Meeting on Combined Vaccine held in May 2000. However, the experts confirmed that these cut-off limits of 1.0 micrograms/ml for unconjugated Hib vaccines and of 0.15 micrograms/ml for conjugated Hib vaccines as minimum levels indicative of protection are questionable. Good clinical efficacy against Hib disease has been observed in populations with lower anti-PRP antibody levels. The anti-PRP level after primary vaccination is today known to reflect only part of the immune response to the conjugated Hib vaccines.

After vaccination with conjugated Hib vaccines, a major protective role is played by persistent cell based immunological memory as indicated by antibody titre response following booster either with unconjugated or conjugated PRP (higher booster response observed in this second case). Maturation of the immune response is indicated by an increased avidity of the antibodies observed after booster challenge. It was concluded that memory lasts longer than measurable antibodies (silent memory) even though it is not known how long. Persistence of memory following the primary immunisation series is to be further studied and the company has provided a concept paper (including timetables) regarding further characterisation of Hib antibodies induced by Hexavac eg. the avidity of antibodies.

There is a notable decrease in antibody titers to HBsAg in Hexavac compared to the monovalent vaccine H-B-Vax II (5 µg), which may have impact on the long term persistency of antibody titres. However, it is noted that the seroconversion rate and GMTs for HBs antibodies after completion of the

primary series with Hexavac appeared comparable to those obtained after two doses of Procomvax, given at 2 and 4 months of age. Procomvax has been licensed recently within the EU. The applicant commits to perform further antibody persistence studies with Hexavac and to also provide data on anti-HBsAg antibody kinetics over long-term follow-up.
At present it cannot be established if the number of Pertussis antigens has any direct consequence in terms of actual protection against severe Pertussis infections. In some studies protection of vaccinated infants was observed five years after vaccination, in spite of the fact that there were no detectable antibody titres 18 months after vaccination. This indicates that antibody titres and duration of antibody response after primary immunisation cannot be considered as surrogates of protection.

Re-emergence of pertussis in vaccinated populations was also discussed and it was considered that the monitoring of the clinical disease as well as the circulation of strains among the vaccinated population should be continued.

A number of post-marketing commitments were undertaken by the company and are designed so as to be a control system that is sufficiently sensitive to monitor the efficacy of Hexavac.

National epidemiologic surveillance systems experienced in monitoring the relation between Hib and pertussis vaccination and (re-)emergence of disease will be involved in the post-marketing surveillance. These studies will be performed in collaboration with the company.

In summary, based on the principles of evaluating quality, safety and efficacy of medicinal products in general and of vaccines in particular, and considering the state of the art knowledge CPMP concluded that the benefit/risk ratio in relation to Hexavac was positive.