SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Infanrix Hexa. This scientific discussion has been updated until 01.11.02. For information on changes after 01.11.02 please refer to module 8B.

1. Introduction

Infanrix hexa is a combined vaccine, which contains

- diphtheria toxoid (**D**), adsorbed
- tetanus toxoid (**T**), adsorbed
- three purified pertussis antigens (pertussis toxoid (**PT**), filamentous haemagglutinin (**FHA**) and pertactin (**PRN**; 69 kiloDalton outer membrane protein), adsorbed
- the purified major surface antigen (**HBsAg**) of the Hepatitis B virus (**HBV**), adsorbed
- three types of inactivated Polioviruses (**IPV** type 1: Mahoney strain; IPV type 2: MEF-1 strain; IPV type 3: Saukett strain)

and

- a conjugate of Haemophilus influenzae type b (**Hib**) capsular polysaccharide and Tetanus toxoid (**PRP-T**), adsorbed.

The first five components are in a liquid aluminium salt adsorbed state (suspension for injection) whereas the Hib component is a lyophilised powder adsorbed onto aluminium salt. Prior to administration, the lyophilised Hib powder has to be reconstituted with the liquid suspension for injection containing the DTPa-HBV-IPV component.
In the following this combination vaccine will be referred to as "Infanrix hexa" or as the "candidate vaccine". The components of the vaccine will be referred to as "DTPa-HBV-IPV component" or "Hib component".

All antigens of Infanrix hexa have already been licensed, either in monovalent vaccines or as combined vaccines in EU member states and are manufactured by the applicant (e.g. Infanrix HepB: D, T, Pa and HBV; Infanrix IPV: D, T, Pa and IPV; Hiberix: Hib). Infanrix hexa is thus a new combination of known and approved antigens.

The rationale for the development of this combination vaccine is : to facilitate the universal vaccination of infants against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive disease caused by Haemophilus influenzae type b, in countries recommending the use of inactivated poliovirus vaccine as well as universal vaccination against hepatitis B and Haemophilus influenzae type b by simplifying vaccine delivery.

The therapeutic indication for Infanrix hexa is "for primary and booster vaccination of infants against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and disease caused by Haemophilus influenzae type b".

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

The composition of Infanrix hexa is given in Table 1.

To potentiate the immune response, D, T, pertussis antigens (PT, FHA and PRN), and HBsAg are adsorbed on aluminium salts (aluminium hydroxide and aluminium phosphate) which are well-known and universally accepted immunopotentiating agents. The IPV component, although not pre-adsorbed for formulation, does adsorb when mixed with the other antigens. The Hib component is adsorbed also.

An antimicrobial agent (2-phenoxyethanol) is added since it is not possible to terminally filter the DTPa-HBV-IPV component and the cloudy appearance of the suspension could mask microbial contamination. This is in accordance with the CPMP Note for Guidance on the Pharmaceutical and Biological Aspects of Combined Vaccines (CPMP/BWP/477/97). Sodium chloride is added to establish isotonicity and Medium 199 is used as a stabiliser during production of IPV component. Lactose is used as a stabiliser for the Hib component.

Table 1: Composition of Infanrix hexa

Composition of the reconstituted DTPa-HBV-IPV/Hib vaccine		
Ingredients	**Quantity/dose(*) (0.5 ml)**	**Function**
Active substances		
1. Diphtheria toxoid, adsorbed (D)	not less than 30 IU	Immunogen
2. Tetanus toxoid, adsorbed (T)	not less than 40 IU	Immunogen
3. Pertussis toxoid, adsorbed (PT)	25 µg	Immunogen
4. Filamentous haemagglutinin, adsorbed (FHA)	25 µg	Immunogen
5. Pertactin (69kDa Outer Membrane Protein - PRN adsorbed)	8 µg	Immunogen
6. r-DNA Hepatitis B surface antigen, adsorbed (HBsAg)	10 µg	Immunogen
7. Inactivated Polio Virus (IPV) Type 1	40 DU	Immunogen
8. Inactivated Polio Virus (IPV) Type 2	8 DU	Immunogen
9. Inactivated Polio Virus (IPV) Type 3	32 DU	Immunogen
10. Conjugate of *Haemophilus influenzae* type b capsular polysaccharide (PRP) and Tetanus toxoid (T), adsorbed (PRP-T)	10 µg of PRP and 20-40 µg of T	Immunogen
Excipients		
1. 2-phenoxyethanol	2.5 mg	Preservative
2. Sodium chloride (NaCl)	4.5 mg	For isotonicity
3. Medium 199 (M199) (including aminoacids)	1.15 mg (0.09 mg)	IPV stabiliser
4. Lactose	12.6 mg	Hib stabiliser
5. Water (H_20) for injections q.s. ad	0.5 ml	Solvent
Adjuvants		
Aluminium 0.5 mg as aluminium hydroxide ($Al(OH)_3$) 0.32 mg as aluminium phosphate ($AlPO_4$)	0.82 mg 0.12mg from Hib	Adjuvant

The vaccine is presented as combination pack consisting of one container of liquid DTPa-HBV-IPV component and one container of lyophilised Hib component. Infanrix hexa is to be administered by intramuscular injection after reconstitution of the lyophilised Hib component with the contents of the liquid sterile suspension of DTPa-HBV-IPV component.

