7 September 2012
EMA/560492/2012
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Hexaxim

diphtheria, tetanus, pertussis (acellular, component), hepatitis b (rdna),poliomyelitis (inactivated) and haemophilus influenzae type b conjugate vaccine (adsorbed)

Procedure No.: EMEA/H/W/002495

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

Ab	Antibody
Abm	Monoclonal Antibody
ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACN	Acetonitrile
acP	acellular Pertussis
ACT	Adenylate Cyclase Toxin
ADH	Adipic acid Dihydrazide
ADP	Adenosine Diphosphate
AE(s)	Adverse event(s)
AFP	Final Purified Hepatitis B Antigen
AFSSAPS	Agence Française de Sécurité Sanitaire des Produits de Santé
Ag	Antigen
AlOOH	Aluminium Hydroxide
ALTE	apparent life-threatening event
AMQ	6-aminoquinoline
AQC	6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
AR(s)	Adverse reaction(s)
AS	Ammonium Sulphate
ASF 4B	Asialofetuin Sepharose
ATP	Adenosine Triphosphate
AUC	Analytical Ultracentrifugation
BCG	Bacille-Calmette-Guérin
	BfR Bundesinstitut für Risikobewertung Deutschland
BG	Bordet-Gengou
BL	Blood sample
BMV	Brome Mosaic Virus
BSA	Bovine Serum Albumin
BSC	Biological Safety Cabinets
	BSE Bovine Spongiform Encephalopathy
CCID	Cell Culture Infectious Dose
CCID50	50% cell culture infective doses (viral infectious units)
CCIT	Container Closure Integrity Test
CDM	Clinical Data Management
CDMS	Clinical Data Management System
CDT	Crude Diphtheria Toxoid
CFU	Colony Forming Unit
CFV	Concentration Factor Volume
cGMP	Current Good Manufacturing Practices
CI	Confidence Interval
CIDS	Congenital immunodeficiency syndrome
cIEF	Capillary Isoelectric Focusing
CIF	Complementary Information Form
Cm	Centimeter
COS	Certificate of Suitability
Cp	Capability
CPE	Cytopathic Effect
CPVS	Concentrated Purified Viral Suspension
CRF	Case Report Form
CRS	Chemical Reference Substance
CSE	Control Standard Endotoxins
CT	Threshold Cycle
CTD	Common Technical Document
CTP	Concentrated Tetanus Protein
CTT	Crude Tetanus Toxoid
CTW	Carbonate-Tween
CZE	Capillary Zone Electrophoresis
D	Diphtheria
dATP	Deoxy Adenosine Triphosphate
DC	Diary Card
DCF	Data Correction Form

dCTP	Deoxy Cytidine Triphosphate
DCW	Dry Cell Weigh
DEAE	Di Ethylamino Ethylen
dGTP	Deoxy Cytidine Triphosphate
DHAS	Dihydroxyacetone Synthase
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DNT	Dermonecrotic Toxin
DP	Drug Product
DPNH	Reduced Diphosphate Pyridine Nucleotic
DSC	Differential Scanning Calorimetry
DT	Diphtheria Toxin
DTaP	Diphtheria, tetanus, and acellular Pertussis
DTCoq/DTwP	Diphtheria, Tetanus, Whole-Cell Pertussis vaccine
DTT	Dithiotreitol
dTTP	Deoxy Thymidine Triphosphate
DTwP	Diphtheria-tetanus-whole-cell pertussis
DU	Arbitrary D-antigen Unit
DUW	Degassed Ultrafiltered Water
ECD	Electron Capture Detector
ECi	Enhanced chemiluminescence
EDAC	1-ethyl-3-(3 dimethyl aminopropyl) carbodiimide
EDC	1-Ethyl-3-[3dimethylaminopropyl] carbodiimide hydrochloride
EDQM	European Directorate for the Quality of Medicine
EDTA	Ethylenediaminetetraacetic acid
EDU	1-ethyl-3(3-dimethylaminopropyl) urea
EF-2	Elongation Factor-2
EIA	Enzyme immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
EM	Electron Microscopy
EPI	Expanded Program on Immunization
ESI	Electrospray Ionization
EU	ELISA units
EWS	European Reference Standard
FA	Formic Acid
FAMA	Fluorescent antibody to membrane antigen
FBP	Final Bulk Product
FCS	Foetal Calf Serum
FDA	Food and Drug Administration
FHA	Purified Filamentous Hemagglutinin
FID	Flame Ionization Detection
FMDH	Formate Dehydrogenase
FP	Filled Product
FPERT	Fluorescent Product Enhanced Reverse Transcriptase
FTIR	Fourier Transform InfraRed
G6P	Gluconate-6-Phosphate
G6P-DH	Glucose-6-Phosphate Dehydrogenase
GC	Gas Chromatography
GCP	Good Clinical Practice
GFC	Gas Filtration Chromatography
GLC	Gas Liquid Chromatography
GLDH	Glutamate Dehydrogenase
GM	Geometric mean
GMP	Good Manufacturing Practices
GMT	Geometric mean of Ab titer
GPVD	Global Pharmacovigilance Department
GPI	Glucose Phosphate Isomerase
GSK	GlaxoSmithKline
GTA	Glutaraldehyde
HA test	Haemagglutination test
HBsAg	Hepatitis B surface Antigen
HD	Human Dose
Hep B	Hepatitis B

Hib	Haemophilus influenzae type b
HIV	Human Immunodeficiency Virus
HK	Hexokinase
HLA	Human Leukocyte Antigen
HMW	High Molecular Weight
HPAEC-PAD	High Performance Anion Exchange Chromatography – Pulse Amperometric Detection
HPLC	High Pressure Liquid Chromatography
HPSEC/LS	High Performance Size Exclusion Chromatography/Light Scattering
HS	Histamine Sensitizing
HSA	Histamine-Sensitizing Activity
HXP	Hydroxyapatite
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IDU	5-Iodo-2'Deoxyuridine
IEC	Ion Exchange Chromatography
IEF	IsoElectric Focusing
IF	Intrinsic Fluorescence
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intra-Muscular
IMD	"Institute Merieux Diphtheria" medium
IPC	In-Process Control
IPV	Inactivated Vero Trivalent Poliovaccine
IR	Infrared
ISL	Intermediate Seed Lot
ITC	Isothermal Titration Calorimetry
ITT	Intent to Treat
IU	International Unit
IUDR	Iodo Uracile DesoxyRibose
IVRP	In Vitro Relative Potency
kDa/Kd	Kilo Dalton
LAL	Limulus Amoebocyte Lysate
LALS	Laser Light Scattering Detector
LC	Liquid Chromatography
LCM	Lymphocytic Choriomeningitis Virus
LDH	Lactate Dehydrogenase
LLOQ	Lower limit of quantitation
LOQ	Limit of quantitation
LMW	Low Molecular Weight
LPC	Lysophosphatidylcholine
LPS	Lipopolysaccharide
Mab	Monoclonal antibody
MAD	Maximum Allowable Deviation
MALDI	Matrix-Assisted Laser Desorption Ionization
MedDRA	Medical Dictionary for Regulatory Activities
ml	Milliliters
mm	Millimeter
MEM	Minimum Essential Medium
MLD	Minimum Lethal Dose
MLE	Marcy l'Etoile
MMR	Measles, mumps and rubella
MMRV	Measles, mumps and rubella vaccine
MoA	Month of Age
MOI	Multiplicity of Infection
MOX	Methanol Oxidase
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MSL	Master Seed Lot
MTT	Methylthiazol Tetrazolium
MW	Molecular Weight
N/A	Not Applicable
NADH	reduced Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	reduced Nicotinamide Adenine Dinucleotide Phosphate

NHS	N-hydroxysuccinimide
NIBSC	National Institute for Biological Standards and Control
NIST	National Institute of Standard and Technologies of the United States of America
NVR	Non Volatile Residues
OD	Optical Density
ODA	Orthodianisidine
OOS	Out Of Specification
OPD	o-Phenylenediamine
OPV	Oral Poliovirus Vaccine
PBS	Phosphate-Buffered saline
PC	Phosphatidylcholine
PCR	Polymerase Chain Reaction
PDA	Parenteral Drug Association
PDL	Population Doubling Level
PDT	Purified Diphtheria Toxoid
PE	Phosphatidylethanolamine
Pediacel	DTaP-IPV-PRP-T (fully liquid combination : Diphtheria, Tetanus, 5-component acellular Pertussis, Poliomyelitis and Haemophilus influenzae type b conjugate vaccine)
PEG	Polyethylene Glycol
Pentaxim/ Pentavac	DTacP-IPV//Hib (Reconstituted combination : Diphtheria, Tetanus, 3-component acellular Pertussis, Poliomyelitis and Haemophilus influenzae type b conjugate vaccine)
PERT	Product Enhanced Reverse Transcriptase
PFU	Plaque forming units
	PGD Phosphogluconate Dehydrogenase
pH	Potential hydrogen
	Ph. Eur. European Pharmacopeia
PI	Phosphatidylinositol
PM	Petit Modèle
PMKC	Primary Monkey Kidney Cells
PMS	Phenazine Methosulfate
ppm	parts per million
PP	Per Protocol
PRN	Pertactin
	PRNT Plaque Reduction Neutralization Test
PRP	Polyribosyl Ribitol Phosphate
PRP-AH	Activated adipic acid hydrazide-PRP
PRP-T	Polyribosyl Ribitol Phosphate Tetanus conjugated (Haemophilus influenzae type b polysaccharide conjugated to tetanus protein)
PS	Phosphatidylserine
PT	Pertussis Toxoid
PTP	Purified Tetanus Protein
PTT	Purified Tetanus Toxoid
PTxd	Purified Pertussis Toxoid
PVDF	Polyvinylidene fluoride
Q	Quadrupole
QC	Quality Control
QL	Quantification Limit
RALS	Right-Angle Light Scattering
RB	Rhein Biotech
rDNA	Recombinant DNA
Rh	Hydrodynamic radius
RI	Refractive Index
	RCDC Reverse Cumulative Distribution Curve
RIA	Radio-immunoassay
RIE	Rocket Immunoelectrophoresis
	RIV RIJKS Instituut voor de Volksgezonheid
RNA	Ribonucleic Acid
rpm	round per minute
RRF	Relative Response Factor
RSE	Reference Standard Endotoxin
RT	Reverse Transcriptase
RU	Resonance Unit
SAE(s)	Serious adverse event(s)

SD	Standard Deviation
SafAS	Safety Analysis Set
	SDS-PAGE Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
SIDS	Sudden Infant Death Syndrome
SO	Original Strain
SOC	System Organ Class
SOP	Summarized Operating Procedure
SPR	Surface Plasmon Resonance
SS-SAT medium	Stainer and Scholte-SAT medium
SUD	Sudden Unexplained Death
SV40	Simian Virus 40
T	Tetanus
TAE	Tris, Acetate,EDTA
TBE	Tris, Buffer,EDTA
TCA test	Trichloroacetic test
	TCID50 50% tissue culture infective doses (viral infectious units)
TCT	Tracheal Cytotoxin Content
TDA	Triple detection Array
TE	Tris-EDTA
TEM	Transmission Electron Microscopy
Tetracoq	DTwP-IPV (Diphtheria, Tetanus, Whole-Cell Pertussis and Poliomyelitis vaccine)
Tetraxim/ Tetravac	DTacP-IPV (Diphtheria, Tetanus, 2-component acellular Pertussis and Poliomyelitis vaccine)
TFA	Trifluoroacetic acid
TFMS	Trifluoromethane sulfonic acid
TLC	Thin Layer Chromatography
TNBS	Trinitrobenzene Sulfonic
TOC	Total Organic Carbon
TOF	Time of Flight
TRIS	Hydroxymethyl aminomethane
TRS	Technical Report Series
TSA	Trypcase Soya Agar
TSB	Trypcase Soya Broth
TSE	Transmissible Spongiform Encephalopathy
TT	Tetanic Toxin
TTC	Toxicological Threshold Concern
USP	United States Pharmacopeia
UV	Ultra Violet
VDR	Val de Reuil
WCL	Working Cell Bank
WER	Weekly Epidemiological Record
WFI	Water For Injection
WHO	World Health Organization
wP	Whole-cell pertussis
WSL	Working Seed Lot
YCB	Yeast Carbon Base
YNB	Yeast Nitrogen Base
YPD	Yeast extract/Peptone

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Sanofi Pasteur S.A. submitted on 23 June 2011 an application in accordance with Article 58 of Regulation(EC) No 726/2004 to the European Medicines Agency (EMA) for a scientific opinion in the context of cooperation with the World Health Organisation for Hexaxim.

The eligibility was agreed upon by the CHMP on 20 January 2011 after having consulted the World Health Organisation (WHO).

Hexaxim is intended for markets outside the Community in accordance with Article 58 of Regulation (EC) No. 726/2004

The applicant applied for the following indication:

"Hexaxim is indicated for primary and booster vaccination of infants from six weeks of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive infections caused by Haemophilus influenzae type b (such as meningitis, septicaemia, cellulitis, arthritis, epiglottitis, pneumopathy, osteomyelitis)."

This application refers to:

By analogy to the European Legislation, it corresponds to an Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Not applicable

New active Substance status

The evaluation of the requirement for New Active Substances in the EU is not considered applicable since Hexaxim is intended for markets outside the Community in accordance with Article 58 of Regulation (EC) No. 726/2004. The requirement for New Active Substances is not applicable to the Art 58 procedure

Scientific Advice

The applicant did not seek scientific advice at the CHMP.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Jan Mueller-Berghaus **Co-Rapporteur**: Pieter Neels

- The application was received by the EMA on 23 Jun 2011.
- The procedure started on 20 July 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 10 October 2011. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 11 October 2011.
- During the meeting on 14-17 November 2011, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 18 November 2011.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 14 February 2012.
- The summary report of the inspection carried out at the following site: Sanofi Pasteur, Calle 8, N° 703 (esquina 5),Parque Industrial Pilar - (1629), Provincia de Buenos Aires, Argentina between 7-10 February was issued on 24 April 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 March 2012.
- The Rapporteurs circulated an updated Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 13 April 2012.
- During the CHMP meeting on 16-19 April 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 16 May 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CHMP members on 6 June 2012.
- The Rapporteurs circulated an updated Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CHMP members on 15 June 2012.
- During the meeting on 18-21 June 2012 the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion to Hexaxim on 21 June 2012.

2. Scientific discussion

2.1. Introduction

Hexaxim has been developed to provide protection against diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B and invasive infections caused by Haemophilus influenzae type b. Hexaxim qualifies for a CHMP scientific opinion according to Article 58 of Regulation (EC) No. 726/2004 by outlining how it meets the criteria of vaccines required for countries outside the Community: namely, prevents diseases of major public interest, meets the immunization schedules of developing countries including the EPI, as single dose fully liquid combination vaccine.

The following sections describe relevant clinical and epidemiological aspects of these infectious diseases, focusing on data about young children internationally, and the need for, as well as the impact of vaccination programs.

Diphtheria

Active immunization in the paediatric population with diphtheria toxoid has markedly altered the epidemiology of diphtheria, reducing the disease to extremely low levels in developed countries and many developing countries,. In developed countries, endemic diphtheria has either disappeared or become extremely rare, with only infrequent cases of imported diphtheria are reported. Immunity is thought to be lifelong following infection; however, waning of adult immunity to diphtheria has been reported. This highlights the need for vaccination programs to continue from birth through adulthood. Variations in the case definition used for reporting of diphtheria cases also exist. The case fatality rate is 3-23%. Diphtheria is rare in infants younger than 6 months owing to the presence of maternal antibody (Ab). The WHO estimates that 4000 of the 5000 annual deaths from diphtheria that occurred worldwide in 2002 were among children less than five years of age. However, marked disparities remain in reported incidence rates between countries. Some developing countries have achieved control of diphtheria comparable to developed countries, some have observed dramatic falls of the disease but still have sporadic outbreaks, and a small number continue to have evidence of widespread circulation of toxigenic strains.

Tetanus

In spite of the availability of a highly effective vaccine, tetanus continues to exert a substantial global health burden. Tetanus is now considered rare in most developed countries due to improved hygiene and childbirth practices, improved wound care, reduction in exposure to C. tetani spores and improved rates of active immunization over many birth cohorts. Worldwide annual deaths from tetanus, in 2002, were estimated by WHO at 213,000 out of which 198,000 (86%) occurred among children under 5 years of age.

The overall tetanus case-fatality rate varies from 10% to 70%, depending on treatment, age and general health of the patient. Without hospitalization and intensive care, fatality is almost 100% among the youngest and the oldest patients. Tetanus affects all age groups and case-fatality rates can be high even where modern intensive care is available. Tetanus in infants and children commonly reflects poor coverage of the national childhood immunization program.

Immunization with tetanus vaccines early in- and throughout-life has remarkably reduced the number of tetanus infections in industrialized countries. While the worldwide elimination of neonatal tetanus by 1995 (one of the targets of the WHO) has not been achieved, the number of countries in which neonatal tetanus occurs is progressively decreasing. In the WHO Europe region, Turkey was the only country still reporting cases of tetanus.

Pertussis

Pertussis is an important cause of infant death internationally and continues to be a public health concern even in countries with high vaccination coverage. Recent estimates from the WHO suggest that, in 2003, about 17.6 million cases of pertussis occurred worldwide, 90% of which were in developing countries, and that about 279,000 individuals died from this disease. It is further estimated that, in 2003, global vaccination against pertussis averted about 38.3 million cases and 607,000 deaths.

In summary, pertussis, although largely preventable by vaccination, still affects many countries in the world, even in countries with high vaccine coverage. The youngest age groups remain the most affected by pertussis infection and with higher morbidity. Thus, continual monitoring, careful surveillance, high vaccine coverage and appropriate booster administration in the paediatric population and adults is needed across the World to reduce incidence and prevent resurgence of this disease.

Poliomyelitis

Since the GPEI was launched in 1988, 3 WHO regions have been certified poliovirus-free: the Americas in 1994, the Western Pacific in 2000 and the European region on June 2002. So far, the global fight against poliovirus diseases is estimated to have saved 5 million persons from paralysis. The total number of cases decreased from an estimated 350,000 in 1988 to less than 2000 cases in 2009, and the number of poliovirus endemic countries from 125 to 4. Until worldwide eradication of poliovirus has been achieved, high levels of vaccine-induced immunity must be maintained in all populations. Use of OPV contains a small risk of poliovirus-like disease caused by one of the 3 Sabin vaccine related poliovirus types; with a risk of vaccine associated paralytic polio (VAPP). VAPP is seen in 1 case out of 1 million vaccinations

In 2009, a total of 23 countries reported at least one poliovirus disease case due to wild-type poliovirus (WPV). Of these, 4 are considered to be poliovirus-endemic (Afghanistan, India, Nigeria and Pakistan) since they have been unable to eliminate indigenous circulation of WPV type-1 and WPV type-3. The remaining countries were previously considered poliovirus-free, but have reported cases and outbreaks caused by imported WPV type 1 or 3. In spring 2010, a new outbreak in Tajikistan has resulted in 452 laboratory-confirmed cases of WPV type 1 and 20 deaths, and at least 7 related cases have been reported in the Russian federation With continued efforts to achieve high rates of vaccination against polio, eradication from the natural environment is anticipated in the years to come.