- The DTPa-HBV-IPV component is presented as 0.5 ml monodose preparations in 1 ml prefilled glass (Type I, Ph. Eur.) syringes. Syringes are presented with separate needles or without needles. Syringes without needles are fitted with grey butyl rubber tip caps. Separate needles are fitted with grey butyl rubber shields. Plunger stoppers are grey butyl rubber. Needles 23G1", 25G5/8" or 25G1" can be used for needleless syringes.

- The adsorbed Hib component is presented as a lyophilised preparation in 3 ml uncoloured neutral glass vials (Type I, Ph. Eur.) with butyl rubber closures suitable for lyophilisation and sealed with either flip-off caps (green aluminium skirt with purple polypropylene cap top) or Bioset caps.

The Bioset cap is a newly developed capping system, which is used as an alternative to the normal aluminium seal used for lyophilised vaccines. The objective of this new system is to provide the user with a facile and safer means of combining liquid and lyophilised components of this combination vaccine.

Bioset caps are received ready sterilised by gamma irradiation. Technical drawings of the cap and a list of control tests applied by both the manufacturer Biodôme and SB Biologicals are provided.

Development pharmaceutics

Compatibility studies regarding DTPa-HBV-IPV component

The compatibility between D, T, Pa and HBV and between D, T, Pa and IPV have been established technically and clinically via licensed formulations containing these antigen combinations (Infanrix Hep B and Infanrix IPV).

The Company provided specific and appropriate data, technical and clinical, to demonstrate the compatability of the DTPa-HBV-IPV combination.

Compatibility between DTPa-HBV-IPV and Hib adsorbed components

In order to demonstrate the compatibility of the DTPa-HBV-IPV and adsorbed Hib components in the candidate vaccine, various parameters were monitored just prior to and following reconstitution of different lots of adsorbed Hib component with different lots of DTPa-HBV-IPV component. Two of the DTPa-HBV-IPV lots and three of the adsorbed Hib component lots had been used in clinical studies.

Appropriate tests, including toxicity tests, were performed on the combination vaccine lots. The *in vivo* potency/immunogenicity of all antigens and *in vitro* immunogenicity for the HBV and IPV antigens were also monitored. Where pertinent, the tests used were the same as those used for routine release of the two final containers. For non-routine tests, references to, or details, of methods were supplied by the applicant.

The results obtained from these studies demonstrate satisfactory compliance with the CPMP guideline CPMP/BWP/477/97 on combined vaccines, which for reconstituted vaccines require demonstration of the compatibility of the two components following reconstitution by testing different lots.

Method of preparation

Finished product

DTPa-HBV-IPV component

For the preparation of the finished product, the sterile adsorbed DT, PT, FHA, PRN and HBsAg concentrates and the IPV component (trivalent bulk) are mixed with a sterile solution of sodium chloride and with water for injections. A sterile solution of 2-phenoxyethanol is added. The adsorbed DTPa-HBV-IPV vaccine is distributed aseptically in sterile glass (type I, Ph. Eur.) syringes.

In-process control consists of checking pH during formulation. During filling, homogeneity of the suspension and filled volume are checked. Environmental monitoring and counting of non-viable particles is carried out. Humidity and temperature of the filling room is monitored.

The aseptic filling system is validated by media fill studies. The entire process is carried out in aseptic conditions that ensure that the final product is sterile.

The formulation process has been adequately described and validated. ELISA tests showed that, within the detection limits of the tests, all the antigens engaged in the formulation process are

adsorbed on aluminium and remain bound to the carrier over time. The IPV component, which is not pre-adsorbed for formulation, does adsorb on aluminium when mixed with the other antigens. Consistency of the production process is highlighted by the results of QC testing on routine production lots.

Hib component

The active ingredient used for the preparation of the final bulk is the adsorbed PRP-T conjugate bulk, which is manufactured in compliance with the Ph. Eur. monograph on Haemophilus influenzae type b conjugate vaccines and with WHO requirements for the same vaccine.

For the preparation of the final bulk, the sterile adsorbed PRP-T conjugate bulk is subsequently added to a sterile concentrated lactose solution and the resulting suspension is stirred and the pH is checked. The final bulk is stored in the sterile formulation tank between +2°C and +8°C and then filled in 3-ml capacity glass vials. Vials are then lyophilised, sealed with aluminium caps or Bioset caps and stored at +2 to +8°C.

All the above operations are carried out in aseptic conditions to ensure the quality of the finished product.

GMP inspection status

The adsorbed DT concentrate and the tetanus toxoid concentrate for the Hib component are prepared by Chiron-Behring (formerly Behringwerke), Postfach 1140, D-3550 Marburg 1, Germany. The adsorbed PT, FHA, PRN, HBsAg concentrates, PRP component and IPV concentrate are prepared by SB Biologicals at Rixensart, Belgium. Both sites have active manufacturing authorisations demonstrating compliance with GMP.

During its meeting on 19-21 October 1999, the CPMP agreed that a GMP inspection of the manufacturing sites was not necessary.

Control of starting materials

D and T

Diphtheria and tetanus toxoids are obtained by formaldehyde treatment of purified *Corynebacterium diphtheriae* and *Clostridium tetani* toxins. The toxoids are produced and controlled by Chiron-Behring, Marburg, Germany as previously described and approved for Infanrix HepB.

PT, FHA and PRN

The acellular pertussis vaccine components are obtained by extraction and purification from phase I *Bordetella pertussis* cultures, followed by irreversible detoxification of the pertussis toxin by glutaraldehyde and formaldehyde treatment, and formaldehyde treatment of FHA and PRN. The antigens are produced according to the methods approved for Infanrix HepB. They comply with the specification limits and are tested as approved for Infanrix HepB.