Invasive Haemophilus influenzae type b disease

Hib disease burden is highest among infants aged 4 to 18 months, but invasive Hib disease is occasionally observed in infants aged < 3 months and among those aged > 5 years. In unvaccinated populations, invasive Hib is the dominant cause of non-epidemic bacterial meningitis during the first year of life. Even with prompt and adequate antibiotic treatment, the case fatality rate of patients with Hib meningitis is 3 to 20%. Where medical resources are limited, fatality rates for Hib meningitis are typically higher, and severe neurological sequelae are frequently observed in survivors (in up to 30 to 40%) Active immunization first of young children with plain vaccines and later of infants of less than 6 months of age with conjugated vaccines has dramatically decreased the incidence of invasive diseases by almost 100%.

Within a few years of the inclusion of Hib vaccine in routine childhood immunization programs in more than 90 countries (e.g., including European, North American, Latin American, South Africa, Saudi Arabia) invasive Hib disease has been practically eliminated. The reported incidence has been decreased between < 1 to 5/100,000 in children less than five year of age. The majority of invasive Hib disease occurs in resource limited settings when Hib conjugate vaccine is not in routine use

Hepatitis B

The need of controlling hepatitis B infection has been recognized as a major public health target. In the 1980's, a strategy limiting vaccination to individuals at high risk of infection failed to reduce the incidence of Hep B possibly because most people concerned were inaccessible for vaccination or could not be

identified as high-risk individuals. In 1992, the WHO assembly endorsed the universal immunization of infants against Hep B. As of 2008, 177 countries had included hepatitis B vaccination in their national immunization program, including most countries in Eastern and Southeast Asia, the Pacific Islands, Australia, North and Latin America, Western Europe, and the Middle East.

Worldwide, an estimated 1 million deaths annually are attributable to Hep B-associated cirrhosis and hepatocellular carcinoma

In the low endemic areas (with a general population prevalence of < 2%), such as the United States and Europe, less than 10% of the total infections are in the perinatal (infants < 1 year of age) and early childhood (1 to 4 years of age) populations. In Europe, Hep B carriage rates are generally 2% to 7% but vary widely, from < 1% in Scandinavia and the United Kingdom (UK) to 18% in Albania

Hepatitis B vaccines are licensed in approximately 75% of all countries and are capable of inducing a protective Ab response in approximately 95% of young healthy subjects after a 3-dose regimen.

About the product

Hexaxim vaccine is a preservative free liquid formulation for intramuscular administration which combines aluminium hydroxide as adjuvant and six Drug Substances as follows:

- Purified Diphtheria Toxoid (PDT);
- Purified Tetanus Toxoid (PTT);
- 2-component acellular pertussis (aP; purified pertussis toxoid and purified filamentous haemagglutinin);
- Inactivated poliomyelitis trivalent concentrate (IPV);
- Hepatitis B surface antigen (HBsAg);
- Haemophilus influenzae type b (Hib) polysaccharide conjugated to tetanus protein.

The vaccine is presented in single-dose type I glass vials or syringes without needle.

Proposed indication (from the applicant at submission):

The therapeutic indication **initially** claimed by the applicant was:

Hexaxim is indicated for primary and booster vaccination of infants from six weeks of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive infections caused by Haemophilus influenzae type b (such as meningitis, septicaemia, cellulitis, arthritis, epiglottitis, pneumopathy, osteomyelitis).

Proposed posology and method of administration (from the applicant at submission):

The posology and method of administration **initially** claimed by the applicant was:

Posology

Primary vaccination:

The primary vaccination schedule consists of three doses of 0.5 ml (such as 6, 10, 14 weeks; 2, 3, 4 months; 3, 4, 5 months; 2, 4, 6 months) to be administered at intervals of at least four weeks, in accordance with official recommendations.

All vaccination schedules including the Expanded Program on Immunisation (EPI; at 6, 10, 14 weeks of age) can be used whether or not a dose of hepatitis B vaccine has been given at birth. Where a dose of hepatitis B vaccine is given at birth, Hexaxim can be used for supplementary doses of hepatitis B vaccine from the age of six weeks. If a second dose of hepatitis B vaccine is required before this age, monovalent hepatitis B vaccine should be used.

Booster vaccination:

After vaccination with 3 doses (e.g. 6, 10, 14 weeks; 2, 3, 4 months; 3, 4, 5 months; 2, 4, 6 months) of Hexaxim, a booster dose should be given preferably during the second year of life, at least 6 months after the last priming dose. Booster dose should be given in accordance with the official recommendations, but, as a minimum a dose of Hib must be administered.

In accordance with the official recommendations, Hexaxim or sanofi-pasteur DTaP-IPV/Hib vaccine (Pentavac/Pentaxim) can be considered for the booster when the subject is primed against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive infections caused by Haemophilus influenzae type b.

Paediatric population

There is no relevant use of Hexaxim in children over 5 years.

Method of administration

Hexaxim should be administered intramuscularly. The recommended injection sites are generally the antero-lateral aspect of the upper thigh in infants and toddlers and the deltoid muscle in older children.

2.2. Quality aspects

2.2.1. Introduction

Hexaxim is a sterile, whitish and cloudy suspension of diphtheria and tetanus toxoids, acellular pertussis components (Pertussis Toxoid and Filamentous Haemagglutinin), inactivated poliomyelitis vaccine (Vero cell origin) types 1, 2 and 3 (IPV), Haemophilus influenzae type b capsular polysaccharide (polyribosylribitol phosphate, PRP) covalently bound to tetanus protein and Hepatitis B surface antigen (produced in yeast Hansenula polymorpha cells by recombinant DNA technology) adsorbed on aluminium hydroxide.

The development of the vaccine is based on a 5-valent vaccine (Pentavac/Pentaxim – DTaP-IPV-Hib) that has been used since 1997. Hexaxim is based on Pentavac/Pentaxim with the addition of a newly formulated Hepatitis B component

In addition to the new Hepatitis B component, the amount of Hib has been changed in relation to the amount used in Pentaxim: 12 µg Haemophilus influenzae type b polysaccharide (polyribosylribitol phosphate) instead of 10 µg are conjugated to 22-36 µg tetanus protein (PRP-T).

Hexaxim should be administered intramuscularly. The recommended injection sites are generally the antero-lateral aspect of the upper thigh in infants and toddlers and the deltoid muscle in older children.

2.2.2. Purified Diphtheria Toxoid

Manufacture

Purified Diphtheria Toxoid (PDT) is manufactured through the fermentation of *C. diphtheriae*, the toxin being harvested and then detoxified by formaldehyde. The resulting Crude Diphtheria Toxoid (CDT) is further purified through a selective precipitation by ammonium sulphate leading to the PDT.

The production of the PDT drug substance is based on a seed lot system: Pre-Master, Master, Intermediate and Working Seed Lots for *C. diphtheriae*. The Diphtheria antigen production process was long ago established and produces a highly immunogenic antigen.

All materials used during the production of PDT are tested according to either the European Pharmacopoeia (Ph. Eur.) or internal specifications. Ruminant raw materials used include bovine milk, ovine blood, bovine milk, skeleton, muscles and heart and comply with the TSE guidance.

The CDT intermediate is stored in a stainless steel tank.

In process controls (IPCs) for the intermediates of the drug substance include tests with specified acceptance criteria and tests to monitor the process. All IPCs applied are in compliance with the bulk purified toxoid part of Ph. Eur. monograph 0443 "Diphtheria vaccine (adsorbed)", and with WHO TRS No. 800 Annex 2 "Requirements for diphtheria, tetanus, pertussis and combined vaccines (adsorbed)".

Process validation is divided based on the main three production steps: Fermentation, Detoxification and Purification. Each part of the manufacturing process has been independently validated.

The PDT drug substance was characterized by SDS-PAGE and mass spectrometry. The results were consistent for three consecutive batches.

As the production of PDT involves the use of culture media containing material of animal origin (bovine/ovine) and as recommended by WHO in section A.3.1.3 of TRS 800, during the initial development of the product, tests for blood-derived substances and bovine serum albumin were performed. None of the toxoid batches (development lots) contained detectable levels of either blood substances. All the purified toxoids (development batches) tested were negative for bovine albumin antisera.

Specification

The tests and specifications for the control of the PDT drug substance are in compliance with the bulk purified toxoid part of Ph. Eur. monograph 0443 "Diphtheria vaccine (adsorbed)", and with WHO TRS No. 800 Annex 2 "Requirements for diphtheria, tetanus, pertussis and combined vaccines (adsorbed)".

Stability

The results of stability studies for three production batches support the claimed shelf life when stored in polypropylene flasks.

Conclusion

In summary, the manufacturing process of PDT is well established and controlled by different IPCs, release and shelf life specifications.

2.2.3. Purified Tetanus Toxoid (PTT)

Manufacture

The manufacturing of Purified Tetanus Toxoid (PTT) is performed at the Sanofi Pasteur S.A. site in Marcy L'Etoile, France.

PTT is a detoxified protein obtained from *Clostridium tetani* Harvard 49205 strain.

Tetanus Toxoid is manufactured through the fermentation of *C. tetani*, the toxin being harvested and then detoxified by formaldehyde. The resulting Crude Tetanus Toxoid (CTT) is further purified through a selective precipitation by ammonium sulphate leading to the PTT.

In-process controls during the production process are well defined in the process schemes and are in accordance with the recommendations of the Ph. Eur. monograph 0452, and with the "Manual for the production and control of vaccines: tetanus toxoid" (WHO document BLG/UNDP/77.2 Rev 1) named in the WHO TRS 800 Appendix 2.

The materials used during the production of the PTT are tested according to either Ph. Eur. or internal specifications. Regarding raw material of animal origin, information on the species and tissue, country of origin and stage in the manufacturing process where each of the raw materials is used, was provided. Materials of biological origin include bovine liver, lung and heart, bovine milk and poultry feathers. Where applicable, certificates were provided. Impurities like blood-derived substances or bovine albumin, appearing from material of animal origin (bovine/ovine), could not be detected in the PTT.

Specification

The specifications for the PTT drug substance are in compliance with the Ph. Eur. monograph 0452 and with WHO TRS 800. Batch Analyses performed on 3 clinical batches as well as on 3 current production batches met acceptance criteria and showed consistency and uniformity.

Stability

Stability data provided on the intermediate Crude Tetanus Toxoid justifies the claimed shelf-life when stored in stainless steel tanks.

Stability studies on the PTT support the claimed shelf-life.

The PTT is distributed for storage in polypropylene flask.

Conclusion

Overall, the PTT manufacturing process is well defined and controlled by in-process controls. In addition, the PTT is monitored by release and shelf-life specifications which are in compliance with Ph. Eur. monograph 0452 and WHO TRS 800.

2.2.4. Acellular Pertussis (adsorbed PTxd and adsorbed FHA)

Manufacture

The drug substance is composed of two antigenic proteins, the Adsorbed Purified Pertussis Toxoid (PTxd) and the Adsorbed Purified Filamentous Haemagglutinin (FHA). These proteins are obtained from *Bordetella pertussis*.

Both pertussis antigens (native purified FHA and native purified Pertussis Toxin) are obtained from the same fermentation process and are separately processed by adsorption chromatography and affinity chromatography. Native purified Pertussis toxin is then detoxified. Purified FHA, which is routinely proved to be completely devoid of toxic activities, is used in its native form. Both antigens (purified Pertussis Toxoid in solution and purified FHA in solution) are then adsorbed separately onto aluminium hydroxide.

Several intermediates are involved in the manufacture of the two-component acellular pertussis drug substance (adsorbed purified Pertussis Toxoid (PTxd) and adsorbed purified FHA). These are native purified FHA, purified FHA in solution, native purified Pertussis Toxin, and purified Pertussis Toxoid in solution. All intermediates are tested with compendial methods or adequately established in house methods. Batch analysis and stability data show that the manufacturing process provides the intermediates in a reproducible manner and allows storage in glass containers. The materials used in production of the acellular drug substance are in compliance with Ph. Eur. and WHO requirements. For materials of animal origin that are covered by the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents, Certificates of Suitability (COS) were provided.

Specification

The tests and specifications for the control of the acellular Pertussis drug substance (adsorbed Pertussis toxoid and adsorbed FHA) are in compliance with monograph Ph. Eur. 1934 on acellular component Pertussis and WHO TRS 878, Annex 2. Batch analyses show that all acceptance criteria were met.

Stability

Stability studies results for the adsorbed Pertussis toxoid and adsorbed FHA support the claimed storage time in glass containers.

Conclusion

In principle, the manufacturing process of the adsorbed Pertussis toxoid and the adsorbed FHA antigens is well established and controlled in order to provide consistent acellular Pertussis drug substances.

2.2.5. PRP-T Drug Substance

The amount of Hib has been changed in relation to the amount used in Pentaxim, which has been used since 1997. Now, 12 µg PRP instead of 10 µg are conjugated to 22-36 µg tetanus protein (PRP-T).

Manufacture

The *Haemophilus* polysaccharide conjugate drug substance (PRP-T) is a polysaccharide prepared from *Haemophilus influenzae* type b, covalently bound after chemical activation to a carrier (tetanus) protein. These two components are produced, extracted and purified separately using their own seed lot systems and manufacturing processes.

PRP-T production is divided into three main production steps: (1) production of the *Haemophilus* type b polysaccharide, (2) production of the tetanus protein and (3) conjugation of the *Haemophilus* type b polysaccharide with the concentrated tetanus protein.

The polysaccharide is precipitated from a culture of *H. influenzae* type b, purified and subsequently activated (PRP-AH) through chemical linkage/activation.

The tetanus protein is prepared by fermentation of *C. tetani* (Harvard strain 49205) and lysis, purification and inactivation of the toxin.

The activated polysaccharide is subsequently covalently bound to the tetanus protein. The conjugate product is purified and diluted resulting in the PRP-T drug substance.

For storage, the Haemophilus polysaccharide conjugate concentrated bulk is filled in polypropylene flasks.

The production of the PRP-T drug substance is based on two seed lot systems: (1) Pre-Master, Master and Working Seed Lots for H. influenzae type b; and (2) Master and Working Seed Lots for C. tetani; control of both seed lot systems is acceptable.

The materials used during the production of PRP-T are tested according to either Ph. Eur. or internal specifications (tests and acceptance criteria). Ruminant raw materials used include bovine milk, bovine heart, porcine skin and pancreas, horse blood, poultry feathers and comply with the TSE guidance. The manufacturing process of the purified Haemophilus type b polysaccharide (PRP) includes an optional reprocessing step, which is performed only once depending on upcoming high endotoxin and pyrogen levels.

The manufacturing stages for PRP-T are driven by production parameters and in-process controls. IPCs for the intermediates of the drug substance include tests with specified acceptance criteria and tests to monitor the process. All IPCs applied during manufacture of PRP-T are considered acceptable. In contrast to WHO TRS 897, purity testing hasn't been performed at the purified polysaccharide stage. Purity and gram staining however is tested as in process control at pre-culture and industrial culture stages.

The results of the validation programs and of the stability studies provide consistency data and show that the process is under control. The specifications of intermediates comply with Ph. Eur. and WHO technical report series. The storage time of intermediates has been demonstrated with stability data.

The process validation is divided based on the main production steps (PRP-AH, CTP, PRP-T). Each part of the manufacturing process has been independently validated. At least three consecutive industrial batches have been involved considering production parameters, in-process controls, Quality Control tests and additional characterization testing (where appropriate). All data recorded met the operating requirements and results of Quality Control testing met the acceptance criteria. The results presented for the process validation of the PRP-T drug substance are satisfactory.

Several modifications have been introduced to the production of the Conjugated Haemophilus b Polysaccharide Bulk: 1) scale-up of the C. tetani industrial fermentation batch size, 2) Renewal of the Seed Lot and 3) Change in the composition of medium. All the assessments made at the different stages confirmed the equivalency of the processes. The results obtained for the production parameters, IPCs and additional tests comply with their acceptance criteria.

Specification

Tests and specifications performed as a part of the routine testing on the Drug Substance are in compliance with Ph. Eur. or WHO technical report series; or a full process validation study has been provided with adequate results. The test on free tetanus content is based on Ph. Eur.2.2 "Physical and Physicochemical methods", 2.2.31 "Electrophoresis" and monograph 1219. The percentage of the free

tetanus protein content relative to the total tetanus protein content is calculated by comparing the intensity of the free tetanus protein band of the sample (after gel staining) to the intensity of the band of the calibration range. In general, the results from the batch analysis of the PRP-T Drug Substance demonstrate consistency and are within the pre-set limits.

According to WHO TRS 897, the absence of specific toxicity of the carrier protein should be tested at the bulk conjugate stage or assessed through validation of the production process. For Hexaxim, the detoxification is controlled by monitoring production parameter and validation data. The absence of toxin (specific toxicity) and irreversibility of toxoid is tested at the CTP stage in guinea pigs and is in line with Ph. Eur. 452 & WHO TRS 800.

Stability

The results of the studies described support the claimed shelf-life for PRP-T when stored in polypropylene flasks.

Conclusion

The PRP-T manufacturing process is well controlled by IPCs, release and shelf-life specifications.

2.2.6. IPV Drug Substance

Manufacture

The IPV trivalent drug substance comprises the three serotypes 1, 2 and 3 and each monovalent is manufactured separately on Vero cell substrate. Following expansion of the Vero cells in bioreactors using microcarriers, the cells are infected by the respective serotype. The virus harvests are clarified, concentrated and purified by chromatography and subsequently inactivated by formaldehyde. The inactivation is conducted in two stages and it is confirmed through control testing according to international requirements. Monovalent lots of each serotype are then blended in specific proportions to formulate the concentrated trivalent batch. In general the manufacturing process of the IPV trivalent drug substance is well established and sufficiently characterized and validated to ensure consistent production. In addition it was shown that process related impurities are effectively and consistently removed by the manufacturing process.

The starting material is defined by internal specifications and for all raw materials of ruminant origin certificates of suitability issued by EDQM are available. The history, generation and control of the Vero cell banks and poliovirus seed lots were well documented and comply with Ph. Eur. and WHO requirements. As preventive measure material of biological origin (i.e. BCS/FBS and trypsin) is tested for adventitious agents and is further gamma-irradiated. The test program covers circoviruses.

Specification

The control of the drug substance and the quality control tests applied are appropriate to confirm product of consistent quality. The quality tests are acceptably validated and well defined reference preparations are used. The quality test program complies with international and European requirements (Ph. Eur. 214).

Stability

The storage period of the IPV trivalent drug substance in glass bottles or stainless steel tanks is justified by stability data.

2.2.7. HBsAg Drug Substance

Manufacture

The HBsAg drug substance manufacture is based strain K3/8-1 of *Hansenula polymorpha*, which was derived by recombinant DNA technology. K3/8-1 has inserted the gene encoding HBsAg, which was isolated from a chronically infected patient in multimeric form in its genome.

The production of the HBsAg by the recombinant strain K3/8-1 consists of several steps including fermentation of the cells to high cell density and induction of gene expression, harvest of the cells and cell disruption to release the antigen followed by purification using mainly chromatography, and maturation of particles.

Information on starting material including raw material of animal origin is available. The source, history and generation of the *Hansenula polymorpha* strain, of the gene encoding the HBsAg and of the expression vector are well described. Following several passages in selection and stabilization media clone K3/8-1 was isolated that has integrated the gene encoding the HBsAg in multimeric form into the host genome and expressed HBsAg in high amounts. Clone K3/8-1 was employed to establish a pre-master seed lot and subsequently the Master and Working seed lots. The seed lots are well characterized and controlled at release and during storage. The MSL and WSLs comply with WHO and Ph. Eur. requirements.