The pertussis antigens comply with the requirements of the Ph. Eur. monograph 1356 (1999 supplement).

Elimination of adenylate cyclase, tracheal cytotoxin and dermonecrotic toxin was demonstrated for all the production scales validated. Absence of residual pertussis toxin is shown on each lot of the three antigens using the CHO cell test. The histamine sensitisation test in mice is not carried out at that stage but is performed on the finished product.

Elimination of detoxifying agents and other reagents has been validated. Polysorbate 80 is the only quantifiable reagent that remains in the bulk antigens (approximately 40 µg/dose).

HBV

The surface antigen of the HBV is produced by culture of genetically-engineered yeast cells *(Saccharomyces cerevisiae)* which carry the gene coding for the major surface antigen of the HBV. The HBsAg is expressed in yeast cells and purified by several physico-chemical steps. The HBsAg assembles spontaneously, in the absence of chemical treatment, into spherical particles of 20 nm in average diameter containing non-glycosylated HBsAg polypeptide and a lipid matrix consisting mainly of phospholipids. Extensive tests have demonstrated that these particles display the characteristic properties of the natural HBsAg.

The final HBsAg bulk is tested for sterility, HBsAg identity, protein content and mercury content.

IPV

The inactivated polio vaccine component is produced on the Vero cell line using poliovirus strains Mahoney (type 1), MEF-1 (type 2) and Saukett (type 3) as seed materials. The origin and history of the polio virus strains are known. Identity was confirmed by seroneutralisation, infectivity measured and microbiological purity demonstrated (tests for mycoplasma, bacteria, fungi and extraneous agents in animals). A test for the detection of Marburg virus was also performed.
Production and controls follow the requirements of WHO and Ph. Eur. Production is based on the seed lot principle: each production starts with inoculation of Vero cells expanded from one ampoule of the manufacturers working cell bank with one ampoule of the virus working seed lots.

The seed lot and cell banking system has been adequately established and characterized.
Production of the vaccine includes the following steps: preparation of cell substrate, virus inoculation, virus harvest, virus purification, virus inactivation, sterile filtration and pool of the monovalent bulks to obtain a trivalent concentrate.

Results of in process and quality control tests indicate that the production process is adequate. Virus yield after culture is reproducible. Purification gives a product of consistent quality from which proteins and VERO cell DNA are virtually eliminated.
Inactivation is performed in standard conditions using formaldehyde and effective inactivation is consistently achieved.

For quality control, all the tests recommended by WHO and Ph. Eur. are performed.

PRP-T

The manufacture and testing of the PRP-T active ingredient of the Hib adsorbed component is described in Ph. Eur. Monograph 1219 on Haemophilus influenzae type b conjugate vaccine (1998) and WHO TRS 814 (note the 1991 version is under revision). It involves the following steps:

- fermentation of Haemophilus influenzae type b (strain 20,752) based on the seed lot principle,
- extraction and purification of PRP,
- activation of PRP with cyanogen bromide and adipic acid dihydrazide,
- coupling to purified tetanus toxoid,
- purification of the conjugate by size exclusion chromatography,
- diafiltration.

Control of intermediate products

Intermediate products are prepared in advance and a shelf life is claimed for them. These are the adsorbed DT concentrate, the adsorbed PT, FHA and PRN concentrates, the trivalent polio concentrate and the tetanus toxoid concentrate used to prepare the purified tetanus toxoid for coupling with the PRP component.

As the adsorbed DT concentrate is prepared at Chiron-Behring, Marburg, Germany, the product is released by them and retested at SB Biologicals prior to use. Each lot of DT concentrate is tested for

aluminium, formaldehyde, sodium chloride and 2-phenoxyethanol content, for pH and sterility, for potency in animals, specific toxicity and for absence of blood group substances. Batch analysis data show consistency of production and quality.

The adsorbed Pa antigen concentrates are prepared at SB Biologicals and are in process tested for pH and sterility after adsorption and prior to use.

The trivalent polio concentrate is tested in conformity with the Ph. Eur. requirement for absence of infectious poliovirus, sterility, antigen content and polysorbate 80 content.

The tetanus toxoid concentrate used to prepare the purified tetanus toxoid for coupling with the PRP component is manufactured by Chiron-Behring. The tetanus toxoid concentrate complies with Ph. Eur. 452 (bulk purified toxoid) and WHO (TRS No. 800, 1990) requirements. The tests performed for release are sterility, antigenic purity, absence of tetanus toxin, reversion to toxicity, formaldehyde content, sulphate content, sodium chloride content and pH. Batch release protocols from Chiron-Behring are provided in the application. This intermediate may be stored at +2°C to +8°C for a designated time before being processed at SB Biologicals. The shelf life is supported by stability data.

Control of finished product

DTPa-HBV-IPV component

For the control of the finished product, tests can be performed either on the final bulk or on the final container. Several final container lots can be filled from the same final bulk. Therefore, tests, which involve animals, are carried out on the final bulk in order to avoid unnecessary use of animals. This principle is considered acceptable.