Data on process validation are available on three processes established during process development. The data generally confirm that the process is capable to yield consistent product which is comparable between the first, second and third generation production batches used in clinical studies. Moreover characterization studies and validation studies confirmed that process related impurities such as host cell DNA and protein are effectively and reproducibly reduced by the purification steps to acceptable levels. Drug substance batches derived from the different manufacturing processes were extensively characterized using biochemical, immunochemical and biophysical methods. It was demonstrated that HBsAg derived from first, second and third production processes had similar properties as regards composition, modification, size and structure.

Specification

The control of the drug substance complies with WHO TRS 786 and Ph. Eur. monograph 1056.

The analytical procedures to determine the HBsAg content, purity as well as protein, carbohydrate and lipids content were validated.

The reference material used was sufficiently characterized. Acceptance criteria for the individual characterization parameters of HBsAg were defined during characterization studies. Upon request a minimum number of tests were defined for calibration of any new reference material.

Stability

The stability data justify the proposed storage time of the HBsAg drug substance.

2.2.8. Finished Medicinal Product

The Hexaxim vaccine is a suspension for injection to be administered by the intramuscular route.

It is a combined vaccine which consists of the following antigens: Purified Diphtheria Toxoid (PDT), Purified Tetanus Toxoid (PTT), 2-component acellular pertussis (purified Pertussis Toxoid (PTxd) and purified Filamentous Haemagglutinin (FHA), Inactivated Poliomyelitis Virus (IPV), Hepatitis B surface Antigen (HBsAg) and Haemophilus influenzae type b polysaccharide conjugated to Tetanus protein (PRP-T). Aluminium hydroxide is added as adsorbant.

The composition of one human dose of the drug product Hexaxim is given below.

Table 1 Composition of Hexaxim vaccine, per human dose of 0.5 ml

Components *	Quantity per dose (0.5 m)	Function
Diphtheria toxoid	≥ 20 IU	Active substance
Tetanus toxoid	≥ 40 IU	Active substance
Bordetella pertussis antigens Pertussis toxoid Filamentous haemagglutinin	 25 µg 25 µg	Active substance
Poliovirus (inactivated): Type 1 (Mahoney) Type 2 (MEF-1) Type 3 (Saukett)	40 DU 8 DU 32 DU	Active substance
Hepatitis B surface antigen	10 µg	Active substance
Haemophilus influenzae type b polysaccharide (polyribosylribitol phosphate) conjugated to Tetanus protein (PRP-T)	12 µg 22-36 µg	Active substance
Aluminium hydroxide, hydrated, for adsorption	0.6 mg Al^{3+}	Adjuvant
Buffer solution Disodium hydrogen phosphate Potassium dihydrogen phosphate Essential amino acids Trometamol Saccharose	15 mg	Neutralization and osmolality adjustment
Water for injections	Up to 0.5 ml	Diluent

Pharmaceutical Development

PDT, PTT, PTxd and FHA, IPV and PRP-T are currently licensed in well-established combination vaccines (*e.g.* Tetravac (DTaP-IPV) and Pentavac (DTaP-IPV/PRP-T)).

The antigen concentrations of these active ingredients per human dose of Hexaxim are similar to those usually used in commercial Sanofi Pasteur paediatric vaccines. The concentration of PDT, PTT, PTxd FHA and IPV are the same as those in Tetravac and Pentavac. The PRP-T concentration was defined according to the formulation of the non-adjuvanted Act-Hib vaccine, for which a concentration of 10µg/dose was confirmed to ensure efficient protection. The PRP-T concentration in the Hexaxim formulation was set at 12µg/dose to compensate the possible amount of PRP-T adsorbed onto aluminium hydroxide, which is expected to be less immunogenic than the non-adsorbed one, and to guarantee similarly at least 8 µg/dose of non-adsorbed PRP-T. Data obtained in phase I studies suggested that PRP-T adsorbed to aluminium hydroxide was less immunogenic than non-adsorbed PRP-T or plain PRP in healthy adult.

Adsorption of conjugate PRP-T onto aluminium hydroxide led to a decrease of antibody responses to PRP. Both the internal data and the findings in the published literature therefore justify the rationale to avoid adsorption of PRP-T in the formulation.

The only new antigen in Hexaxim is Hepatitis B surface antigen produced by the recombinant yeast Hansenula polymorpha. The HBsAg concentration was based on previous internal and external experiences: safe and immunogenic hepatitis B vaccines are commercially available since several decades. Hepatitis B antigen-containing vaccines have been formulated to contain 3 µg to 40 µg of HBsAg protein per millilitre (ml). For the infant/toddler targeted vaccines, hepatitis B content range from 1.5µg/dose to 10µg/dose. Dose response studies and randomized comparative trials between two yeast-derived recombinant HBsAg vaccines have shown repeatedly that a dose of 10 µg of recombinant HBsAg is the optimal antigen content to use for the infant/toddler immunization. For all hepatitis B antigen-containing combination vaccines evaluated in humans, the HBsAg, when used at the same content as with hepatitis B stand-alone vaccines, remains sufficiently immunogenic to elicit protective levels of anti-HBs. In addition, the two phase III clinical studies performed using the Sanofi Pasteur hepatitis B antigen, demonstrated its good immunogenicity performance in adolescents with a content of 10µg/dose. This HBsAg concentration of 10µg/dose has therefore been chosen in animals and in humans.

The appearance of the vaccine is a whitish and cloudy suspension with a pH value within 6.8-7.5 and an osmolality value between 300mOsmol/kg and 400mOsmol/kg. The physico-chemical and biological properties of the medicinal product are determined by the release tests.

To develop an immunogenic and stable hexavalent vaccine, an initial formulation of Hexaxim was defined. The formulation process and composition were then improved from the initial formulation to the optimized formulation. In parallel, the manufacturing process has also evolved with respect to internalization of the site of production of the FBP and FP and a manufacturing up-scale from 50L to industrial scale of 250L. The FBP and FP manufacturing process improvements or changes from the initial formulation to the optimized formulation at industrial scale were described and justified in detail.

Hexaxim vaccine is presented in single-dose glass vials or syringes (type I, Ph.-Eur) without needle.

Glass container (vials and syringes) is of type I grade. During product development the initial elastomeric closures were changed to a more inert plunger stopper/stopper. Several compatibility studies (physicochemical and biological tests, extractable studies and available stability studies) demonstrate the compatibility between Hexaxim vaccine and the chosen new container closure system.

Adventitious agents

All raw materials of ruminant origin used for the manufacture of DTacP-IPV-HepB-PRP-T vaccine comply with Ph. Eur. monographs 1483 and 5.2.8.

Certificates of suitability issued by EDQM were provided for all raw materials of ruminant-origin, or raw materials that contain materials manufactured from ruminant-origin.

All culture media containing raw materials of animal origin used in the manufacture of D, T, P, Hib, HepB and IPV drug substances and which are considered to be the main potential source of viral contaminations are heat steam sterilized or heat treated. These culture media can be considered free of adventitious agents.

In the IPV process, calf serum, cholesterol and trypsin are used, that are the main potential source of viral contamination. These raw materials of animal origin are tested by the manufacturer and are specifically treated to ensure the virus safety. In addition the manufacture of the trivalent concentrated bulk includes an inactivation step.

Manufacture of the product

The manufacturing process for the Hexaxim Drug Product consists in three principal steps:

- Manufacture of the Final Bulk Product;
- Filling of the Final Bulk Product;
- Secondary packaging of the Filled Product.

Critical steps during the manufacture of the Final Bulk Product and the filling of the Final Bulk Product (FBP), are monitored by process parameters applied to ensure that all quality attributes of manufactured vaccine met the acceptance criteria.

FBP is formulated by sequential addition of the individual drug substances and excipients in a specific order to achieve a homogeneous and consistent formulation prior to filling (into vial or syringe). Sterility is tested at release and is ensured by means of validated aseptic process for the introduction of the aluminium gel and the FHA/PTxd during the formulation and by means of validated sterilizing filtrations for the other components.

Hexaxim vaccine can be filled in syringes without attached needle or in vials. The filling equipment is appropriately prepared before steam sterilization using sterilization cycle parameters set to ensure final sterility. FBP is kept at +5°C ± 3°C in a stainless steel tank where it is stirred continuously during the filling step. The tank is connected to the filling machine that is supplied with the sterilized primary packaging components (syringes, plunger stoppers and tip caps or vials, stoppers and flip off caps). The filling process is described in detail and in-process controls for filling volume and homogeneity are applied. The filled product (FP) is inspected for container closure integrity.

Shipment is performed at controlled temperature and is subjected to adequate monitoring (check of sealing, temperature recording).

Validation data of critical manufacturing steps of Hexaxim vaccine drug product demonstrate that the Final Bulk Product batches (MLE site) and the Final Product batches (MLE, VDR and Anagni sites) are consistently manufactured with the required quality attributes whatever the manufacturing sites.

Pharmacopoeial grade excipients used in the manufacture of Hexaxim vaccine are tested according to Ph. Eur.

Non Pharmacopoeial grade excipients are adequately controlled. Each essential amino acid is separately compliant with their respective Ph. Eur. Monograph.

No excipients from human or animal origin and no new excipients are used for the formulation of Hexaxim vaccine.

Product specification

The control of the drug product complies with European requirements.

The tests and methods used to control the Final Bulk Product (FBP) and the Filled Product (FP) are presented hereafter:

Table 2 Specifications of the Final Bulk Product

Tests	Ph. Eur./Methods
Osmolality measurement	Ph. Eur. 2.2.35 Physico-chemical method

Tests	Ph. Eur./Methods
Free formaldehyde content	Based on Ph. Eur. 2.4.18 Colorimetric assay
Bacterial and fungal sterility test	Ph. Eur. 2.6.1 Membrane filtration
Histamine-Sensitizing Activity (HSA)	Ph. Eur. 2067 Injection of the vaccine into mice by intraperitoneal route followed by the injection of an histamine base solution
Non-adsorbed Polyribosyl Ribitol Phosphate (PRP)	Ph. Eur. 2.2.29 High Performance Anion Exchange Chromatography - Pulse Amperometric Detection (HPAEC-PAD)
Depolymerized PRP	
Percent adsorption - Diphtheria toxoid	Rocket immunoelectrophoresis method
Percent adsorption - Hepatitis B	Ph. Eur. 2.7.1 ELISA Method
Diphtheria potency	Ph.Eur.2.7.6 Intradermal challenge test in guinea-pigs (injection of the vaccine into animals by intradermal route)
Tetanus potency	Ph. Eur. 2.7.8 Challenge test in mice (injection of the vaccine into animals by subcutaneous route)
Pertussis immunogenicity	Ph. Eur. 2.7.16 Immunogenicity test in mice (serological assay: ELISA method)
D-antigen content	Ph. Eur. 2.7.1 ELISA method
Hepatitis B *In Vitro* Relative Potency (IVRP)	Ph. Eur. 2.7.15 ELISA method

Table 3 Specifications of the Filled Product

Tests	Ph. Eur./Methods
Appearance	Ph. Eur. 2.9.20 Visual inspection
pH measurement	Ph. Eur. 2.2.3 Potentiometric method
Extractable volume	Ph. Eur. 2.9.17 Volume = mass/density

Tests	Ph. Eur./Methods
Aluminium content	Based on Ph. Eur. 2.5.13 Complexometry assay (EDTA)
Bacterial and fungal sterility test	Ph. Eur. 2.6.1 Membrane filtration
Pyrogen test	Ph. Eur. 2.6.8 Measuring rise of body temperature in animals
Diphtheria identity	Ph. Eur. 2.7.1 Luminex method Or as alternative Ouchterlony double gel diffusion
Tetanus identity	Ph. Eur. 2.7.1 Luminex method Or as alternative Ouchterlony double gel diffusion
Pertussis identity	Ph. Eur. 2.7.1 Luminex method Or as alternative Ouchterlony double gel diffusion
Poliomyelitis identity	Ph.Eur.2.7.1 Luminex method Or as alternative ELISA method
Hepatitis B identity	Ph.Eur.2.7.1 Luminex method Or as alternative ELISA method
Haemophilus identity	Ph. Eur. 2.7.1 Luminex method Or as alternative Ouchterlony double gel diffusion

Most Analytical Procedures for FBP and FP testing are compendial methods and are in line with Ph. Eur. requirements. Since all in vivo assays are compendial methods, they were not specifically validated for Hexaxim release testing for ethical reasons. Compendial tests for osmolality and bacterial fungal sterility (FBP) as well as pH and bacterial fungal sterility have been validated.

Non compendial tests (Free formaldehyde content; Non-adsorbed PRP/Depolymerized PRP; Percent adsorption - Diphtheria toxoid (Rocket); Percent adsorption - Hepatitis B (ELISA); Hepatitis B In Vitro Relative Potency (IVRP) and D-antigen content (for FBP stage) as well as Aluminium content and Identity tests (for FP) were validated according to ICH Q2 (R1).

Initial formulation batch analysis data for 4 FBP lots and 7 FP lots were presented. For the optimised formulation batch analysis data for 6 FBP and 6 FP lots (vials and syringes) are available. The results presented demonstrate that all batches from the initial and optimized formulation comply with the defined specifications and therefore fully support manufacturing consistency.

The justifications of the release profile for FBP and FP commercial batches and its associated specifications are based on international requirements (Ph. Eur. monograph 2067, Ph. Eur. monograph 0153 and TRS 927), statistical analysis of batch results and the company's experience with licensed vaccines such as

Tetravac (DTacP-IPV), Pediacel (DTaP-IPV-PRP-T) and Act-Hib. All results obtained with the optimized formulation batches meet these acceptance criteria.

Diphtheria potency limits set for Hexaxim are: Activity ≥ 30 IU/dose, Lower fiducial limit (P = 0.95) of the estimated potency ≥ 20 IU/dose. The diphtheria component of Hexaxim therefore is considered compliant with both WHO (Technical Report Series No. 927,2005) and Ph. Eur. requirements [monograph 01/2008:2067, Diphtheria, Tetanus, Pertussis (Acellular, component), Hepatitis B (rDNA), Poliomyelilitis (inactivated) and Haemophilus influenza type b conjugate vaccine (Adsorbed)].

Stability of the product

Stability studies were conducted to support the comparability of the initial and the optimized formulation.

In general, the results of the five stability studies support the shelf-life of the FBP and the FP and the storage conditions as defined in the SPC.

The studies were conducted using FBP manufactured at Marcy l'Etoile (MLE) and Drug Product filled in single-dose syringes without needle at MLE and in single-dose vials at Val de Reuil (VDR) and Anagni. The design and test program of the stability studies was in general satisfactory and the FBP and FP met the relevant requirements supporting the proposed shelf-life of the vaccine of 36 months when stored at +5°C ± 3°C.

2.2.9. Discussion on chemical, pharmaceutical and biological aspects

No major objections were raised during the assessment of the quality part of the dossier.

The Applicant has responded satisfactorily to all of the other quality concerns and questions identified in the Day 120 List of Questions and in the Day 180 List of Outstanding Issues.

IPV Drug Substance

Due to recent findings of PCV-1 and 2 contaminations in vaccines produced from Vero cells, a risk assessment as regards adventitious agents possibly introduced by starting materials but not detected by classical adventitious agents testing and the confirmation of absence of circovirus contamination in Vero cell banks, seed viruses and the IPV drug substance, were requested. The Applicant confirmed that the test program for the trypsin raw material covers circoviruses. Data demonstrating the absence of PCV-1 and 2 contaminants in working cell banks and seed lots were provided and specific tests were implemented as release tests.

HBsAg Drug Substance

The purity assay is performed as in-process test and as release test for the HBsAg bulk component. Additional validation data on linearity and accuracy were provided by the Applicant and confirmed that the assay is accurate and linear in a 90-100% range.

The lipids content test is performed as a release test for the HBsAg bulk component. The amount of lipids may be important for the immunogenicity of the vaccine and the HBsAg lots used in the clinical studies should be representative for the proposed lipid content acceptance criteria. This point was clarified by the Applicant and the proposed specification limits for the lipid content were shown to be clinically validated.

Drug Product

The chosen acceptance criteria for percent adsorption of Diphtheria Toxoid (at FBP), percent adsorption-Tetanus Toxoid and the test for Non-Adsorbed PTxd and Non-Adsorbed FHA by ELISA were clarified by the

Applicant. Although no upper specification limit is intended to be introduced for percent adsorption of Diphtheria Toxoid in the FBP, an upper control limit for internal monitoring will be established. Likewise, no specification limit is intended to be introduced for percent adsorption of Tetanus Toxoid in the FBP.The test for non-adsorbed PTxD and non-adsorbed FHA by ELISA are considered as a characterization test to be performed on the filled product in case of a process change that may impact the adsorption.

Additional information was provided to justify the chosen stability limits. The end of shelf-life specification for depolymerised PRP was further justified and shown to be clinically validated.

In conclusion, information on development, manufacture and control of the drug substances and drug product has been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

2.2.10. Conclusions on the chemical, pharmaceutical and biological aspects

The manufacturing process of Hexaxim is considered to be well controlled. In-process controls, release and shelf life specifications indicate the high quality of the drug substances and the drug product.

The Quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Data has been presented to give reassurance on viral/TSE safety.

It is recommended that batch compliance control of individual batches be performed by an independent control laboratory before release on to the market in third countries.

2.2.11. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

- DS – DT: The CHMP recommends replacement of the currently approved pre-ranges by definitive operating ranges for the fermentation and detoxification process of Diphtheria purified toxoid, when data on 30 batches are available.
- DS - HBsAg: The CHMP recommends the applicant to assess the HCP content on a large number of batches (minimum of 30 batches) by ELISA. If relevant, specification for the drug substance should be updated.
- DS – PRP-TT : The CHMP recommends the applicant to revise the specification limit for residual cyanide once 100 PRP-AH batches are produced.

2.3. Non-clinical aspects

2.3.1. Introduction

Non-clinical pharmacological and toxicology studies were undertaken on Hexaxim based on

- the CPMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95),
- the Note for Guidance on Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products (CPMP/ICH/386/95).

Based on these guidelines secondary pharmacodynamic, pharmacodynamic drug interaction, pharmacokinetics, genotoxicity and carcinogenicity studies were not considered necessary to be performed on Hexaxim.

2.3.2. Pharmacology

To address the non-clinical pharmacology of Hexaxim, the immunogenicity evaluation of each active substance was assessed in release tests or characterization tests, in suitable animal models following the Ph. Eur. requirements.

Primary pharmacodynamic studies

Release tests or characterization tests with final bulk products

For each Drug Substance, their potency/immunogenicity was assessed at the Final Bulk Product (FBP) stage through *in vivo* studies as release tests or as characterization tests. Overall, four FBP batches of the optimised formulation of Hexaxim were tested, which were considered representative of the vaccine to be marketed. The results were all conform for the batches and are summarised below. For details about the tests, please refer to the section on Quality aspects discussed above.

Diphtheria Potency in Guinea Pigs

The criterion for acceptance based on statistical evaluation of the immune response is that the activity must be not less than 30 IU per 0.5 ml single human dose and that the lower confidence limit (p = 0.95) must be not less than 20 IU Diphtheria Toxoid per dose, when compared to the Diphtheria reference standard.

The results for diphtheria potency assay in guinea pigs of 42 (34-52) IU, 57 (43-82) IU,

76 (57-113) IU and 41 (28-58) IU were determined, respectively, for the four FBP batches tested.

Tetanus Potency in Mice

The criterion for acceptance based on statistical evaluation of the immune response is that the lower confidence limit (p = 0.95) must be not less than 40 IU Tetanus Toxin per dose, when compared to the Tetanus reference standard.