The following in vivo and in vitro tests are carried out:

- Specific toxicity test for diphtheria and tetanus performed according to Ph. Eur. 444.
- Potency for diphtheria and tetanus, performed according to Ph. Eur. requirements 2.2.7 and 2.2.9 respectively.
- Potency for pertussis antigens in mice (in-house method based on Ph. Eur. 214, supplement 1999)
- Test for residual pertussis toxin activity in mice (in-house method).
- Potency for IPV component in rats (in house method)
- In vitro potency assay for IPV component by ELISA (final bulk)
- In vitro potency assay for HBV component

The other tests performed on each final bulk vaccine are pH, sterility, 2-phenoxyethanol content and formaldehyde content. Each final container lot is tested for appearance, identity for all antigens, volume, pH, aluminium content and as indicated above, for HBV and IPV content (in vitro potency).
Validation data for these methods are presented in the application. The specification limits and tests performed are in accordance with Ph. Eur. monograph 153 (1999 supplement) “Vaccine for Human use”, where applicable.

Hib component

Control tests are performed on the final bulk lot and on the final container lot following reconstitution in water. The final bulk is tested for sterility. The final container is tested for visual aspect, pH, identity, PRP content, aluminium content, residual moisture, sterility, and pyrogens (endotoxin content). The routine testing is thus in compliance with Ph. Eur. 1219 except for the absence of a test for immunogenicity in mice. The applicant justifies the absence of the mouse potency test from the release specifications by validation data comprising 10 batches. These data show a satisfactory immunogenicity of the Hib component in mice (i.e. at least 50% of inoculated mice show seroconversion).

DTPa-HBV-IPV/Hib (reconstituted vaccine)

A potency test is performed on the reconstituted DTPa-HBV-IPV/Hib.

Stability

Stability tests on active substances

Infanrix hexa is formulated using the same bulk antigens as for other licensed DTPa-based combination vaccines (Infanrix DTPa, Infanrix Hep B and Infanrix IPV). Therefore the applicant has made reference to the data generated on the above mentioned vaccines to support the shelf life claimed for the active ingredients.

- The stability data presented in the application support all agreed storage periods for the active ingredients.

Stability tests on the finished product

DTPa-HBV-IPV component

Three vaccine lots presented as mono-dose vials and three lots presented in pre-filled syringes are included in the stability studies.

The potency of the vaccine (Ph. Eur. assay for D, T & Pa, mouse immunogenicity for HBV and rat immunogenicity for IPV), through the whole shelf-life, meets the specifications applied to routine quality control tests conducted before each vaccine lot release. After 24 and 36 months of storage, the vaccine is also tested for appearance, identity, volume, pH, aluminium content, 2-phenoxyethanol, formaldehyde, endotoxin, sterility, general safety, D and T specific toxicity and residual pertussis toxin activity. Antimicrobial effectiveness was performed after 12 months and will be done after 36 months.

24 months stability studies are presented in the dossier. The results indicate that a shelf life of 24 months with storage at +2°C to +8°C is acceptable.

Hib component

Twelve final container lots were included in stability studies. Based on the data presented, a shelf life of 3 years at 2-8°C for the Hib component is acceptable.

DTPa-HBV-IPV/Hib (reconstituted vaccine)

After reconstitution, it is recommended to inject the vaccine promptly and not later than 8 hours. Therefore, long-term stability studies for the reconstituted product are not relevant. The compatibility results demonstrate the stability of the reconstituted DTPa-HBV-IPV/Hib vaccine for up to 24 hours stored at ambient temperature (approx. 21°C). The maximum storage period of 8 hours indicated is practical and ensures that no reconstituted vaccine is stored over night for use the following day.
Infanrix hexa will be supplied as a package consisting of one container of DTPa-HBV-IPV component and one container of adsorbed Hib component. The shelf life for each container will be calculated as follows:

- for Hib component: start of validity period (36 months) on the filling date
- for DTPa-HBV-IPV component: start the validity period (24 months) on the date of first valid potency tests.

Each container will be labelled with the calculated expiry date based on the shelf life of its contents. The expiry date on the outer carton will correspond to the shorter of the two expiry dates for the separate constituents. This approach is considered acceptable.

TSE risk assessment

Comprehensive information on the origin and preparation of substances of animal origin used in master and working seed lots and in routine production is included in the dossier. The applicant has switched when possible from materials of bovine origin to materials of either synthetic or non-ruminant origin.

Animal derived material used in the manufacture of Infanrix hexa complies with the requirements of the CPMP Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy via medicinal products (Revision April 1999, CPMP/BWP/1230/98).

Conclusion on chemical, pharmaceutical and biological aspects

Infanrix hexa is obtained by adding the entire contents of the supplied container of the liquid DTPa-HBV-IPV component to the vial containing the lyophilised adsorbed Hib component.

The two constituents of Infanrix hexa are prepared according to the procedures described in the established Ph. Eur. and WHO guidelines for the different components. Starting materials of adequate quality are used. The vaccine is prepared according to Good Manufacturing Practices rules and meets the WHO requirements for the manufacture of biological substances. Consistency of production is demonstrated. No novel excipient is used in the finished product.

The vaccine complies with approved specifications and is tested according to Pharmacopoeia methods where applicable. Methods developed in house are validated. Packaging materials are the same as those used for other vaccines manufactured by the applicant (except for the Bioset cap). The separate DTPa-HBV-IPV and Hib adsorbed components vaccines are stable during storage at +2°C to +8°C and following reconstitution for up to 24 hours at ambient temperature (approx. 21°C).

Several outstanding quality issues will be addressed by the applicant on an ongoing (post-approval) basis.

3. Part III: Toxico-pharmacological aspects

Toxicology

As indicated in the CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95), studies on reproductive function, embryo/foetal and perinatal toxicity are not necessary for paediatric vaccines and studies on mutagenicity and carcinogenicity are normally not needed for vaccines. The absence of perinatal toxicity data in the application is thus justified on the basis that Infanrix hexa is intended for paediatric use only. The absence of mutagenicity and carcinogenicity data in the application is justified on the basis that the product is a vaccine and none of the active ingredients or excipients are novel or known to induce mutagenic or carcinogenic effects.