The results for tetanus potency assay in mice of 556 (280-853) IU, 584 (413-795) IU and 705 (485-1017) IU were determined respectively for three FBP batches. The tetanus potency of an additional batch was analyzed with the former lethality method which was replaced by the Ph. Eur. and gives comparable results i.e. 893 (584-1243).

Pertussis Immunogenicity in Mice

The criterion of acceptance is that the anti-PTxd and anti-FHA antibody titres induced by the test vaccine are not significantly different (p = 0.95) than that of the reference vaccine.

The results for Pertussis Toxoid (PTxd) and Filamentous Haemagglutinin (FHA) assays in mice were all conform for the four batches tested.

Activity of Pertussis Vaccine on Bacterial Challenge

Protective effect of Hexaxim was consistently shown for all three batches in this challenge model, with bacterial CFU counts in the lungs lower in Hexaxim-vaccinated mice than in the non-immunized mice.

Poliomyelitis Immunogenicity in Rat

The potency was calculated by comparing the numbers of responders for the test vaccine to the number of responders for the reference vaccine (Pediacel).The IPV potency in protecting units/dose of the four batches was not considered to be significantly less than the reference vaccine.

Haemophilus Immunogenicity in Mice

The criterion of acceptance is that not less than half the vaccinated mice show a titer not less than four time that of the pooled control serum. To be conformed, the batches must induce a humoral response in more than half of the mice.

The mice immunized with the different batches were all responders. The batches met the criterion of acceptance and were considered conform.

Hepatitis B Potency in Mice

The ED50 (efficient dose in µg that enables a 50% seroconversion at D42 after immunization) relative to the reference vaccine was determined. The criterion of acceptance is that the upper confidence limit (p=0.95) was not less than 1.0.

All four batches of Hexaxim met this criterion.

Assessment of antigenic interference in mice

Study Objective and Design:

To investigate the possible antigenic competition between HBsAg and PRP-T by following the magnitude of humoral response elicited against each of these two antigens.

Rationale: HBsAg and PRP-T were selected because 1) within the Hexaxim formulation HBsAg was considered the only new antigen produced from a novel source (*Hansenula polymorpha* yeast), and 2) both antigens were identified as the most susceptible to antigenic interference based on literature review.

In parallel, to assess:

- the effect of the aluminium hydroxide on the HBsAg and PRP-T immune responses
- the polarization and persistence of immune responses induced by both antigens

Group Definition and Treatment:

One hundred NMRI mice (7 weeks, female) were distributed in 10 groups of 10 mice. Each group received either HBsAg and/or PRP-T, alone or mixed with D, T, aP, IPV antigens, with or without AlOOH as adjuvant (Table below). An additional group of 10 randomized naive mice of the same delivery was used to collect blood samples for the establishment of a baseline for all ELISA titrations.

Table 4

Group (Mouse #)	Inoculum	Total Al^{+++} / human dose
1 (1 to 10)	HBsAg without AlOOH	0
2 (11 to 20)	PRP-T without AlOOH	0
3 (21 to 30)	HBsAg + PRP-T without AlOOH	0
4 (31 to 40)	HBsAg + AlOOH	0.6 mg
5 (41 to 50)	PRP-T + AlOOH	0.6 mg
6 (51 to 60)	HBsAg + PRP-T + AlOOH	0.6 mg
7 (61 to 70)	Hexavalent vaccine (D, T, aP, IPV, Hepatitis B, Hib + AlOOH)	0.6 mg
8 (71 to 80)	Hexavalent vaccine without AlOOH	0.046 mg brought by adsorbed PTxd and FHA
9 (81 to 90)	(HBsAg + AlOOH) + (PRP-T + D, T,aP ,IPV + AlOOH) **in separate sites**	1.2 mg (0.6 mg / injection site)
10 (91 to 100)	(PRP-T + AlOOH) + (HepB + D, T ,aP, IPV + AlOOH) **in separate sites**	1.2 mg (0.6 mg / injection site)

These different products under test contained the same amount of active ingredients as in the hexavalent vaccine. Their formulations were also identical to that of the hexavalent vaccine, except for AlOOH content in groups 8, 9 and 10, as indicated above.

Immunization was implemented by injection three times at 3-week intervals by intramuscular route. The kinetics of anti-HBsAg and anti-PRP-T specific IgG antibody responses were monitored over a 16 week period of time. These immune responses were compared in the presence or absence of aluminium hydroxide adjuvant, and in combination or not with the other vaccine antigens (D, T, aP, IPV).

Results:

Humoral immune response to HBsAg – effects of AlOOH and PRP-T and other Antigens

AlOOH increased significantly the anti-HBsAg IgG antibodies (especially IgG1 levels). Mixing HBsAg with PRP-T and D, T, aP, IPV increased the specific IgM and IgG responses to HBsAg as well, although PRP-T alone failed to do so . This adjuvant-like positive effect of the antigens was not observed anymore if AlOOH was present, but no negative interferences could be noticed either. In a vaccine formulation containing AlOOH, the addition of PRP-T and/or D, T, aP, IPV antigens resulted in stronger IgG2a immune responses specific for the Hepatitis B antigen.

Th1 / Th2 Polarization of the anti-HBsAg Responses

The addition of AlOOH significantly increased the levels of anti-HBsAg IgG1 (but not of IgG2), resulting in a more Th2 biased response. In the complete mixture, the Th2 polarizing effect of AlOOH was partially balanced by the addition of the PRP-T antigen, which by itself, increased more specifically the anti-HBsAg IgG2a levels (Th1-like polarizing effect). Therefore, the overall IgG1 / IgG2a ratio was not significantly modified, but the titres of both anti-HBsAg IgG1 and IgG2a were significantly increased (0.5 log) by AlOOH and by PRP-T in the final combination vaccine. Overall, an "adjuvant-like" effect of PRP-T on the HBsAg specific IgG2a titres could be observed, when PRP-T was added to HBsAg alone or mixed with the other hexavalent antigens.

HBsAg Antibody Persistence over Time

Anti-HBsAg IgG (including IgG1 and IgG2a) reached a peak on week 8, and then only, very slowly decreased during the following weeks, whereas a more rapid decline of anti-HBsAg IgM titres is observed. The anti-HBsAg IgG titres observed at week 16 always remained high and superior to 4 log except for group 1 (HBsAg without AlOOH), suggesting the induction of an anti-HBsAg memory response in all groups including the one of the hexavalent vaccine.

Humoral immune response to PRP-T – effects of AlOOH and HBsAg and other Antigens

The presence of the AlOOH did not seem to modify anti-PRP-T IgG titres when it was injected alone, but tended to increase the anti-PRP-T response in the presence of HBsAg and the other antigens. In particular, anti-PRP-T IgG titres elicited by the hexavalent vaccine increased more rapidly and reached higher levels than those induced by the PRP-T administered alone. In addition, the hexavalent formulation emerges as the best over time. A similar trend for an increase in anti-PRP-T titres when other antigens were added to the vaccines was also observed for IgG1.

Th1 / Th2 Polarization of the anti-PRP-T Responses

PRP-T injected alone without AlOOH induced a slightly Th2 biased response (measured via IgG1 / IgG2a ratio). The addition of AlOOH moderately increased the anti-PRP-T IgG1 titres, but more markedly when PRP-T was mixed with HBsAg and other antigens. In absence of AlOOH, this increase in IgG1 due to addition of HBsAg and/or of the other antigens was less efficient. Therefore, addition of HBsAg and or of the D, T, aP, IPV, PRP-T combination increased anti-PRP-T IgG1 and Th2 polarization in presence of AlOOH.

PRP-T Antibody Persistence over Time

Anti-PRP-T IgG antibodies decreased less rapidly and were more stable when the PRP-T was injected in the presence of AlOOH and with HBsAg and all other antigens.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were conducted as no specific risks were identified with the candidate vaccine in line with the EMA "Note for guidance on preclinical pharmacological and toxicology testing of vaccines" (CPMP/SWP/465/95)).

Safety pharmacology programme

No dedicated safety pharmacology study was performed with Hexaxim as no cardiotoxic, respiratory or neurotoxic specific risks were identified in line with the EMA "Note for guidance on preclinical pharmacological and toxicological testing of vaccines" (CPMP/SWP/465/95).

Pharmacodynamic drug interactions

No pharmacokinetic studies were performed, which is in accordance with Regulatory Guidelines quoted above.

2.3.3. Pharmacokinetics

No pharmacokinetic studies were performed, which is in accordance with Regulatory Guidelines quoted above.

2.3.4. Toxicology

The nonclinical safety of Hexaxim was evaluated in three rabbit studies: two repeat-dose toxicity studies, which included systemic toxicity evaluation and a local tolerance assessment and evaluated both the initial and optimized vaccine formulations and an investigative local tolerance study (with limited assessment of systemic toxicity), which was conducted to follow up on some local lesions observed in batch release tests in guinea pigs.

Single dose toxicity

A single dose toxicity study was not considered necessary as the vaccine is intended to be used with repeated administrations.

Repeat dose toxicity

Repeated-dose Intramuscular Study in New Zealand White Rabbits

The study was designed to determine the toxicity of Hexaxim (final bulk product), when administered 5 times at 2-week intervals by intramuscular route to male and female New Zealand White Rabbits, and to evaluate the recovery of potential effects after a two-week treatment-free period.

New Zealand White rabbits (8 animals/sex/group, approximately 12 weeks old) randomly assigned to study groups received a 0.5 ml intramuscular injection of 0.9% saline (Group 1) or Hexaxim (equivalent to one human dose; Group 2) on Study Day (SD) 1, 15, 29, 43, and 57. Injections rotated between sites in the right and left thighs (dose sites 1 and 2, respectively). Four animals/sex/group were sacrificed each on SD 58 and 71. Parameters evaluated included mortality, clinical and cage side observations (≥ 2 daily), dermal Draize observations (immediately following each dose, daily for the three days after each dose (daily observations continued for each injection site noted with findings), and weekly in between), body weights (study Day 1, weekly thereafter, and at termination (fasted)), food consumption (daily, unless interrupted for study related events), ophthalmologic examinations (Prior to first dose, SD 3, and within 5 days of sacrifice), clinical pathology (SD3, 58, and 71), immunogenicity (anti-Diphtheria antigen only, SD58), organ weights, gross pathology, and histopathology (SD58, SD71).

Results:

Under these study conditions, repeated intramuscular injections of Hexaxim in New Zealand White Rabbits did not result in toxicologically relevant changes in mortality, clinical observations, body weights, body weight gains, food consumption, or organ weights.

Treatment did result in a slightly increased level of Draize observations following the last injection, variations in some clinical pathology parameters probably linked to the inflammatory and immune reactions induced by a vaccine which are generally reversible, and gross pathology findings at the injection sites associated with histopathology findings of inflammation were still observed at the end of the treatment-free period. No sign of recovery of local injection site reactions was observed at the end of 14-day recovery period, suggesting a need for longer period of time for reversibility

Repeated-dose Intramuscular Study in New Zealand White Rabbits

The objective of the study was to evaluate the local tolerance and the potential systemic toxicity of the test item, HEXAXIM, after five intramuscular injections at 2-weekly intervals in New Zealand White rabbits, followed by a 1-day or 14-day observation period.

The batch used for this study, which was evaluated in this final stage of Hexaxim development, was representative of the vaccine to be marketed. The study aims to bridge the first repeat-dose toxicity study, to confirm the nonclinical safety profile, and eventually to support the safety of this optimized formulation.

The study design was the same as the first repeat-dose toxicity study presented above.

In addition, immunogenicity of Diphtheria, Tetanus, and Hep B antigens was assessed for all animals with blood samples collected prior to treatment, on SD58 and SD 71.

Results:

Five intramuscular injections of HEXAXIM vaccine at 2-week intervals were clinically well tolerated in the male and female rabbit. Toxicological findings were restricted to a persistent inflammatory reaction at the injection sites associated with a transient increase in neutrophil counts. Stimulation of the lymphoid tissues was also noted. These observations are consistent with the results typically recorded after the administration of an aluminium hydroxide adjuvanted vaccine.

The study was in general considered adequately designed, although the 14-day recovery period was not long enough for this study to see a sign of reversibility of findings of lymphoid tissue stimulation and histology findings at injection sites. The species was relevant and exposed to the vaccine as suggested by immunogenicity data.

Overall, the study with optimized formulation of Hexaxim did not raise major safety concerns.

Genotoxicity

Genotoxicity of the new process residues in association with Hep B manufacturing was investigated based on literature search [i.e., using information from marketed vaccines, regulatory guidance and available toxicity data]. None were identified at levels of toxicological concern which could pose risk for the infant/toddler population after intermittent use in a vaccine product. A dedicated genotoxicity study was therefore not required in line with relevant regulatory Guidelines quoted above.

Carcinogenicity

In accordance with EMA "Note for guidance on preclinical pharmacological toxicological testing of vaccines" (CPMP/SWP/465/95), carcinogenicity studies were not considered necessary as the exposure to the vaccine is short term.

Reproduction Toxicity

In accordance with EMA "Note for guidance on preclinical pharmacological toxicological testing of vaccines" (CPMP/SWP/465/95) and WHO guidelines on nonclinical evaluation of vaccines no reproductive or

developmental toxicity studies were conducted with Hexaxim as the target population is infants and toddlers only. Information on reproductive organs effects was obtained during the repeat dose toxicity studies and no evidence of toxicity was observed.

Toxicokinetic data

Not Applicable

Local Tolerance

Investigative local tolerance and repeated-dose study in the Female Rabbit following 4 administrations by I.M. Route

The objective of this investigative study was to determine the systemic toxicity and the local tolerance of three different batches of Hexaxim following four intramuscular administrations at two-week intervals to the Female New Zealand White rabbits.

The design of this investigative rabbit study was similar to that of the first repeat dose toxicity study but the focus was on local tolerance. There were some minor differences in design, which were as follows: four, not five, doses were administered intramuscularly; the injection sites were in the dorso lumbar area instead of the thigh (allowed four separated sites, instead of two); only the sites of injection and any abnormal tissues were examined microscopically, and the last sacrifice time was extended to 30 days post the last dose.

Four groups of 10 females received 0.5 ml of batches of Hexaxim or saline control via intramuscular injection on days 0, 14, 28 and 42.

All animals were observed for morbidity/mortality at least twice daily and for clinical signs and local reactions at the injection sites at least once daily. A full clinical examination was performed at least weekly. Ophthalmological examinations were performed pre-test and on days 2 and 43 (two days after the first injection and one day after the last injection, respectively). The recovery animals were also examined on day 56 (two weeks after the last injection). All animals were weighed weekly. Food consumption was measured daily for each animal. Clinical pathology samples were collected for clinical laboratory determinations from all remaining rabbits once pre-test and on days 2, 43, 57 and 72. Five females from each group were sacrificed one day after the last dose (day 43); the remaining animals were sacrificed 30 days after the last dose (day 72). Selected organs were weighed and a full tissue list was taken and preserved. Histopathology examinations were performed on the injection sites and any organ/tissue with gross lesions.

Results

Four intramuscular injections of all three batches of Hexaxim at 2-week intervals were clinically well tolerated in the female rabbit. Toxicological findings were confined to inflammatory reactions at the injection sites with a transient increase in neutrophil counts noted one day after the last injection. There was no sign of reversibility of these reactions 30 days after treatment, and the severity of inflammatory reactions differed between the batches slightly.

Histological changes were noted at the injection sites in all treated groups and were mainly characterized by inflammatory infiltrate with foam cell aggregate (mainly macrophages), presence of amorphous material, cell debris and mixed inflammatory cells. The mixed inflammatory cells appeared to be slightly more severe in animals that received vaccine from two of the three batches tested. The inflammatory reactions (foam cell aggregate) were still present in the treated groups 30 days after treatment, suggesting the absence or a slow reversibility of these findings. Other inflammatory changes considered to be treatment-related, such as amorphous material with cell debris and mixed inflammatory cells were seen

very infrequently and with a low severity, suggesting these changes were not entirely reversible after 30 days.

The patterns of noted abnormalities, expected or unexpected (e.g., mean globulin levels and A/G ratios, mean cholesterol level, heart weight, etc.), appear to differ between this study and the above two standard studies.

Overall, this investigative study using I.M. route of administration in rabbits did not reveal unexpected local reactions (as seen in a release test in guinea pigs using subcutaneous route).

Since for Alum-adjuvanted vaccines, the I.M. route is a preferred route of administration, the results of this rabbit study were considered predictive of human reactions.

Other toxicity studies

Not applicable.

2.3.5. Ecotoxicity/environmental risk assessment

No toxicity to the environment is expected for the components of Hexaxim. The justification of the applicant for not carrying out the studies for an environmental Risk Assessment (ERA) was considered acceptable.

2.3.6. Discussion on non-clinical aspects

The immunogenicity of the new HBsAg antigen was further demonstrated in a dedicated pharmacological study in NMRI female mice where experimental batches were used. In this study, antibody response to HBsAg was significantly augmented in the presence of AlOOH adjuvant (0.6 mg in 0.5 ml vaccine formulation), with some extent of adjuvanting effect also shown for the PRP-T antigen. Furthermore, the addition of AlOOH did not alter the persistence and IgG1/IgG2a balance of humoral responses to these two antigens. However, open question remains as to whether the 0.6 mg of AlOOH is representing an optimal amount (or resulting in optimal adjuvant/antigens ratio(s)). However it was considered that this question could be addressed in a clinical setting if necessary based on the outcome of the clinical data submitted.

Of note, a potential difference of vaccine materials may not have contributed to the discrepancy among the studies, as the Applicant clarified that the experimental vaccine lot used in the repeat dose toxicity study is considered representative to the vaccine to be marketed.

Also noteworthy is that the new Hep B antigen was demonstrated compatible with the PRP-T antigen and did not undergo any negative interference from any component antigens of Hexaxim in the presence or absence of AlOOH adjuvant. To answer whether the presence of HBsAg antigen could reduce the immunigenicities of D, T, aP and IPV antigens within Hexaxim, the Applicant addressed this issue by presenting 2-year persistence data from on-going A3L26 clinical trial revealing similar antibody or protective responses to antigens D, T and aP, indicating the absence of significant interference.

The nonclinical safety of Hexaxim was evaluated in three repeated dose and local tolerance toxicity studies (all GLP-compliant) in NZW rabbits. The animals developed specific antibodies against Hexaxim's antigens analysed (including the new Hep B antigen), thus verifying animal exposure as well as the relevance of the model. Notably, these studies were designed to well reflect clinical exposure, such as the use of I.M. route of vaccine administration, full human dose, and 5x dosing in two standard toxicity studies. The use of

reduced dosing intervals (2-weeks) in these studies also aligns with the WHO Guideline, and can be considered appropriate even from a booster response viewpoint, for the last injection(s). Other aspects of the study designs (endpoints, timing of blood sampling, recovery groups, etc.) as well as the use of final bulk product (initial or optimized formulation) also well meet regulatory expectations.

The vaccine-related effects, normally expected or indicative of immune stimulation and inflammatory responses, have been noted, including clinical signs of erythema and/or oedema at injection sites (minimal intensity) in two studies increases in WBC (neutrophils) in all three studies and increased globulin levels associated with lower A/G ratios in two studies, the increased lymph node weight and the development of germinal centres (minimum to slight) in spleen and lymph nodes in one study, and the chronic active inflammation in histology (mainly macrophage infiltrate, minimum to slight in intensity) at injection sites in all three studies. In addition, two studies indicated relative heart weight increase at the end of a 14-day recovery period. However, historical control values of relative mean heart-to-body weight ratio of two testing facilities showed that the observed changes lie within historical range or are broadly comparable to historical control values. This was therefore considered of no relevance and no further studies/data are considered necessary.

The immune reactions- or inflammation-related effects were generally reversible with the exception for lymphoid tissue stimulation and for injection site inflammation, where no sign of reversibility was noted after 14-day or up to 30-day recovery, respectively. Notably, a healing process following inflammation or onset of recovery was suggested by the presence of fibroplasia / fibrosis in interstitium/fascia and myofiber regeneration (minimum intensity) noted in one study, or the presence of very scarcity of amorphous material with cell debris and mixed inflammatory cells and with a low severity noted in another study. Whether the absence or the slow reversibility of these microscopical changes has any impact on the safety/tolerability of Hexaxim in a clinical setting is actually a matter of clinical scrutiny. In this regard, further nonclinical studies aiming to expand this finding/effect on reversibility would not be expected to provide additional information and are therefore deemed unnecessary for this initial MAA.

Similarly, further immunotoxicity study following routine tiered approach is not applicable to vaccine products and is therefore not needed. However, the fact of persistence of the chronic inflammation, together with unexpected cutaneous lesions observed in Guinea pigs, although after subcutaneous administration, calls for some doubts about the optimum of the amount of 0.6 mg of AlOOH arbitrarily selected for 0.5 mg AL per Hexaxim dose, and questions why a reduced amount of AlOOH was not better in the immunogenicity/reactogenicity balance context. No nonclinical study was conducted to determine an optimal antigen/adjuvant (aluminium) ratio for Hexaxim, and the 0.6 mg quantity of aluminium per 0.5 ml Hexaxim dose was selected empirically. However as outlined further below, a comparable finding of persistent localised inflammation could not be observed in the available clinical data

2.3.7. Conclusion on the non-clinical aspects

Overall, the release/characterization tests have demonstrated immunogenicity or potency of each active substance of Hexaxim in suitable animal models, using four final bulk product batches of the optimized formulation. Either the pre-defined acceptance criteria were met, or Hexaxim was noted to be similar to a reference vaccine, in these tests.

The general toxicity studies did not reveal vaccine-related systemic effects that are considered to be of toxicological significance.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

A GCP inspection was undertaken in Mexico and Peru for study sites involved in study A3L04. No major or critical findings were reported, GCP compliance was attested. Regarding nonclinical aspect, no inspection was required.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

All clinical trials were carried out outside of the European Union.

Table 5 Tabular overview of clinical studies

Study Identifier	Title	Trial Period (FVFS to LVLS)	Third Country
A3L01	Phase-I Safety of a Booster Dose of Either the Investigational DTaP-IPV-HB-PRP~T Combined Vaccine or HEXAVAC in Healthy Argentinean 16- to 19-Month-Old Toddlers	19 January 2004 - 04 March 2004	Argentina
A3L02	Phase II Immunogenicity Study of a DTaP-IPV-HB-PRP~T Combined Vaccine Compared with PENTAXIM and Engerix B PEDIATRICO at 2, 4, and 6 Months of Age in Healthy Argentinean Infants	26 October 2004 - 10 November 2005	Argentina
A3L16 (Booster phase of A3L02	Immunogenicity Study of the Antibody Persistence and Booster Effect of PENTAXIM at 18 Months of Age Following a Primary Series of DTacP-IPV-HepB-PRP-T Combined Vaccine or of PENTAXIM and ENGERIX B PEDIATRICO at 2, 4, and 6 Months of Age in Healthy Argentinean Infants	15 February 2006 – 02 November 2006	Argentina
A3L04	Large Scale Safety Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine, in Comparison to Tritanrix-Hep B/Hib and OPV Administered at 2, 4, and 6 Months of Age in Latin American Infants	17 July 2006 – 02 January 2008	Peru - Mexico
A3L10	Immunogenicity of DTaP-IPV-Hep B-PRP-T Combined Vaccine Compared with PENTAXIM and ENGERIX B at 2-3-4 Months Primary Schedule in	01 June 2006 – 18 June 2007	Turkey

Study Identifier	Title	Trial Period (FVFS to LVLS)	Third Country
	Healthy Turkish Infants		
A3L22 (Booster phase of A3L10)	Immunogenicity and Safety Study of a Booster Dose of DTaP-IPV-Hep B-PRP-T Combined Vaccine at 15 to 18 Months of Age Following a Primary Series at 2, 3 and 4 Months of Age in Healthy Turkish Infants	14 December 2007 – 07 July 2008	Turkey
A3L11	Lot-to-Lot Consistency Study of DTaP-IPV-Hep B-PRP-T Vaccine Administered at 2-4-6 Months of Age in Healthy Mexican Infants	14 November 2006 – 13 June 2008	Mexico
A3L21 (Booster phase of A3L11)	Immunogenicity Study of the Antibody Persistence and Booster Effect of the DTaP-IPV-Hep B-PRP-T Combined Vaccine at 15 to 18 Months of Age Following a Primary Series of DTaP-IPV-Hep B-PRP-T or Infanrix hexa Administered at 2, 4, and 6 Months of Age in Healthy Mexican Infants	26 March 2008 – 28 May 2009	Mexico
A3L12	Immunogenicity Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to Infanrix hexa, Both Concomitantly Administered with Prevnar at 2, 4, and 6 Months of Age in Thai Infants	22 October 2006 – 19 November 2007	Thailand
A3L15 (Primary Series)	Immunogenicity Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to CombAct-Hib Concomitantly Administered with Engerix B Paediatric and OPV at 6, 10, and 14 weeks of Age in South African Infants	28 August 2006 – 27 November 2007	Republic South Africa
A3L15 (Booster Phase)	Immunogenicity Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to CombAct-Hib Concomitantly Administered with Engerix B Paediatric and OPV at 6, 10, and 14 weeks of Age in South African Infants	28 January 2008 – 04 February 2009	Republic South Africa
A3L17	Immunogenicity Study of DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to Infanrix hexa, at 2-4-6 Months of Age in Healthy Peruvian Infants	23 May 2008 – 12 May 2009	Peru

2.4.2. Pharmacokinetics

As mentioned in the Note for Guidance on Clinical Evaluation of New Vaccines (CHMP/VWP/164653/2005), "Pharmacokinetic studies are usually not required for vaccines. However, such studies might be applicable when new delivery systems are employed or when the vaccine contains novel adjuvants or excipients". As Hexaxim is an aluminium hydroxide adjuvanted vaccine for intramuscular (IM) injection and contains an established amount of active drug substances, it was found acceptable that the applicant did not conduct pharmacokinetic (PK) studies during the clinical development of Hexaxim.

2.4.3. Pharmacodynamics

Hexaxim is adjuvanted with an established adjuvant, aluminium hydroxide, which enhances the immune response. The quantity of aluminium within Hexaxim (600 µg Al+3/0.5 ml dose) does not exceed that of other marketed vaccines, which may contain up to 1.25 mg per dose in accordance with European Pharmacopoeia monograph 0153 requirements.

According to available literature, antigenuria has been detected in some instances following receipt of a vaccine containing Hib antigen. The only clinical implication is that urine antigen detection may not have

diagnostic value in suspected cases of Hib disease occurring within 2 weeks of immunization. No specific evaluation has been performed for the Hexaxim file as this finding has no clinical significance.

The pharmacological profile of Hexaxim is represented by its immunogenicity profile evaluated in the clinical trials submitted. No dose-response effect study has been generated through this program as knowledge for dosing of almost all the antigens constituting Hexaxim is well established through the clinical and post-marketing experiences with Pentaxim.

No dose-finding study was performed for the new Hep B antigen. Hep B containing vaccines are usually formulated to contain 3 to 40 µg of rHBsAg per millilitre, and for the infant/toddler targeted vaccines their content ranges from 1.5 to 10 µg per dose.

Dose response studies and randomized comparative trials between 2 yeast-derived rHBsAg vaccines reported in the literature have shown repeatedly that a dose of 10 µg of rHBsAg is the optimal antigen content to use for the infant and toddler vaccines. In addition, for all Hep B valence containing combination vaccines evaluated in humans, the HBsAg, when used at the same content as with Hep B stand-alone vaccines, remains sufficiently immunogenic to elicit protective levels of anti-Hep B antibodies.

2.5. Clinical efficacy

The applicant claims consistency of the clinical development programme with the WHO recommendations and covers different primary vaccination schedules including EPI schedule as well as booster vaccination and concomitant use studies (MMRV and Prevenar). Additionally, the difference of vaccine efficacy with as well as without Hepatitis B birth dose has been tested. Concomitant use together with Meningococcus vaccine, Rotavirus vaccine (study is on-going) or the additional application of HB IG has not been evaluated.

Studies have been conducted in countries of all continents and covering all major ethnicities (Hispanic, Asian, African and Caucasian).

For control acellular as well as whole cell Pertussis vaccines have been used for the Hepatitis B component stand-alone as well as combination vaccines containing Hepatitis B. For the polio-component control vaccines included inactivated as well as live-Polio vaccines.

There are no formal efficacy studies, and all studies evaluating efficacy use established immunogenicity correlates or surrogates of protection.

In the primary vaccination studies base-line blood draws were only made for the assessment of antigens specified in the primary (or secondary) endpoints but all booster studies have pre-vaccination blood draws.

Two studies are either still on-going or have been finalised recently: A3L24 (lot-to-lot consistency and optimized excipient formulation and coadministration with rotavirus and PCV7) and A3L26 (long-term antibody follow-up to 3.5 and 4.5 years of age); no data from either study was provided for the initial assessment. Both were considered confirmatory and not expected to change either immunogenicity or safety profile of Hexaxim. As part of the answers to the D120 LoQ the applicant supplied preliminary data for study A3L24 to support the concomitant use claim for Rotarix and Prevenar and preliminary data for the study A3L26 long term antibody follow-up to 3.5 years of age. The CHMP recommended awaiting the final data (especially for safety reasons) and filing the data from study A3L24 as a variation to claim the concomitant use.

2.5.1. Dose response studies

No formal dose response studies have been made as most valences in the vaccine are identical to other licensed multivalent vaccines by this company. Only HepB and PRP have been increased (PRP) or newly formulated (HepB).

2.5.2. Main studies

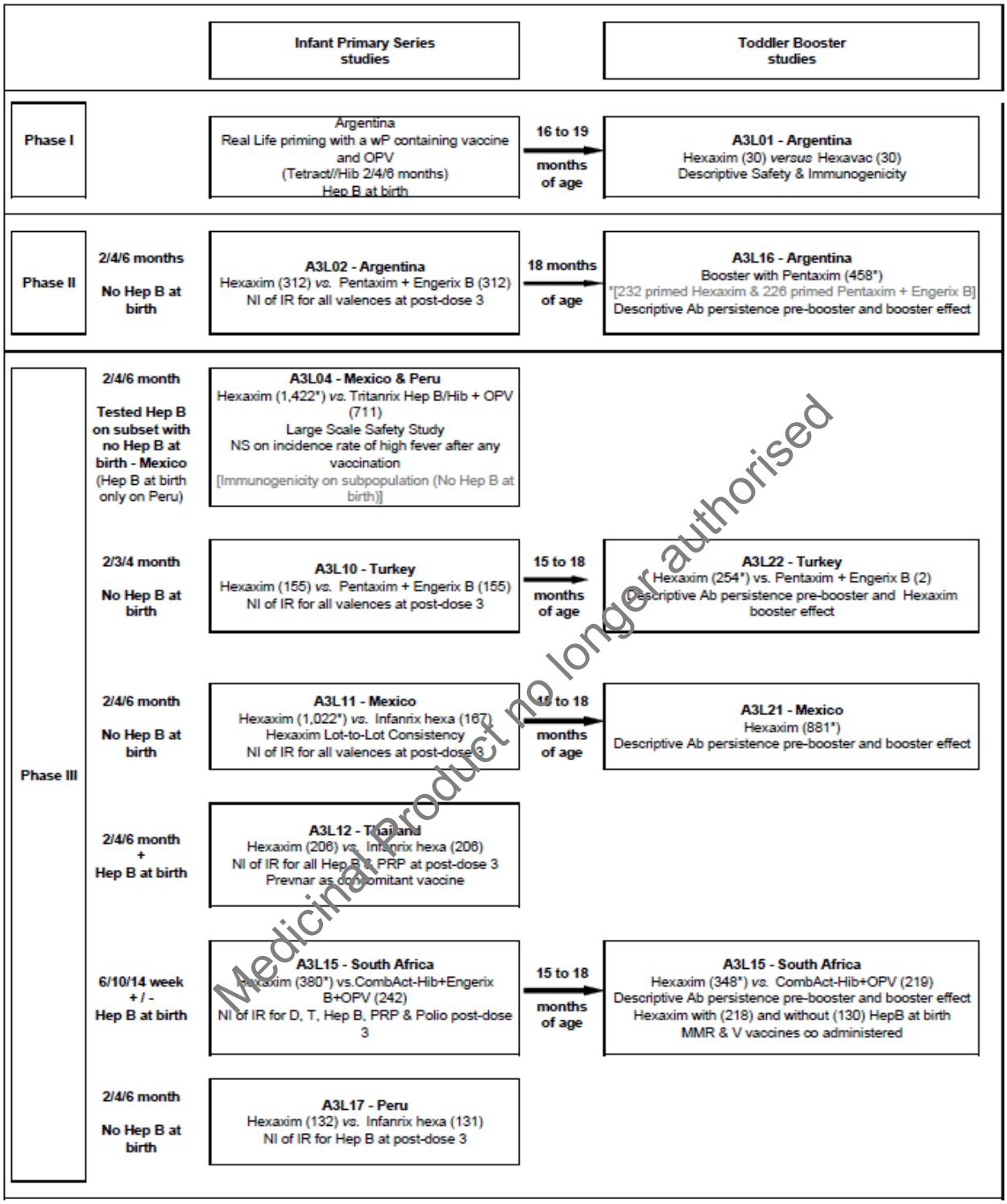
In the figure and table below all 12 studies submitted for this application are presented. All together 3424 infants received 3 doses in the primary series and 1511 toddlers received a booster dose. Different immunization schedules and different vaccines for comparison have been used; in some studies subjects had received an additional HepB dose at birth. In some studies BCG was given according to local standards.

Table 6 Schematic Overview of the Clinical Development Plan of Hexaxim

Clinical study	Primary series					Booster		
	IMMUNOGENICITY				SAFETY	IMMUNOGENICITY		SAFETY
	Dosing schedule	Non-inferiority	Lot to lot consistency	Co-adm.		Dosing schedule	Co-adm.	
A3L01	NA				NA	√ 16-19 m n=30	NA	√ n=30
A3L02/A3L16	2, 4, 6 m No Hep B at birth	√ n=260	NA	NA	√ n=312	NA (Pentaxim booster only)		NA
A3L11/A3L21	2, 4, 6 m No Hep B at birth	√ n=695	√ n=695	NA	√ n=1022	√ 15-18 m n=177*	NA	√ n=881
A3L17	2, 4, 6 m No Hep B at birth	√ n=132	NA	NA	√ n=132	NA		NA
A3L12	2, 4, 6 m + Hep B at birth	√ n=189	NA	n=189 Prevenar	√ n=206	NA		NA
A3L04	2, 4, 6 m No Hep B at birth	√ n=183	NA	NA	√ n=1423 (Non-superiority) + / - Hep B at birth	NA		NA
A3L10/A3L22	2, 3, 4 m No Hep B at birth	√ n=145	NA	NA	√ n=155	√ 15-18 m n=114*	NA	√ n=252
A3L15	6, 10, 14 weeks + / - Hep B at birth	√ n=220	NA	N.A	√ n=380	√ 15-18 m n=320	√ n=320 MMR & V	√ n=348
TOTAL	-	1844	695	189	3630	641	320	1511†

Immunogenicity data: PP; Safety data: SaTAS √ parameter studied; * Hexaxim primary series group boosted with Hexaxim; † or 1276 if excluded subjects primed with control vaccine during primary series and boosted with Hexaxim

Figure 1 Summary of Hexaxim clinical development Plan



Nb of subjects are presented on ITT: NI : Non inferiority; NS : Non superiority; IR : Immunoresponse

Seven studies (4 primary, 3 booster) were conducted in Hispanic infants and toddlers. 2 studies each (1 primary and 1 consecutive booster study) were conducted in African and Caucasian infants and toddlers. One study was conducted in Asian infants (primary vaccination).

The new drug substance HBsAg (produced in Hansenula polymorpha yeast) has been tested in a monovalent investigational vaccine in two Phase III studies (PAL 02 and PAL03, 10 µg for adolescent and

20µg/dose 16 to 45 years of age). These studies were randomized, comparative, blind-observer designs: one in Argentina (344 participants aged 10–15 years) and one in Uruguay (344 participants aged 16–45 years). The clinical results of these studies confirmed the safety and immunogenicity profiles of the new stand-alone Hep B antigen using both dose schedules. Even if no study reports are available for these two studies, taking into account the studies with the multivalent candidate vaccine already, they were not considered relevant for the assessment of Hexaxim.

The studies included in this application consist of the following:

Primary vaccination studies

Phase II:

- Study **A3L02** in Argentina also uses acellular Pertussis and inactivated Polio components in the comparator. No BCG was given at birth. Corresponding booster study**: A3L16.**

  Non-inferiority for all valences*.*

Phase III:

- Study **A3L04** contains one study arm with infants that have been vaccinated against Hepatitis B at birth (Peru sites only). Here again OPV is used in the comparator group. This is the largest study with safety as primary objective.

  Descriptive immunological results for HepB in subset (no HepB at birth) only.

- Study **A3L10** is the only European study. It uses acellular Pertussis and inactivated Polio components in the comparator. BCG vaccination at birth was allowed. Corresponding booster study: **A3L22.**

  Regarding primary vaccination: Non-inferiority for HepB, descriptive immunological results for all other valences.

  - Study **A3L11** assessed consistency in the production of Hexaxim. Here, three batches were tested against the comparator Infanrix hexa (acellular Pertussis and inactivated Polio components). BCG vaccination at birth had been given. Corresponding booster study: **A3L21.**

    Regarding primary vaccination: Non-inferiority for D, equivalence testing for3 batches descriptive for all antigens.

- Study **A3L12** aimed to assess the concomitant use of Hexaxim with Prevenar 7. It used the comparator Infanrix hexa (acellular Pertussis and inactivated Polio components). The impact of the concomitant use on the Prevenar serotypes was not assessed.

  Non-inferiority for HepB and PRP, descriptive immunological results for all other valences except Prevenar-serotypes.

- Study **A3L15*ps*** uses OPV and a whole-cell Pertussis containing vaccine as a comparator to Hexaxim. It is the only study in Africa. BCG vaccination at birth had been given. Corresponding booster study: **A3L15*bo.***

  Regarding primary vaccination: Non-inferiority for D, T, HepB, PRP + Polio, descriptive immunological results for FHA and PT.

  - Study **A3L17** assessed the immunogenicity and safety of Hexaxim close to the end of shelf-life. Additionally, the immunological effect of the local practice, to vaccinate pregnant women against Diphtheria and Tetanus, on infants in Peru is looked at. The comparator Infanrix hexa (acellular Pertussis and inactivated Polio components) is used. BCG vaccination at birth had been given.

Non-inferiority for HepB only, descriptive immunological results for D and PRP.

Booster studies

Phase I

- In **A3L01**, is a small study (Phase I), where a booster of Hexaxim has been compared to a booster of Hexavac.

  Descriptive immunological results for all valences pre- and post-booster.

Phase II

- In study **A3L16**, follow-up study of A3L02 (Hexaxim vs. Pentaxim +Engerix), the booster was Pentaxim.