The preclinical testing included a repeated dose toxicity study reflecting the clinical use of the vaccine. Local tolerance was also evaluated as required by the CPMP Note for Guidance. The vaccine was well tolerated and no significant toxicological reaction was observed.

Data were presented from in vivo safety studies for inactivated trivalent polioviruses demonstrating the safety of these components.

The adsorbed Hib component of the vaccine was tested for pyrogenicity according to Ph. Eur. (2.6.8). All three lots of Hib adsorbed final container tested complied with the specifications. It should be noted that for routine QC release, the adsorbed Hib component final containers are tested for endotoxin by the Ph. Eur. in vitro LAL method.

Other data, which provide assurance with respect to the safety of the candidate vaccine come from routine release in vivo tests which are performed on the DTPa-HBV-IPV/Hib vaccine. These are the specific toxicity test for diphtheria and tetanus (Ph. Eur. 444) and the test for residual pertussis toxin activity (histamine sensitisation test) which are performed on the DTPa-HBV-IPV component. General safety (abnormal toxicity) testing is no longer required by Ph. Eur. for routine release. However, data were presented in the application. All lots met the requirements of this test.

Some final bulk lots of Hib adsorbed component were also tested to demonstrate that the conjugation reaction does not influence the irreversibility of the toxoiding for the tetanus toxoid used as protein carrier.

In addition, the applicant addressed the question of potential toxicity and/or potential allergenicity for 2-phenoxyethanol, which is contained as a preservative in the DTPa-HBV-IPV component (2.5mg/dose). The same preservative is used at the same concentration in other vaccines manufactured and commercialized by the applicant. The results of a comparison of the adverse event profile of a vaccine containing 2-phenoxyethanol and another vaccine without 2-phenoxyethanol gave no reason for concern.

Pharmacology (Immune response in animals)

The applicant only presents primary pharmacologic data. The absence of secondary pharmacodynamic data (safety pharmacology) is justified because all the vaccine components are well known and are described in either Ph. Eur. or WHO monographs. Furthermore, any undesirable pharmacological activities would have been revealed in the repeated dose toxicity study, the safety studies for IPV or other routine toxicity tests.

It is indicated in the CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines that pharmacokinetics studies are normally not needed and this is considered to be the case for Infanrix hexa.

Potency tests for diphtheria and for tetanus demonstrated a satisfactory potency response for the concerned components. A satisfactory immune response for the IPV component was demonstrated in immunogenicity tests. The vaccine elicited a satisfactory immune response for the HBV component in an in vivo immunogenicity test. For the pertussis component, the immunogenicity has been demonstrated in mice. The protective capacity of the anti-pertussis antibodies was demonstrated using a lung clearance activity test in a *Bordetella pertussis* intranasal challenge model of infection in mice. An in vivo immunogenicity test in mice for the adsorbed Hib component was performed on some of the adsorbed Hib final container lots.

The applicant has also provided data demonstrating that the reconstitution of Hib adsorbed component with the DTPa-HBV-IPV component does not influence the potency/immunogenicity of the different vaccine antigens in animals.

Environmental risk assessment

The applicant has indicated that although the hepatitis B component of the candidate vaccine is derived from a genetically modified yeast strain, the final vaccine preparation does not contain any genetically modified organisms as the HBsAg undergoes extensive purification following its extraction from the yeast. With respect to the risk associated with use of the vaccine, the applicant has stated that no drug substance or identifiable metabolite will be introduced into the environment in quantities that merit concern.

Conclusion on toxico-pharmacological aspects

In conclusion, the applicant has performed adequate preclinical toxicity testing in accordance with the CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines .The testing shows that the vaccine was well tolerated in animals with no significant toxicological reaction

or abnormality.

The applicant has provided adequate primary pharmacodynamic data to demonstrate that Infanrix hexa elicits a satisfactory potency/immunogenicity response in animals with respect to all the vaccine components. The pharmacological properties of all the vaccine components have been shown to be satisfactory.

No environmental hazard meriting concern has been identified as being associated with use of this vaccine.

4. Part IV:

4.1 Clinical aspects at the time of initial marketing authorisation

Overview of clinical documentation

Complete reports of 9 primary vaccination studies are presented in the application. A total of 7 different lots of the DTPa-HBV-IPV component and 6 different lots of the Hib component were used. During the development programme, the manufacturing process was modified. This was addressed in the clinical development programme:

The primary vaccination studies include

- 2 feasibility studies.
- 1 lot to lot consistency study.
- 1 double blind bridging study from the old to the new manufacturing process.
- 5 open randomised studies evaluating the safety and/or immunogenicity of the candidate vaccine according to various primary vaccination schedules including the Expanded Programme on Immunization (EPI) schedule.

These studies included a total of 4970 infants of more than 6 weeks of age, of which 3145 received the candidate vaccine and were included in the according to protocol cohort for analysis of reactogenicity, and 1352 were included in the ATP cohort for analysis of immunogenicity.

In addition, 5 booster studies are included in the initial application, and data from 3 additional booster studies assessing Infanrix hexa as a fourth dose during the second year of life following priming with the same vaccine, were submitted with the responses to the consolidated list of questions.

The procedures used in these studies were in accordance with the Declaration of Helsinki. The protocols were designed according to the Good Clinical Practice guidelines.