  Descriptive immunological results for all valences pre- and post-booster.

Phase III

- In study **A3L22** it has been evaluated whether a booster with Hexaxim is similarly immunogenic even if the priming has been done with Pentaxim plus Engerix.

  Descriptive immunological results for all valences pre- and post-booster.

- In **A3L21** it has been evaluated whether a booster with Hexaxim is immunogenic even if the priming has been done with Infanrix hexa.

  Descriptive immunological results for all valences pre- and post-booster.

- In **A3L15** 4 doses of Hexaxim have been compared to 4 doses of CombActHib + 3 doses of Engerix (no Engerix booster in the second year of life). Concomitant use of MMRV.

  Descriptive immunological results for all valences pre- and post-booster.

Immunogenicity was used as a primary endpoint in all 12 studies, except for A3L04 (safety study).

Seven studies have been performed in healthy infants (priming) and 5 in healthy toddlers (booster studies).

It was the aim of the development programme to compare Hexaxim to currently licensed vaccines (Infanrix hexa, Pentaxim, Hexavac, CombAct-Hib, Tritanrix-HepB/Hib and Engerix B, OPV) with different primary vaccination schedules. Primary studies A3L15 and A3L10 used the most condensed vaccinations schedules (EPI and 2,3 and 4 months), which is optimal for accelerated disease control. In all other studies subjects have been vaccinated at month 2, 4 and 6, which has advantages regarding development of immunogenic responses. Additionally a booster in the second year of life (15-19 months), the effect of a hepatitis B vaccination at birth and the co-administration with PCV7 has been evaluated.

In general the used assays, thresholds of protection and methods to explore the new antigens HepB and PRP (higher amount) were considered acceptable. The concordance of both anti-D assays and both HepB assays were shown.

The study using Prevenar concomitantly (A3L12) did not evaluate a possible interference to the immunogenicity of the Prevenar serotypes'.

The Assays used in the studies of this application were as follows:

- **Diphtheria**
  - Micrometabolic Inhibition Test using Vero cells and a pH indicator for development (MITpH)

- Micrometabolic Inhibition Test using Vero cells and a crystal violet stain for development (MIT-CV)

- **Tetanus**
  - ELISA
- **Pertussis**
  - Pertussis toxin (PT) and FHA ELISAs
- **Poliovirus**
  - Micrometabolic Inhibition Test using wild type poliovirus and Vero cells (MIT-WT)
  - Micrometabolic Inhibition Test using Sabin poliovirus strains and HEp2 cells (MIT-Sa)
- **Hepatitis B**
  - Radioimmunoassay (RIA)
  - anti-HBs ECi
- **Haemophilus influenzae type b**
  - Polyribosylribitol Phosphate (PRP) RIA
  - PRP ELISA
- **Measles, Mumps, Rubella, and Varicella (MMR and V)**
  - anti-measles IgG ELISA
  - anti-mumps IgG ELISA
  - anti-rubella IgG ELISA
  - anti-varicella IgG ELISA
  - Anti-measles and anti-mumps Plaque Reduction Neutralization Test (PRNT)
  - Varicella-Zoster Virus Fluorescent Antibody to Membrane Antigen (FAMA) Assay

Table 7 Correlates of protection and surrogates for protection used in the studies

Antigen	Antibody titre as level of protection	Priority
Diphtheria	≥0,01 IU/ml (short-term) ≥0,1 IU/ml (long-term)	Established correlate
Tetanus	≥0,01 IU/ml (short-term) ≥0,1 IU/ml (long-term)	Established correlate
Polio 1,2,3	≥8 (1/dil)	Established correlate
PRP (Hib)	≥0,15 µg/ml (short-term) ≥1µg/ml (long-term)	Established correlate
Hepatitis B	≥10 IU/ml ≥100 IU/ml	Established correlate
PT, FHA (Pertussis)	≥4 fold titer increase from baseline to post dose 3	Accepted surrogate
Measles	≥ 300 mIU/ml Anti-measles Neutralizing Ab titer ≥ 120mIU/ml	accepted surrogate≥ 120mIU/ml
Mumps	≥500 U/ml by ELISA or Neutralization ≥ 60 l/dil	Not defined
Rubella	≥10 mIU/ml	accepted surrogate
Varicella	≥300 mIU/ml ≥4 l/dil (FAMA)	Accepted surrogate ≥1/64 dilution; ≥5 IU/ml

The use of accepted correlates of protection was considered appropriate.

Main inclusion criteria used in the studies:

- The child had to be of the age defined by the vaccination scheme, term born and healthy
- Informed consent signed by legal guardian and independent witness if illiterate guardian
- Able to attend all visits of the study and comply with procedures of the study

Main exclusion criteria used in the studies:

- Current or planned participation in another clinical trial during the respective study's time
- Suspected/proven immunodeficiency, chronical illness, HepB or C infection or other severe health affliction (including thrombocytopenia and bleeding disorders or seizures)
- Known hypersensitivity to any of the antigens present in Hexaxim or to any of the excipients
- Specified SAEs after prior use of similar vaccines (e.g. encephalopathy after pertussis vaccination, hypotonic-hyporesponsive episode or afebrile seizures after any previous vaccination)
- Use of blood or blood derived products
- Use of other vaccines with similar content as Hexaxim prior to study or planned application of other vaccines during the study time
- History of infection with pertussis, tetanus, diphtheria, poliomyelitis, Hib or HepB

- Fever and acute illness at time of inclusion (usually a temporary contraindication)

The inclusion and exclusion criteria used are commonly used in vaccine trials and standard of care and commonly used in clinical trial in the EU.

All studies discouraged the prophylactic use of antipyretics.

The statistic considerations of the studies:

- Sample size

The method described by Farrington Manning was used in the primary series studies (except study A3L11) for immunological parameter to determine the sample size for non-inferiority with regard to the difference in proportion of seroprotected / seroconverted subjects. Pre-defined non-inferiority margins were applied (HBs, diphtheria, tetanus, PRP: 10%, polio: 5%, PT, FHA: 10%). The sample size was calculated applying a (one-sided) type I error of .025 in order to achieve a global power of about 90% with regard to the primary immunological parameters in the different studies. In study A3L11 simulation was applied for sample size calculation.

For safety study A3L04 sample size was calculated according the method by Blackwelder in order to assess whether the DTaP-IPV-HepB-PRP-T-vaccine is non-inferior to the comparator with respect to the risk of severe fever following vaccination. No formal sample size calculation was done for the booster studies. The methods applied for sample size calculation are comprehensible.

- Randomisation

Permuted block randomisation was used in the primary series studies.

The method applied for randomisation is considered acceptable. However, specific information e.g. on block size was not included in the application.

- Blinding (masking)

All studies were performed open label. In some studies (e.g. A3L04, A3L11, A3L12, A3L17) endpoints were assessed by a blinded observe. It is acknowledged that blinding these vaccination studies was not feasible. The CHMP highlighted that safety assessment should have been done ideally by a blinded observer in all trials in order to minimise a possible assessment bias.

- Statistical methods

With regard to the primary immunological endpoints the aim of the trials (except A3L11) was to assess whether DTaP-IPV-HepB-PRP-T was non-inferior to the corresponding control. Non-inferiority with regard to a specific immunological endpoint was to be concluded if the if the lower limit of the two-sided 95% confidence interval for the difference in seroprotection / seroconversion rates between DTaP-IPV-HepB-PRP-T and control was above -0.1 (anti-Hep Bs, anti-diphtheria, anti-tetanus, anti-PRP,PT/FHA) and -0.05 IPV (parameter) respectively. The trials were considered successful if non-inferiority could be shown for all primary immunological endpoints simultaneously. Lot-to-lot consistency in study A3L11 was concluded if all 90% CI for the pair wise differences in seroprotection / seroconversion rates (between the 3 lots) for all primary valences were within the pre-specified equivalence ranges. The Wilson-score method without continuity correction was used to calculate confidence intervals for the difference of proportions.

Secondary immunological endpoints were analysed descriptively by means of appropriate statistical characteristics (e.g. continuous data: GMT including 95%-CI; categorical data: absolute and relative frequencies including 95%-CI).

The non-inferiority of DTaP-IPV-HepB-PRP-T to the comparator with regard to the risk of severe fever was to be concluded if the upper limit of the 95% CI for the relative risk of severe fever was below 3.

Descriptive analyses were used to analyse the primary series and booster studies.

In general the statistical analyses method applied were considered acceptable.

Primary vaccination studies:

Most primary vaccination studies assess safety and immunogenicity of the vaccination scheme 2, 4 and 6 months of age (A3L04, A3L11, A3L12 and A3L17). One study each assessed the EPI – 6, 10, and 14 weeks – (A3L15ps) and the "accelerated" vaccination scheme -2, 3, and 4 months (A3L10).

Additionally, the studies have different focuses or specialities:

Study A3L15ps (6,10,14 weeks of age)

This study assessed the most condensed schedule which is recommended in the Republic of South Africa (RSA):

"Immunogenicity Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to CombAct Hib Concomitantly Administered with Engerix B Paediatric and OPV at 6, 10, and 14 Weeks of Age in South African Infants"

For the time being a HepB dose at birth is not recommended in RSA. Nevertheless, the study included a third arm where this has been assessed. All Ag contained in Hexaxim were tested for non-inferiority.

Methods

This study has been conducted in 715 South African Infants as a PIII multicentre trial following the EPI schedule. A monovalent hepatitis B vaccine (Engerix B) had been given at birth.

This study part consists of visits 0 – 6 (safety 6 months after last vaccination and measles vaccination). The booster dose part of the study is described further in the respective section further below (Study A3L15bo).

Study subjects had to be healthy (mothers sero-negative for HIV), full-term born infants. All infants had already received one dose of BCG at 0-3 days of age.

Study Participants

The ITT population consists of 622 subjects. There was a comparably high amount of drop-outs between the two allocation steps.

Treatments

All subjects were to receive one dose of the investigational or reference vaccines at 6, 10, and 14 weeks of age. In addition, subjects in Group 3 were to receive one dose of Engerix B Paediatric vaccine at birth.

Objectives

Primary objective: Non-inferiority of immune response against tetravalent wP combined vaccine (CombActHib) + OPV + Engerix B one month after the three-dose primary vaccination for D, T, polio, Hep B and PRP.

Secondary objective: To describe in each group the immunogenicity parameters for each primary series vaccine component 1 month after the third dose of the primary series.

Overall, non-inferiority is analysed versus commonly used products in this area and schedule. This induces the difference for the pertussis components: Hexaxim uses acellular Pertussis antigens whilst the comparator uses a whole-cell formulation. For Polio non-inferiority is analysed for an inactivated (IPV-component) versus a live vaccine (OPV). It is feasible for the intended indication to prove the appropriateness of the new vaccine against established components.

Outcomes/endpoints

Primary serological endpoints 1 month after the third dose of the primary series (i.e. at 18 weeks of age) with seroprotection being defined as:

- Anti-T antibody (Ab) titres ≥0.01 International Unit (IU)/ml
- Anti-D Ab titres ≥0.01 IU/ml
- Anti-Hep B Ab titres ≥10 mIU/ml
- Anti-PRP Ab titres ≥0.15 µg/ml
- Anti-polio 1, 2, and 3 Ab titres ≥8 (1/dil)

The differences in seroprotection rates between Group 1 (DTaP-IPV-Hep B-PRP-T group, without Hep B at birth) and Group 2 (CombAct-Hib +Engerix B Paediatric and OPV group, without Hep B at birth) were calculated (Group 1 – Group 2). The clinically relevant limit for non-inferiority was –10% for the D, T, Hep B, and PRP antigens and 5% for the polio antigens. The statistical method was based on the lower bound of the two-sided 95% confidence interval (CI) of the difference between the seroprotection rates.

Secondary endpoints were Anti-T, anti-D Ab, Anti-HBsAg Ab, Anti-PRP Ab, Anti-pertussis toxoid (PT), anti-filamentous haemagglutinin (anti-FHA) Ab and Anti-polio 1, 2, and 3 Ab titres including different cut-off levels than those considered for the primary endpoints.

As the comparator used included a whole-cell formulation of Pertussis non-inferiority of the immune response for the aP formulation included in Hexaxim would not have been feasible. A descriptive analysis for Pertussis is included in the secondary endpoints, which was also acceptable.

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above

Results

Participant flow

Of the 715 subjects initially randomized, 93 withdrew prior to group allocation. Thus, the ITT consists of 622 subjects. All subjects are accounted for.

Recruitment

A two-step subject allocation to the different groups was used. This was followed by vaccination at defined ages of the subjects and a blood-draw-visit one month after the third vaccination. All subjects were followed-up for safety 6 months after the last primary vaccination.

Conduct of the study

The following amendments were made and approved by IECs and MCC:

- An increase in the sample size (to compensate for an unexpectedly high drop-out rate between V01 and V02), and an increase in the expected attrition rate from 10% to 20%.
- The time of BL storage and clotting was amended according to new sample preparation procedures, and 'height' was removed from demographic characteristics recorded at V01.
- The addition of MMR and varicella vaccinations at 15 to 18 months of age, and a change in the timing of the booster dose to 15 to 18 months
- Amendment to the ICF, and addition of inclusion criteria for booster phase (namely, signing of ICF addendum, plus subject's age)
- The collection of information on injection site events / reactions for the MMR and varicella vaccines during the booster phase, and addition of extensive limb swelling after the booster vaccination as a solicited AE
- Clarification of the relevant vaccine for each immunogenicity endpoint, and the analyses to be performed
- The addition of a secondary endpoint to allow the optimal analysis of immunogenicity results from aP components constituting the investigational vaccine
- Anti-polio Ab titres assay changed from Hep2 cell culture to mammalian cell culture
- Anti-PRP Ab titres assay changed from enzyme immunoassay (EIA) to RIA, and the LOQ changed from 0.065 µg/ml to 0.06 µg/ml
- The addition of "Subjects present at V01" or "Subjects present at V02" as a study population defined for statistical analysis
- The addition of five further protocol violation criteria for the PP Analysis Set (three for the primary series and two for the booster series): "no definite contraindication present at the time of vaccination with any dose and no development of a relevant exclusion criterion that may affect immunogenicity assessment during the entire trial period", "6 weeks of age (42 to 49 days old) at V02", and "BL2-V05 (D126) drawn or with any measurement available" for the primary series; and "BL2-V05 (D570) drawn or with any measurement available" and "no contraindications to the study vaccine Nos. 3 to 7, no contraindications to MMR Nos. 2 to 5, and no contraindications to varicella Nos. 2 to 5" for the booster phase. The following violation criterion: "Use of vaccine declared not usable due to cold chain break" was also used for the booster phase.
- Update on the assessment method for testing Haemophilus influenzae antigen (PRP). The ELISA technique was replaced by RIA.

Baseline data

In the ITT Analysis Set, the mean age was similar in all groups and there was a similar distribution of males and females in each group. The same results were observed in the PP Analysis Set. The majority of subjects were black. The groups were still considered comparable despite the high number of drop-outs.

Numbers analysed

In study A3L15 622 subjects have been randomized to three different groups. For the exact allocation see Table below

Table 8

	Hexaxim (N= 243)	CombAct-Hib + Engerix B + OPV (N= 242)	Hexaxim with Engerix B at birth (N= 137)
Sex			
M	243	242	137
Male: n (%)	112 (46.1)	124 (51.2)	69 (50.4)
Female: n (%)	131 (53.9)	118 (48.8)	68 (49.6)
Ethnic origin			
M	243	242	137
Asian: n (%)	1 (0.412)	2 (0.826)	1 (0.730)
Black: n (%)	239 (98.4)	238 (98.3)	136 (99.3)
Caucasian: n (%)	1 (0.412)	1 (0.413)	0 (0)
Hispanic: n (%)	0 (0)	0 (0)	0 (0)
Other: n (%)	2 (0.823)	1 (0.413)	0 (0)
Age (weeks) at first dose			
M	243	242	137
Mean (SD)	6.26 (0.231)	6.27 (0.243)	6.27 (0.235)
Minimum; Maximum	5.57; 7.14	5.43; 7.14	5.71; 7.14

N: number of subjects analyzed according to the ITT Analysis Set
M: number of subjects with available data for this characteristic
n: number of subjects
%: percentages are calculated according to the number of subjects with available data for the characteristic

Outcomes and estimation of A3L15

The thresholds defined for long-time immunogenicity are reached for all antigens in the majority of cases (for tabulated results, please see the respective table in section "Summary of main studies" below). Significantly more subjects achieved very high titres for anti-D in both Hexaxim groups. Anti-T shows no significant difference to the comparator vaccine. The lower GMT of Hexaxim for anti PRP - is seen here as well in the lower number of subjects with long-term protective titres.

All primary endpoints concerning non-inferiority were met: Hexaxim was shown to be non-inferior compared to priming with CombAct-Hib +Engerix+OPV for D, T, PRP, HepB and Polio.

D-, T- and Pertussis antibodies were considered satisfactory for Hexaxim and for D the correlate for long-term protection (≥0,1 IU/ml) is achieved by more than twice the subjects than those who had been given CombActHib

Anti-PRP (Hib) GMTs are lower for Hexaxim subjects but the non-inferiority criterion would even have been met if δ had been halved. Thus, the results for this antigen are acceptable as well.

Reverse cumulative distribution curves (RCDCs) show only a marginal effect of the birth HepB dose on antibody titres against D, T, PRP and PT, FHA.

However, there is, as expected, a clear effect of the birth Hep B dose (Engerix B) on the titer of HepB-antibodies (GMT: 330 for group 1 vs. 1319 for group 3). The specific effect of a HepB dose given at birth is particularly explicit when considering seroprotection rates with a threshold of ≥ 100mIU/ml. Regarding this

threshold 78.8% of subjects were protected after priming with three doses of Hexaxim when no HepB birth dose has been given. If HepB was administered at birth 96.9% of subjects were seroprotected after priming with Hexaxim. However, at the ≥10mIIU/ml level, which is an established correlate of protection against HepB, 95.7% of subjects without a HepB dose at birth were seroprotected.

Anti-Polio GMTs post vaccination for all three types were significantly higher than needed for protection (approximately between 500 and 1000 MN-1/dil after use of Hexaxim). Based on the seroprotection rate (≥8 1/dil) 1 month after the third vaccination Hexaxim was shown to be non-inferior to the control vaccines

In general, GMTs to poliovirus types 1, 2 and 3 were higher in the Hexaxim group (1) compared to the CombAct-Hib + Engerix B + OPV group (2) demonstrating better immunogenicity of IPV compared with OPV

Study A3L10 (2,3,4 months schedule)

This Phase III (mono-centre, open-label, randomized, active-control) trial was conducted in order to evaluate immunogenicity and safety of Hexaxim compared to Pentaxim (DTaP-IPV/Hib) plus Engerix-B Pediatrico in 310 infants. It is also (together with the corresponding booster study A3L22) the only study in Europe:

"Immunogenicity of DTaP-IPV-Hep B-PRP-T Combined Vaccine Compared with PENTAXIM and ENGERIX B at 2-3-4 Months Primary Schedule in Healthy Turkish Infants"

The primary objective of this study focused on anti-Hep B immunogenicity responses and the secondary objective on the safety of this combined formulation.

The booster study for this vaccination scheme is Study A3L22 described further below.

This is the only study conducted in the EU.

Methods

Study Participants

This study has been conducted in one centre in 310 infants in Turkey using a 2, 3, 4 months schedule. Two blood draws were made (baseline and one month after the last vaccination). Safety follow-up was 6 months after last vaccination. BCG vaccination at birth was allowed.

Treatments

3 doses of Hexaxim or Pentaxim+ Engerix B were given.

Objectives

Primary Objective is the non-inferiority of the Hep B antigen of Hexaxim compared to the combination Pentaxim + Engerix B one month after vaccination.

Secondary objective is the description of the other antigens' immunogenicity.

Outcomes/endpoints

Primary endpoint:

- Anti-Hep B surface antigen antibody (HBsAg Ab) titres ≥10 mIU/ml assessed at Day 90 (D90; 1 month after the third dose of the primary series).

The primary parameter was the difference in seroprotection rate in Hep B antigen (HBsAg) between the two groups (DTaP-IPV-Hep B-PRP-T and PENTAXIM + ENGERIX B). The clinically relevant limit for non-inferiority was 10%. The statistical method was based on the lower bound of the 95% two-sided confidence interval (CI) of the difference in the seroprotection rate between the two groups.

Secondary endpoints were Anti-T, anti-D Ab, Anti-Hep Bs Ab, Anti-PRP Ab, Anti-pertussis toxoid (PT), anti-filamentous haemagglutinin (anti-FHA) Ab and Anti-polio 1, 2, and 3 Ab titres including different cut-off levels than those considered for the primary endpoints.

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above

Results of A3L10

Participant flow

302 of 310 subjects completed the study. All subjects are accounted of.

Conduct of the study

No relevant changes were made to the protocol.

Baseline data

Both groups are comparable.

Numbers analysed

Table 9 Subject Disposition for Immunogenicity Analyses According to Randomization - Full Analysis Set and Per Protocol Set; A3L10

	DTaP-IPV-Hep B-PRP-T (N=155)		PENTAXIM™ and ENGERIX B® PEDIATRIC (N=155)		Total Randomized (N=310)	
	n	%	n	%	n	%
Full Analysis Set	155		155		310	
Per Protocol Analysis Set	145	93.5	141	91.0	286	92.3
Subjects excluded from the PP Analysis Set	10	6.5	14	9.0	24	7.7

N: number of subjects analyzed according to Full Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in Full Analysis Set; Subjects could be excluded for more than one reason;

In this study a double-blind design was not possible as there were two injections in Group 2 but only one in Group 1.

Outcomes and estimation of A3L10

The seroprotection rates to anti-Hep B elicited by Hexaxim fulfilled the statistical criteria of non-inferiority to Pentaxim+Engerix one month after priming.

The results of the secondary objectives are presented below:

Anti-diphtheria and anti-tetanus antibody responses

At the ≥ 0.01 IU/ml level, seroprotection rates were similar for both groups for D and T antigens. At the ≥ 0.1 IU/ml level, Ab titres were similarly high in both groups for T (≥ 98.6%), but tended to be lower in the Hexaxim group for D. GMTs were similar in both groups for both D and T.

Anti-PT and anti-FHA antibody responses

Both seroconversion rates and vaccine responses for PT and FHA were similar in both groups. For PT, GMTs were similar in both groups; for FHA, they were higher in the Hexaxim group than in the Pentaxim+Engerix B group.

Anti-poliovirus antibody responses

The majority of subjects in both groups (94.0%–100%) had titres ≥ 8 (1/dil) for all poliovirus. GMTs were similar in both groups.

Anti-Hep B antibody responses

GMTs were lower in the Hexaxim group than in the Pentaxim+Engerix B group; however, as high seroprotection rates were achieved at the ≥ 10 mIU/ml level, there is no clinical significance to the difference observed for GMTs.

Non-inferiority of Hexaxim was shown for Hepatitis B

Anti-PRP antibody responses

Seroprotection rates (titres ≥ 0.15 µg/ml) for Hexaxim were high (≥ 90.7%) but tended to be lower than those for Pentaxim+Engerix B. GMTs were similar in both groups. The data confirmed the similarity of both vaccines in terms of antibody thresholds (correlate/surrogates of protection).

Overall, seroconversion/seroprotection rates of all antigens were similar between both groups. As seen in study A3L15 the PRP seroprotection rate for Hexaxim is slightly (but not significantly) lower than for the comparator, GMT rates are comparable.

Anti poliovirus response rates measured with the MIT-SA assay in this study are more than 2 dilution steps lower compared to the MIT-WT assay used in all other studies. However, as response rates by far exceed the minimum protection threshold this finding has no clinical relevance. Sufficient seroprotection rates for all three Polio-types have been reached in both vaccination groups (94-100%).

Regarding HepB, one month after the third vaccination, similar percentages of subjects acquired seroprotection (threshold ≥10mIU/ml); the statistical criterion for non-inferiority of Hexaxim compared to Pentaxim+Engerix has been fulfilled. However, after administration of Engerix B (group 2) anti-Hep B GMTs were considerably higher than in the Hexaxim group (265 vs. 149, respectively). Likewise, the percentage of subjects with anti-HepB titres ≥100mIU/ml is clearly higher in the Engerix group compared to Hexaxim (78% vs. 65 %, respectively). This could have an influence on the duration of protection and should be followed up carefully.

Study A3L02 (2,4,6 months schedule)

In this trial the immunogenicity of Hexaxim in 624 infants born to HBsAg seronegative mothers was compared to one of the current standards in Argentina:

"Phase II Immunogenicity Study of a DTaP-IPV-HB-PRP-T Combined Vaccine Compared with PENTAXIM and Engerix B PEDIATRICO at 2, 4, and 6 Months of Age in Healthy Argentinean Infants"

This study was also powered to demonstrate non-inferiority of Hexaxim.

The booster study A3L16 following this study is described further below.

Methods

Study Participants

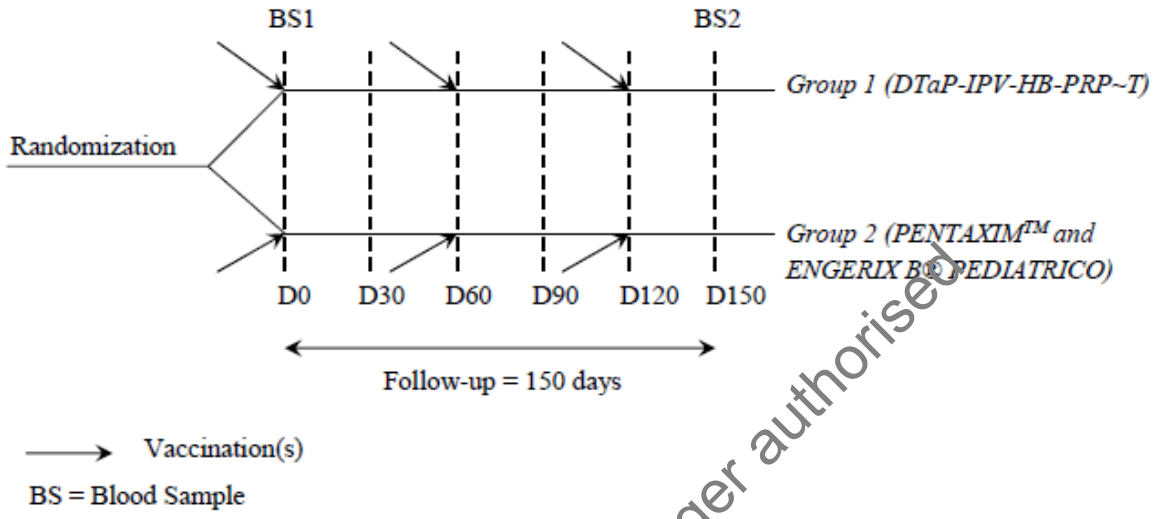
624 infants were vaccinated in a single centre in Argentina. The inclusion and exclusion criteria are similar to those of the other studies (healthy children). There was no BCG vaccination at birth.

There were two blood-draws (baseline and one month after last vaccination) and a safety follow-up of 1 month after the last vaccination.

Treatments

Three doses of Hexaxim or Pentaxim + Engerix B.

Figure 3 **Schedule of vaccination/Treatment and Specimen collection; A3L02 (Figure from study report**



Objectives

The primary objective was non-inferiority of all antigens of Hexaxim versus Pentaxim + Engerix B one month after the last vaccination. The secondary objective is the descriptive analysis of the antigens' immunogenicity.

Outcomes/endpoints

Primary endpoints :

- Anti-T and anti-D antibody (Ab) titres ≥0.01 IU/ml
- Anti-HBsAg Ab titres ≥10 mIU/ml)
- Anti-PRP Ab titres ≥0.15 µg/ml
- Anti-pertussis toxoid (PT) and anti-filamentous haemagglutinin (FHA) Ab titres 4-fold increase
- Anti-polio 1, 2, and 3 Ab titres ≥8 (1/dil)

Secondary endpoints were Anti-T, anti-D Ab, Anti-Hep Bs Ab, Anti-PRP Ab, Anti-pertussis toxoid (PT), anti-filamentous haemagglutinin (anti-FHA) Ab and Anti-polio 1, 2, and 3 Ab titres, including different cut-off levels than those considered for the primary endpoints90

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above

Results of A3L02

Participant flow

604 of 624 subjects completed the study. All subjects are accounted of.

Conduct of the study

No relevant changes were made to the protocol.

Baseline data

In the ITT Analysis Set, the mean age was similar in both groups, and there were similar distributions of males and females. All subjects in both groups were Caucasian. The same results were observed in the PP Analysis Set. Overall, the groups were comparable.

Numbers analysed

Out of 624 subjects who entered the trial 604 completed. 93 subjects were excluded from the PP Analysis Set due to protocol deviations.

Overall, only 260 subjects were included in the per protocol analysis set for the DTaP-IPV-Hep B-PRP-T group and so the planned number of 265 evaluable subjects was not met for this group. However, the conclusions based on the statistical analyses are considered to be valid.

Outcomes and estimation of A3L02

Similar percentages of subjects reached the established thresholds of protection for each antigen in both vaccination groups. GMTs for anti-T, anti-D, anti-PRP, anti-FHA and anti-PT neither show any significant differences between the two vaccine groups.

Overall, non-inferiority for all antigens of Hexaxim against Pentaxim +Engerix B was met.

The results of the secondary objectives are presented below:

Anti-diphtheria and anti-tetanus antibody responses

For diphtheria, 64.2% of subjects in the Hexaxim group and 67.9% of the subjects in the control group achieved the ≥ 0.1 IU/ml level. For tetanus, all subjects (100%) achieved the ≥ 0.1 IU/ml level. For T, GMTs were higher in the Hexaxim group than in the control group; for D, GMTs were similar in both groups.

Anti-PT and anti-FHA antibody responses

For PT, GMTs were lower in the Hexaxim group than in the control group; for FHA, they were higher in the Hexaxim group.

Anti-poliovirus antibody responses

GMTs for all poliovirus were similar in both groups, although they tended to be higher in the Pentaxim+Engerix B group than in the Hexaxim group for poliovirus 3.

Anti-Hep B antibody responses

GMTs were similarly high in both groups. Tends to be higher for Hexaxim

Anti-PRP antibody responses

GMTs were similar in both groups.

The GMTs and RCDCs for anti-T, anti-D, anti-PRP, anti-FHA and anti-PT were similar in both groups.

As in study A3L15, the anti-Hep Bs response (GMTs) at V06 (Day 150) was slightly higher in the Hexaxim group compared to Engerix B (RIA-Test: 1148 and 850 mIU/ml, respectively). In all other studies where Engerix B or Tritanrix-HepB/Hib was used as a comparator, GMTs were higher in the control groups compared to Hexaxim. 99.2% of subjects in group 1 (Hexaxim) versus 100% of subjects in group 2 (Engerix B) were seroprotected after the primary series.

Anti-Polio Type 3 GMTs were slightly lower in the Hexaxim group compared to Pentaxim + Engerix. Seroprotection rates were sufficient for all three Polio types (100% for all groups).

Study A3L04 (2,4,6 months schedule)

This study was conducted to generate a large number of safety data and focuses for immunogenicity on the Hepatitis B component of Hexaxim (in a subset). Here it aims to show non-inferiority against the established vaccine of both countries, Peru and Mexico, (Tritanrix-HepB/Hib) concomitantly given with OPV. A total of 2133 subjects were included in the trial, as planned: 1422 subjects were randomized to the Hexaxim group (which was further divided into three subgroups of 474 subjects who were to receive different batches), and 711 subjects were randomized to the Tritanrix-Hep B/Hib. + OPV group:

"Large Scale Safety Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine, in Comparison to Tritanrix-Hep B/Hib and OPV Administered at 2, 4, and 6 Months of Age in Latin American Infants"

Methods

Study Participants

In total, 2133 healthy infants were vaccinated in this multi centre study in Peru and Mexico. Safety follow-up after the last vaccination was 6 months. There were two blood draws to determine baseline titres and titres 1 month after the last vaccination for Hepatitis B antibodies. Inclusion and exclusion criteria are similar to those of the other studies. BCG vaccination had been given at birth. In Peru only Hepatitis B vaccine had been given at birth.

Treatments

Three doses of Hexaxim (three different batches) + Placebo-OPV (distilled water) or Tritanrix-HepB/Hib + OPV.

Tritanrix-Hep B/Hib contains the same valences as DTaP-IPV-Hep B-PRP-T (Hexaxim), with the exception of poliovirus (polio) types 1, 2, and 3.

Objectives

This is primarily a safety study. Still, for secondary objective the immune response concerning the HepB component is described in a subset (306 subjects) of participants.

Outcomes/endpoints

- Anti-hepatitis B surface (HBs) Ab titres and seroprotection (anti-Hep Bs ≥10 mIU/ml and anti-Hep Bs ≥100 mIU/ml) at Day 150.
- To perform descriptive analysis of the three batches of DTaP-IPV-Hep B-PRP-T vaccine and the control vaccines on the anti-Hep Bs Ab seroprotection rates and the geometric mean titer (GMT) at Day 150 (30 days after last vaccination)

In this study, the assessment of immunogenicity focuses on the Hepatitis B component of Hexaxim.

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above.

Results of A3L04

Participant flow

1998 of 2133 subjects completed the trial. All subjects are accounted of.

Conduct of the study

No relevant changes were made to the protocol.

Baseline data

In the ITT Analysis Set, for the subset of subjects, mean age was the same in both groups. There were more males than females in the Hexaxim group, and more females than males in the control group. The same results are observed in the PP Analysis Set. The study groups in both countries were otherwise comparable.

Numbers analysed

Table 10 Summary of Subjects Excluded From the PP Immunogenicity Analysis Set Due to Protocol Deviations; A3L04

	DTaP-IPV-Hep B-PRP-T n (%)	Tritanrix-Hep B/Hib™ + OPV n (%)	Total n (%)
ITT Immunogenicity Analysis Set, N	192	95	287
PP Immunogenicity Analysis Set, N	183 (95.3)	94 (98.9)	277 (96.5)
Subjects excluded from PP Immunogenicity Analysis Set:	9 (4.7)	1 (1.1)	10 (3.5)

Subjects could be excluded for more than one reason; N: number of subjects analyzed according to ITT or PP Immunogenicity Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in ITT Analysis Set for ITT Analysis Set data

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above.

Outcomes and estimation of A3L04

In the ITT Analysis Set, all subjects in the DTaP-IPV-Hep B-PRP-T and the Tritanrix-Hep B/Hib. + OPV groups met the ≥10 mIU/ml anti-Hep Bs threshold for seroprotection. Similar numbers in each group also met the ≥100 mIU/ml anti-Hep Bs threshold for seroprotection (96.2% and 98.9%, respectively). However, GMT titres in the DTaP-IPV-Hep B-PRP-T group were lower than in the Tritanrix-Hep B/Hib. + OPV group (for tabulated results, please see the respective table in section "Summary of main studies" below).

The Anti-Hep B GMTs were similar for all three batches. The proportion of subjects meeting the ≥10 mIU/ml anti-Hep Bs threshold for seroprotection was 100.0% for all three batches of Hexaxim.

This study's outline and conduct was considered adequate to compare the immunogenicity of the Hepatitis B component with Tritanrix-HepB/Hib. Comparing the immunogenicity of Hexaxim and Tritanrix-HepB/Hib, threefold higher GMTs for Tritanrix compared to the hexavalent candidate vaccine have been found (3364 vs. 1075, respectively); however, based on the anti-Hep Bs thresholds of 10 and 100 mIU/ml, sufficient seroprotection rates in both groups one month after the third vaccination were observed.

Study A3L11

The purpose of this trial was to provide clinical confirmation that the manufacturing process of the second Drug Product generation of the investigational DTaP-IPV-Hep B-PRP-T vaccine was consistent between three industrial scale batches, in terms of immunogenicity and safety.

In this four-arm Phase III study three manufacturing consistency lots of Hexaxim (Lot S4009, Lot S4106 and Lot S4107) were used and compared with one arm receiving Infanrix hexa:

"Lot-to-Lot Consistency Study of DTaP-IPV-Hep B-PRP-T Vaccine Administered at 2-4-6 Months of Age in Healthy Mexican Infants"

Immunogenicity was assessed at V06, 1 month after the third dose of the primary series.

Methods

Study Participants

1189 healthy infants were part of this multi centre study in Mexico.

Hep B vaccination at birth was an exclusion criterion. The other inclusion and exclusion criteria are similar to the other studies. Safety follow-up time was 6 months after the last vaccination. BCG vaccination had been given at birth. There were two blood-draws (baseline and one month after the last vaccination).

The booster study A3L21 following this study is described further below.

Treatments

The participants received either three doses of Hexaxim or Infanrix hexa.

Objectives

Primary objective of this study was to show equivalence of three batches of Hexaxim in terms of seroprotection rates and seroconversion rates (Pertussis) one month after the last vaccination.

Secondary objective was the description of the immune responses (all antigens) and to show non-inferiority against Infanrix hexa for anti-D only.

Outcomes/endpoints

Primary endpoints:

- Anti-T and anti-D antibody (Ab) titres ≥0.01 IU/ml
- Anti-HBsAg Ab titres ≥10 mIU/ml
- Anti-PRP Ab titres ≥0.15 µg/ml
- Anti-pertussis toxoid (PT) and anti-filamentous haemagglutinin (FHA) Ab titres 4-fold increase
- Anti-polio 1, 2, and 3 Ab titres ≥8 (1/dil)

Three paired equivalence tests on seroprotection/seroconversion rates according to the valence were performed 1 month after the third dose of the DTaP-IPV-Hep B-PRP-T vaccine in order to demonstrate consistency. Equivalence among the three batches would be demonstrated if the global null hypothesis for all valences is rejected (D, T, polio types 1, 2, and 3, Hep B, PRP, PT, and FHA). The statistical methodology was based on the use of the two-sided 90% confidence interval (CI) of the differences between pairs of batches for the seroprotection/seroconversion rates.

Secondary endpoints were Anti-T, anti-D Ab, Anti-HBsAg Ab, Anti-PRP Ab, Anti-PT, anti-FHA Ab and Anti-polio 1, 2, and 3 Ab titres including different cut-off levels than those considered for the primary endpoints. In addition, response to pertussis (PT, FHA) antigens defined as anti-PT or anti-FHA ≥4 EU/ml in initially seronegative infants, or at least persistence (post-titer ≥ pre titer) of the Ab titer in initially seropositive infants (titer ≥4 EU/ml) were included.

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above.

Results of A3L11

Participant flow

1056 of 1189 subjects completed the trial. All subjects are accounted of. The number of subjects per batch-group is comparable.