Clinical efficacy

The immunogenicity of the candidate vaccine was evaluated by measuring the antibody response elicited by each vaccine component. All assays were performed blinded to vaccine treatment using validated procedures with adequate controls.

Descriptive analysis of each vaccine group was provided for all studies. The statistical methodology used in most studies to evaluate the immunogenicity and reactogenicity of the candidate vaccine was equivalence or non inferiority testing based on a 90% CI. The pre-specified limits for non-inferiority were defined by the sponsor prior to analysis.

Primary vaccination

Feasibility studies

The reactogenicity and immunogenicity of the candidate vaccine was evaluated in comparison with the separate administration of licensed vaccines according to the same schedule (2, 4 and 6 months) in

an open randomized trial including 2 groups. There was no clinically relevant difference in tolerability between the candidate vaccine and the separate administration of commercial vaccines. In terms of seroprotection or vaccine response rates, the candidate vaccine was at least as immunogenic as the commercial vaccines administered at different sites for all antigens other than PRP. When anti-PRP titres >1.0 mcg/ml were considered, the lower limit of the 90% CI of the difference in vaccine response rate was below the limit for clinical equivalence. The clinical significance of the response to the Hib component in mixed administration (that is after administration of the combined candidate vaccine) in terms of vaccine efficacy will be discussed further in this report.

Assessment of the candidate vaccine compared to the separate administration of its components

Additional studies compared the reactogenicity and immunogenicity of the candidate vaccine to that of the separate administration of its DTPa-HBV-IPV and Hib components according to 2 different schedules.

In these studies the reactogenicity profile of the DTPa-HBV-IPV co-administered with the Hib component at a different site was similar to that of the candidate vaccine. The seroprotection and vaccine response rates and the GMTs were similar in the groups. When analyzed according to the predefined clinical plan, the proportion of subjects with anti-PRP antibodies > 1.0 mcg/ml was higher in the separate injections group. The clinical significance of the response to the Hib component in mixed administration in terms of vaccine efficacy will be discussed further in this report.

Lot to lot consistency

The lot-to-lot consistency of the candidate vaccine was evaluated in a double-blind randomized study in which infants received vaccine from 1 of 3 different lots mixed with 1 of 3 different lots of the Hib component, according to a 3, 4 and 5 months schedule.

There was no difference between the 3 groups in terms of incidence of local reaction and fever. The immune responses elicited were not different according to the pre-specified limits for equivalence, except for the anti-PRP response. However when the reverse cumulative distribution curves for anti-PRP antibodies were plotted, the curves for each of the 3 groups closely aligned.

To address the bridging of the manufacturing process used during early development of the product and the final process, a randomized study evaluated the reactogenicity and immunogenicity of one lot of DTPa-HBV-IPV/Hib from each process. There were no differences between the 2 lots in terms of incidence of local reaction and fever. Also in this trial, the immune response elicited by the 2 lots were not different according to the pre-specified limits for equivalence, except for the anti-PRP response. The difference for anti-PRP was considered to reflect the stringency of the non-equivalence criteria rather than any clinically significant difference.

Primary vaccination studies according to various vaccination schedules

The following schedules have been investigated:

- 2, 3 and 4 months
- 3,4 and 5 months
- 2, 4, and 6 months
- 3, 5 and 11 months
- $1^1/_2$, $2^1/_2$ and $3^1/$ months with one dose of HBV vaccine at birth (EPI schedule))

In one of these studies, the candidate vaccine was compared to a commercial combination including a whole cell pertussis vaccine (DTPw-IPV/Hib, *Pentacoq*), in others with a licensed acellular pertussis-inactivated poliovirus -Hib vaccine (*Infanrix IPV Hib*).

2-3-4, the 3-4-5 and the 2-4-6 months schedules

Overall, one month after completion of the primary vaccination schedule >99.8% of subjects had protective titres against diphtheria and tetanus, over 98.6% had protective anti-HBs titres, 95.7% had neutralizing antibodies to each of the 3 polio antigens, and vaccine response to any of the pertussis antigens was >95.9%. Anti-PRP titres above the 0.15 mcg/ml level were present in more than 96% of the subjects, above 1 mcg/ml in more than 61.9% of the subjects. A schedule effect was seen for vaccine response rates and GMTs: values tended to be higher for schedules in which the first dose of vaccine was given later in infancy and in which the interval between doses is longer.

In an open randomized study comparing the candidate vaccine with a combination vaccine containing whole cell pertussis vaccine (*Pentacoq*) administered with a licensed HBV vaccine at another site, the immune response was similar for the candidate vaccine and the licensed comparator group except for anti-PRP antibodies. The clinical significance of the response to the Hib component in mixed administration in terms of vaccine efficacy will be discussed further in this report.

3-5-11 months schedule

The 3, 5 and 11 months schedule was investigated: DTPa-HBV-IPV/Hib was compared to DTPa-IPV/Hib+HBV administered at another site. At the time of submission, only month 6 data (blood samples tested one month after the second dose) were included in the registration file. The data analysis showed that the candidate vaccine was not inferior to the commercially available vaccines used as comparators. For HBsAg, a higher seroprotection rate (96.5% versus 82.6%) and higher GMTs (582 versus 82 mIU/ml) was seen with the candidate vaccine than with the licensed monovalent hepatitis B vaccine. As requested by the CPMP, the applicant provided further data on reactogenicity and immunogenicity obtained one month after the third vaccine dose, which confirmed the results obtained after 2 doses of vaccine.

6-10-14 weeks schedule (Expanded Programme of Immunization / EPI schedule)

In another study, 2 groups of infants received the candidate vaccine according to the EPI schedule, one of the groups receiving an additional dose of hepatitis B vaccine at birth. This additional dose of hepatitis B vaccine had no effect on the reactogenicity profile of the candidate vaccine. The candidate vaccine was immunogenic when administered according to this schedule. However, for anti-HBsAg, a seroprotection rate of 77.7% was obtained in the group of infants who did not receive a dose of HBV at birth, compared to 98.5% in those who did receive the additional dose. This indicates that, for subjects who will be vaccinated with the candidate vaccine according to the EPI schedule, an additional dose of HBV vaccine should be given at birth.

No issues specific to the Pa component of the candidate vaccine were identified, other than those already relevant for the licensed DTPa vaccines.

Persistence of antibodies up to booster vaccination and booster vaccination

The initial clinical documentation contained only studies evaluating the candidate vaccine as a booster in children primed with other vaccines. At the time of submission, data derived from 5 clinical trials were presented in the registration dossier to support the use of the candidate vaccine as a booster dose. From these studies, it became apparent that the candidate vaccine induces lower anti-PRP titres than the conjugate Hib vaccine administered separately. Following the request to provide complete immunogenicity data of the candidate vaccine after primary vaccination, the applicant submitted with the response to the consolidated list of questions the results from 3 additional trials evaluating the candidate vaccine when used as a fourth dose in the second year of life. In addition, results from 6 trials were submitted to support the booster use of the pentavalent component administered concomitantly with various licensed Hib vaccines.

The results of two comparative trials are of particular interest. One study was an open, randomised trial aimed at assessing the immunogenicity and reactogenicity of various booster regimens. Non-

inferiority as per pre-defined criteria for pre-booster seroprotection/ seropositivity rates of the candidate vaccine compared with the pentavalent component plus separate administration of Hib could not be established for some of the antigens, including PRP, for which lower titres were observed for the group which received the candidate vaccine. Following booster administration, important increases in antibody levels were seen for all components in both groups. Anti-PRP levels tended to be lower for the group which received candidate vaccine. There was a trend towards lower anti-PRP immune response with regard to pre- and post- booster titres of the mixed candidate vaccine compared with the separate administration of Hib.

In the other study, pre-booster antibody levels to all vaccine antigens after primary vaccination with the pentavalent component given either in separate injections or combined with Hib component were compared to a combination vaccine containing whole cell pertussis vaccine (*Pentacoq*). In this study, the rates of subjects assumed to be seroprotected against diphtheria and anti-PRP were lower compared to Pentacoq the seroprotection rates for antipolio and GMTs for pertussis components were higher for the candidate vaccine. As for the first study, the antibody titres after the booster dose were substantially increased with respect to prevaccination levels for all components and in all study groups.

Based on the experience with already licensed vaccines in EU Member States, it is known that combination of Hib valences with acellular Pertussis components is associated with an interference phenomenon: a reduction in the antibody titres to the PRP. This phenomenon is also observed for the present candidate vaccine. The applicant was therefore asked to address this issue in an oral explanation. In particular, the applicant was asked to demonstrate that the lower PRP titres do not have any clinical impact on the protection conferred.

Analysis of the clinical studies included in the application showed that one month after completion of the primary vaccination course, the GMTs of antibodies against the Hib component ranged from 1.52 to 3.53 µg/ml, with between 93.5 and 100% of the subjects reaching antibody titres ≥0.15 µg/ml. One month after the booster dose given in the second year of life, the GMTs ranged from 19.1 to 94.0 µg/ml, with 99.5 to 100% of the subjects reaching antibody titres ≥0.15 µg/ml. These GMTs are lower with respect to separate administration of the Hib component, but they are not different from those elicited by licenced DTPa/Hib and DTPa-IPV/Hib vaccines.

The humoral immune response (as measured by serum antibody levels) is complemented by the induction of a cellular immune response (or immune memory), which has been shown to be present as early as four months after completion of the primary immunisation schedule with Infanrix hexa. Data from field studies in the United Kingdom have shown that Hib vaccine efficacy remains high for at least 6 years after primary vaccination, despite low levels of serum antibodies and without administration of a booster dose. Immune memory has thus been proposed as an important mechanism in the long-term protection against invasive Hib disease.

The effectiveness of the applicant's Hib component (when combined with DTPa or DTPa-IPV) has been confirmed through an extensive post-marketing surveillance study conducted in Germany over a 2 year follow-up period, where the effectiveness of three primary doses of DTPa/Hib or DTPa-IPV/Hib was 98.8%.

Concerns regarding the lower anti-Hib antibody levels with Pa/Hib-containing combinations were also addressed in an Ad hoc Expert Group Meeting which was convened at request of the CPMP in order to clarify the risks and advantages of new combination vaccines presently under CPMP evaluation. The experts confirmed that the historically accepted 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 may be questioned. Good clinical efficacy against Hib disease has been observed in populations with lower anti-PRP antibody levels (e.g. in Finland). 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 immunological memory as indicated by antibody titre response following boosting either with unconjugated or conjugated PRP. Maturation of the immune response is indicated by an increased

avidity of the antibodies prior to and after booster challenge. It was concluded that memory lasts longer than measurable antibodies (silent memory) even though it is not known how long. Therefore, adequate surveillance studies are necessary to further study persistence of memory following the primary immunisation series.

Clinical safety

The safety of the vaccine and the reactogenicity profile was evaluated on the day of the vaccination and on the 3 following days on the basis of a checklist of solicited local and general signs and symptoms. Non solicited symptoms could be recorded on the diary cards. The parents were instructed to immediately inform the investigators of the occurrence of any serious adverse event occurring during the study period and investigators had to notify the sponsor within 24 hours.

Primary vaccination

For primary vaccination, the reactogenicity of the candidate vaccine was comparable to other licensed DTPa vaccines.

Booster vaccination

After the booster dose the incidence of grade 3 fever appears to be increased compared to the primary vaccination (grade 3 fever up to 4.4% in one study). In another study with a higher sample, size grade 3 fever was recorded in 4.0 %) of subjects. Consequently, the incidence of grade 3 fever (> 39.5°C) appears to be higher than that observed with licensed vaccines.

The applicant was requested to address the reactogenicity of the candidate vaccine after boosting. In particular, the company was asked to show that the high fever induced by the candidate vaccine is not a matter of concern and has no influence on the vaccine acceptability.

The applicant performed an analysis of fever after 4 different boosters (DTPw-IPV/Hib+HBV; Infanrix hexa, DTPa-IPV/Hib+HBV; DTPa-IPV/Hib). The data were pooled regardless of priming. Furthermore, the duration of fever above 37.5°C was compared following 4 different boosters (DTPa+Hib; DTPa/Hib; DTPa-IPV/Hib; Infanrix hexa). From the data it can be concluded that there was a similar incidence and distribution of high fever and a similar duration of fever episodes in the various booster groups.

The incidence of high fever above 39°C observed with Infanrix hexa after boosting was also discussed in the Ad hoc Expert Group Meeting on combined vaccines. It was concluded that concerns are still present with reference to high fever, even though such reactions have been observed also with other licensed combined vaccines. An appropriate statement on this subject is present in the SPC. Further detailed evaluation of such severe adverse reactions is needed with post-marketing studies based on well-defined protocols and conformity in data measurements across study centres.

Serious adverse events

In total, 99 subjects reported a serious adverse event after receiving a dose of the candidate vaccine. Only 3 reports were considered as related or probably related to vaccination.

In the booster trials, all SAEs were assessed as unrelated to vaccination, except one considered suspected to be related to vaccination. This subject recovered without sequelae.

The applicant performed a review of all serious neurological events observed in temporal relationship to vaccination with the candidate vaccine. An independent review of all cases confirmed the initial reports of the investigators in concluding that it is unlikely that these events are causally related to immunisation. Importantly and irrespective of the relationship to vaccination, the incidences of the events reported following administration of the candidate vaccine were not different from what has been previously observed with either DTPa or other DTPa-based combinations. This additional information was considered acceptable.

Conclusions on clinical efficacy and safety

The clinical investigation of the candidate vaccine followed a well conducted programme developed according to comprehensive design. Infanrix hexa has shown to be immunogenic in defined conditions of use (specific primary and booster vaccination schedules).

On the basis of the current scientific knowledge of immune response against conjugated Hib containing multivalent vaccines and of effectiveness observed in field studies, the lower anti-PRP antibody levels observed after vaccination in clinical trials with Infanrix hexa is considered not to be an issue preventing the granting of a marketing authorisation. Nevertheless adequate surveillance studies are recommended to confirm long-term effectiveness of this vaccine as well as to further explore immune response patterns.

The observed tendency towards higher reactogenicity following a booster dose of Infanrix hexa was not considered to be a barrier to a positive scientific opinion for this vaccine. Further detailed evaluation of severe reactions and their impact on vaccines acceptance will be monitored via well-defined post-marketing studies.

4.2 Clinical aspects from the post-authorisation phase

Adverse Drug Reactions:

Since the Marketing Authorisation was granted, new safety data have been received which led to changes in the product information. These data concerned the following adverse drug reactions: convulsions, skin reactions (rash dermatitis, eczema), anaphylactoid reactions (urticaria), and thrombocytopenia.

5. Overall conclusions on quality, efficacy and safety and benefit/risk assessment

The quality of Infanrix hexa has been acceptably documented. The applicant has agreed to solve remaining quality issues by providing additional data on an ongoing basis after approval.

Following evaluation of the toxicological/pharmacological documentation, it was concluded that the applicant has performed adequate preclinical toxicity testing in accordance with the CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines. The testing shows that the vaccine was well tolerated in animals with no toxicological significant reaction or abnormality. The pharmacological properties of all the vaccine components have been shown to be satisfactory.

The clinical documentation presented by the applicant conforms to ICH guidelines and is adequate from a GLP and GCP point of view. Infanrix hexa was shown to be immunogenic when administered according to a variety of vaccination schedules. Comprehensive data have been submitted to characterise the reactogenicity profile of Infanrix hexa. The lower anti-PRP antibody levels as well as the observed tendency towards higher reactogenicity of Infanrix hexa as a booster (incidence of high fever) was not considered to be a barrier to a positive scientific opinion for this vaccine. The applicant is asked to perform adequate surveillance studies to confirm long term effectiveness of Infanrix hexa and to further monitor severe adverse reactions and their impact on vaccine acceptability.

From the practical point of view, Infanrix hexa is a further contribution to decrease the number of injections required for primary and booster vaccination in infants. The vaccine will assist in increasing vaccination compliance and in further simplifying vaccination schedules and programmes.

Benefit/Risk assessment

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Infanrix hexa is favourable for the primary and booster vaccination of

infants against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and disease caused by Haemophilus influenzae type b.