Conduct of the study

Some relevant changes were made to the protocol:

- Amendment 1 (Protocol Version 7.0, dated 02 August 2006) was produced mainly because the current practice in Mexico was to immunize pregnant women with vaccines containing T and D during pregnancy. Transmission of maternal anti-D Abs to the infant may influence the infants' immune response to the vaccination. Consequently, non-inferiority of anti-D seroprotection was added as a

secondary objective, and anti-D Ab titres above cut-off were added as secondary endpoints. Maternal vaccination history was also to be collected

- Prior to the full primary series analysis, a sequential analysis of immune responses to HBsAg and PRP antigens was performed on the whole population
- For indicative purposes, assessment of lot-to-lot consistency using 95% CIs of the difference in seroprotection/seroconversion rates between batches, 1 month after the third dose of the primary series
- Non-inferiority testing of pooled batches of DTaP-IPV-Hep B-PRP-T versus Infanrix hexa, using the 95% two-sided CI for the differences in anti-D seroprotection rates (defined by a titer ≥0.01 IU/ml), 1 month after a third dose of the primary series

Routine monitoring revealed a mistake with subject allocation for the first 22 subjects at one site. Due to using the wrong randomization list 15 subjects received the wrong vaccine and had to be excluded from the PP analysis set but are still included in the ITT and safety analysis.

Baseline data

In the ITT Analysis Set, the mean age was similar in both groups, and there was a similar distribution of males and females in each group. The same results were observed in the PP Analysis Set (All subjects in both groups were Hispanic. The four groups were considered comparable in terms of demographics.

Numbers analysed

A total of 1189 subjects were randomized and received a vaccine injection at V01. Therefore these subjects were included the ITT Analysis Set. Of these, 1022 subjects received the DTaP-IPV-Hep B-PRP-T vaccine (batch 1: 340 subjects, batch 2: 343 subjects, batch 3: 339 subjects), and a total of 167 subjects were randomized to receive the control product Infanrix hexa. The percentages and types of exclusion were similar in the different groups. 288 subjects per treatment group were specified in the protocol. Fewer subjects have been evaluable for the PP Immunogenicity Analysis Set.

Outcomes and estimation of A3L11

Based on 95% CIs, no differences between paired batches of Hexaxim were observed (for tabulated results, please see the respective table in section "Summary of main studies" below). Therefore, equivalence of the three Hexaxim batches was concluded based on the 95% CIs of the difference in seroprotection/seroconversion rates using the same margin (5% for polio, 10% for other valences).

Secondary objective included the demonstration of non-inferiority of pooled Hexaxim batches versus Infanrix hexa based on the anti-D seroprotection and the descriptive analysis of GMTs

Comparative immunogenicity (seroprotection/seroconversion) of three batches investigated show no significant differences and equivalence between the different Hexaxim batches was concluded for all valences. Despite the smaller number of analysed subjects, the endpoints were still met.

As a minor exception, some differences in anti-Hep B GMTs were observed between individual Hexaxim batches: batch 2 was associated to higher GMTs (1566) compared to batches 1 and 3 (935 and 1009, respectively), based on non-overlapping 95%CIs. However, the GMTs were sufficiently high for all batches and no relevant differences in seroprotection rates have been found. Consequently, differences reported in this batch to batch consistency study are not clinically relevant.

Comparing pooled batches, the seroprotection rate for Hepatitis B based on the ≥100 mIU/ml threshold criterion one month after the third dose is higher in the Infanrix hexa group (99.2%) compared to the Hexaxim group (91.7%). Likewise, anti-Hep B GMTs were higher in the Infanrix hexa group compared to Hexaxim (ITT: 1545 vs. 1044, respectively). This may have an influence on the duration of protection.

Anti-D seroprotection of Hexaxim vaccinated infants was non-inferior to that of Infanrix hexa vaccinated infants. The GMTs for anti-T, anti-D, anti-PRP, anti-FHA and anti-PT show similarity of Hexaxim and Infanrix hexa. The anti-PRP GMT is significantly better for the pooled Hexaxim groups. Seroprotection and seroconversion results are similar between the three lots of Hexaxim and Infanrix hexa. Of note are the relatively high baseline GMTs of anti-D in all vaccination groups.

For polio types 1, 2, and 3, the Hexaxim pooled batches were associated to lower observed GMT values compared to Infanrix hexa (PP: 882, 1655 and 1106 vs. 1370, 2337 and 2186 respectively). However, seroprotection rates were sufficiently high for all polio-types and for all batches (99.6-100%).

Study A3L12 (2,4,6 months schedule)

The aim of this study in Asia was to show that infants (who have received one dose of Hep B at birth) can be administered Prevenar (7-valent) concomitantly during the priming with Hexaxim:

"Immunogenicity Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to Infanrix hexa, Both Concomitantly Administered with Prevnar at 2, 4, and 6 Months of Age in Thai Infants"

The study focused on specific immunogenicity endpoints (seroprotection rates with anti-Hep B antibody titres ≥10 mIU/ml and anti-PRP antibody titres ≥0.15 µg/ml) of Hexaxim compared to Infanrix hexa.

Methods

Study Participants

412 healthy infants were vaccinated in this multi-centre study in Thailand. Two blood-draws were made (baseline and one month after the last vaccination). Safety follow-up time was again 6 months after the last vaccination. Inclusion and exclusion criteria were similar to the other studies. Hep B vaccination had been done at birth. No information was available on BCG vaccination.

Treatments

The participants received three doses of Hexaxim + Prevenar (7-valent) or Infanrix hexa + Prevenar(7-valent).

Objectives

Primary objective is the demonstration of non-inferiority of the immune response against Hexaxim HepB and PRP antigens versus those of Infanrix hexa.

Secondary objective is the description of the immune response against each antigen of Hexaxim and Infanrix hexa.

The objectives focus on the two "critical" antigens of Hexaxim (Hep B and PRP). It is to be noted that the concomitantly given Prevenar was not evaluated for its serotype immune reaction with the two vaccines.

Outcomes/endpoints

Primary endpoints:

- Anti-HBsAg antibody (Ab) titres ≥10 mIU/ml
- Anti-PRP Ab titres ≥0.15 µg/ml

Secondary endpoints were Anti-T Ab, anti-D Ab, Anti-HBs Ab, Anti-PRP Ab, Anti-pertussis toxoid (PT), anti-filamentous haemagglutinin (anti-FHA) Ab and Anti-polio 1, 2, and 3 Ab titres including different cut-off levels than those considered for the primary endpoints. In addition, Vaccine response to pertussis (PT and FHA) antigens at V06 defined as: anti-PT or anti-FHA in EU/ml ≥LLOQ (=2 EU/ml) in initially seronegative infants, or at least persistence (post-titer ≥pre-titer) of the Ab titer in initially seropositive (titer in EU/ml ≥LLOQ (=2 EU/ml)) were included.

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above

Results of A3L12

Participant flow

393 of 412 subjects completed the trial. All drop-outs are accounted of.

Conduct of the study

No relevant changes were made to the protocol.

Baseline data

The two groups were comparable.

Numbers analysed

The number of subjects with protocol deviations was similar in both vaccine groups.

Table 11 Subject Disposition for Immunogenicity Analyses According to Randomization - ITT and PP Analysis Sets; A3L12

	Group 1: DTaP-IPV-Hep B-PRP-T + Prevnar™ (N=206)		Group 2: Infanrix hexa™ + Prevnar™ (N=206)		Total randomized (N=412)	
	n	%	N	%	n	%
ITT Analysis Set	206	100	206	100	412	100
Per Protocol Analysis Set	189	91.7	190	92.2	379	92.0
Subjects excluded from the PP Analysis Set	17	8.3	16	7.8	33	8.0

N: number of subjects analyzed according to ITT Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in ITT Analysis Set for ITT Analysis Set part and Reason for exclusion from Per Protocol Analysis Set, and percentages are calculated according to the subjects in Per Protocol Analysis Set for Per Protocol Analysis Set part

Outcomes and estimation of A3L12

Anti-Hep B seroprotection rates at 1 month after the third dose of the primary vaccination series were 99.5% for both the Hexaxim+ Prevnar group and the Infanrix hexa + Prevenar group (–0.01% observed difference, two-sided 95% CI: -2.46; 2.43). As the lower limit of the 95% CI was greater than –10, the null hypothesis was rejected and the non-inferiority criterion was met (minimum threshold used to define seroprotection: ≥10 mIU/ml).

Anti-PRP seroprotection rates at 1 month after the third dose of the primary vaccination series were non-inferior for Hexaxim + Prevenar versus Infanrix hexa + Prevenar.

Immune responses to other antigens (D, T, polio, pertussis) and other immunogenicity parameters to Hep B and PRP antigens of the test vaccine vs. Infanrix hexa were analysed as secondary end-points.

The proportions of subjects meeting surrogate correlates of seroprotection for each valence were similar in the two groups, based on overlapping 95% CIs.

The non-inferiority criteria were met for HepB and Hib. As this study was focussed on investigating the immunological response against the Hep B antigen when given concomitantly with Prevenar, it should be noted that non-inferior anti-Hep B seroprotection rates (threshold ≥10mIU/ml) and similar GMTs were observed compared to the study arm receiving Prevenar and Infanrix hexa concomitantly.

GMTs of both vaccines are very similar for most antigens, with the following exemptions:

- Anti-PRP GMT is significantly higher for Hexaxim than for Infanrix hexa vaccinated subjects. The reverse cumulative distribution curve (RCDC) shows a pronounced difference beyond 0,1 IU/ml but the clinical consequences are unknown.
- Anti-Tetanus GMT is significantly lower at visit 6 for Hexaxim compared to Infanrix hexa. The difference is not considered clinically significant taking into account the small difference and that seroprotection levels (long- and short-term) were achieved by all subjects. RCDC for Anti-Tetanus again shows a pronounced difference beyond 1 IU/ml but the clinical consequences are unknown.
- In this concomitant use study (with 7-valent Prevenar) anti-Polio1, 2 and 3 GMTs are significantly lower (approximately 50%) for subjects in the Hexaxim group compared to subjects in the control group one month after priming. Nevertheless, at that timepoint (at an age of 7 months) high anti Poliovirus antibody titer (types 1, 2 and 3) and sufficient seroprotection rates were measured in this study population. Additionally, according to the SmPC, after three doses of the vaccine given during

the first year of live, a booster in the second year is foreseen. For that reason it can be concluded, that concomitant administration of Prevenar dose not have a clinically relevant influence on the immunogenicity of Hexaxim components.

This study was not aimed to shown an impact of Hexaxim on the immunogenicity of the serotypes present in Prevenar 7. Thus, as nothing is known of a potential influence of Hexaxim on the immunogenicity of Prevenar, a concomitant use cannot be claimed in the Product Information.

Study A3L17

This study assessed the immunogenicity of one Hexaxim lot close to the end of shelf-life. It also assesses the immunological effect of the local practice, to vaccinate pregnant women against Diphtheria and Tetanus, on infants in Peru:

"Immunogenicity Study of DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to Infanrix hexa, at 2-4-6 Months of Age in Healthy Peruvian Infants"

Methods

Study Participants

263 healthy infants were vaccinated in one single centre in Peru.

Two blood-draws were made (baseline and one month after the last vaccination).

Safety follow-up time was again 6 months after the last vaccination.

Inclusion and exclusion criteria were similar to the other studies.

BCG vaccination had been done at birth. Immune status (sero-negative) of mothers concerning HepB was of importance.

Treatments

The participants received three doses of either Hexaxim or Infanrix hexa.

According to the sponsor the batch of Hexaxim was close to end of shelf-life (30-32 months). This should be used to determine any negative effect on immunogenicity.

Objectives

Primary objective was the demonstration of non-inferiority of the immune response against Hexaxim HepB antigen versus those of Infanrix hexa.

Secondary objective was the description of the immune response against D, PRP and Hep B. The titre for D was also measured at both visits.

Of note, the measurement of D at both blood-draw visits was triggered by the local standard of DT vaccination for pregnant women.

Outcomes/endpoints

Primary endpoint:

- Anti-Hep B antibody (Ab) titres ≥10 mIU/ml

Secondary endpoints:

- Anti-D Ab titres at V01, and Ab titres for D, PRP, and Hep B at V06 (7 months of age).
- Ab titres above a cut-off (V01):
    - Anti-D Ab titres ≥0.01 IU/ml, ≥0.1 IU/ml
- Ab titres above a cut-off (V06):
    - Anti-D Ab titres ≥0.01 IU/ml and ≥0.1 IU/ml
    - Anti- HBsAg Ab titres ≥100 mIU/ml
    - Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
    - Ab individual titres ratios for anti-D (V06/V01).

Sample size, Randomisation, Blinding (masking) and Statistical methods

See introduction section above.

Results of A3L17

Participant flow

All subjects completed the study.

Conduct of the study

Some relevant changes were implemented in the protocol:

- Change of PRP assessment method to RIA
- Updated descriptions for anti-Hep B, anti-D, and anti-PRP assessment methods
- The Hep-B threshold of ≥10 mIU/ml had not been specified as a secondary endpoint in the protocol. However, since this threshold was a primary endpoint, and had been included as a secondary endpoint in other protocols within the clinical trial program, it was decided that the secondary endpoint tables would be constructed using both ≥10 mIU/ml and ≥100 mIU/ml thresholds.
- Clarification of the PP Analysis Set to include the following information: "No definite contraindication present at the time of vaccination with any dose and no development of a relevant exclusion criteria that may affect immunogenicity assessment during the entire trial period".
- Following a change in internal standards used for the randomization process in Phase III studies, the allocation of subject inclusion numbers, assignment to vaccine groups, and emergency unblinding were performed using the IVRS system.
- The descriptive analysis of secondary endpoints was performed on the PP Analysis Set as well as the ITT Analysis Set.

Baseline data

Both study groups were comparable in terms of demographics.

Numbers analysed

Table 12 Subjects Disposition for Immunogenicity Analyses According to Randomization - ITT and PP Analysis Sets; A3L17

	Group 1: DTaP-IPV-Hep B-PRP-T (N=132)		Group 2: Infanrix hexa™ (N=131)		Total randomized (N=263)	
	n	%	N	%	n	%
ITT Analysis Set	132	100.0	131	100.0	263	100.0
PP Analysis Set	132	100.0	130	99.2	262	99.6
Subjects excluded from PP Analysis Set	0	0	1*	0.8	1*	0.4

N: number of subjects analyzed according to ITT Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in ITT Analysis Set for ITT Analysis Set part and Reason for exclusion from Per Protocol Analysis Set, and percentages are calculated according to the subjects in Per Protocol Analysis Set for Per Protocol Analysis Set part; * Reason for exclusion: BL2-V06 not drawn or no measurement;

Outcomes and estimation of A3L17

Overall, non-inferiority for Hep B was met. The GMTs were comparable for Hep B and D for both vaccines (for tabulated results, please see the respective table in section "Summary of main studies" below). No negative effect on immunogenicity was seen for the Hexaxim batch being near the end of shelf-life compared to other studies.

Of note is the effect of the local standard to vaccinate pregnant women with DT vaccine. This obviously affects the GMTs but the thresholds of seroprotection are still reached after the three vaccinations. As in the previous study A3L12 the anti-PRP GMT for Hexaxim is slightly higher than for Infanrix hexa.

Regarding the anti-Hep B response slightly lower GMTs and a lower seroprotection rate based on the ≥100mIU/ml threshold criterion were observed for Hexaxim compared to Infanrix hexa (GMTs: 986 vs. 1139; ≥100mIU/ml: 93.9% vs. 99.2%, respectively). These results are similar to those from studies A3L011, A3L04 and A3L10.

Comparison of Hepatitis B results of all primary vaccination studies

As summarized in the table below, sufficient seroprotection rates have been achieved in all studies (shadowed in yellow). For the more condensed vaccination schedules (A3L15 and A3L10) lower GMTs have been found compared to the less condensed schedules.

Comparing the different Hep B vaccines (Engerix, Tritanrix or Infanrix hexa or Hexaxim) used in 4 out of 7 priming studies (A3L10, A3L04, A3L011 and A3L017) higher GMTs have been found in the control groups compared to the Hexaxim groups (highlighted in yellow). Moreover, taking into account the ≥ 100mIU/ml threshold, higher seroprotection rates have been found for the control groups compared to the Hexaxim groups (A3L10: 64.9% vs. 78.1%; AL304: 96.2% vs. 98.9%; A3L011: 91.7% vs. 99.2% and A3L17: 93.9% vs. 99.2%, respectively). It is known that higher anti-HBs concentrations will take longer to decline below the minimum threshold for protection of ≤ 10mIU/ml. Lower GMTs might therefore indicate a shorter persistence of protection, which should be followed up post authorisation.

Table 13 Comparison of all GMTs and seroprotection rates regarding Hep B for all priming studies (PP Analyses; one month post vaccination)

Study	A3L15			A3L10		A3L02		A3L04(Hep B at birth only on Peru)		A3L011		A3L012(Hep B at birth) Plus Prevenar		A3L017	
Hepatitis-Vaccine	H	E (Group 2)	H (Hep B at birth)	H	E	H	E	H	T	H	I	H	I	H	I
GMT	330	148	1913	149	265	1148	840	1075	3376	1142 (935; 1566; 1009; batch 1,2 and 3 respectively)	1576	2477	2442	986	1139
% ≥10mIU/ml	95,7	95,4	99.0	94,0	96,1	99,2	100	100	100	98,3	100	99,5	99,5	99,2	100
%≥100mIU/ml	78,8	65,5	96.9	64,9	78,1			96,2	98,9	91,7	99,2	98,4	99,5	93,9	99,2
Non-inferiority testing for HepB	yes			yes		yes		Not done		Not done		yes		yes	
Assay	Ortho-ECI			Ortho-ECI		RIA		Ortho-ECI		Ortho-ECI		Ortho-ECI		Ortho-ECI	
Vaccination-schedule in month	1,5 - 2,5 - 3,5 (most condensed)			2 – 3 – 4 (most condensed)		2 – 4 - 6		2 – 4 - 6		2 – 4 - 6		2 – 4- 6		2 – 4 - 6	

H - Hexaxim; Control vaccines: E - Engerix B, I - Infanrix hexa T-Tritanrix-HepB/Hib

Booster vaccination studies

For the majority of clinical booster trials, only Hexaxim was administered as a booster dose.

In one study, **A3L15**, 4 doses of Hexaxim have been compared to 4 doses of CombActHib + 3 doses of Engerix (no Engerix booster in the second year of life). In this part of the report only the booster part of the study is presented (A3L15bs).

In study **A3L22** it has been evaluated whether a booster with Hexaxim is immunogenic even if the priming has been done with Pentaxim plus Engerix.

In **A3L21** it has been evaluated whether a booster with Hexaxim is immunogenic even if the priming has been done with Infanrix hexa.

In study **A3L16**, follow-up study of A3L02 (Hexaxim vs. Pentaxim +Engerix), the booster was Pentaxim. Here no evaluation of the Hepatitis B immunogenicity has been performed.

A3L01 is a small study (Phase I), where a booster of Hexaxim has been compared to a booster of Hexavac.

Study A3L15bo

MMRV vaccines are largely implemented in vaccination calendars during the second year of life. The aim of this study was to show that toddlers can be administered Trimovax and Varilrix concomitantly with Hexaxim.

Methods

Study Participants

Study subjects from the primary study phase were boostered in this study at the age of 15-18 months of age. The same inclusion and exclusion criteria applied. Additionally, the toddlers infectiological status now was of interest (HIV, HepB, HepC). This part consisted of **visits 7** (pre-booster Blood-draw and vaccination) and **8** (one month after vaccination blood-draw).

Treatments

One booster dose of Hexaxim (Groups 1 and 3) or CombActHib + OPV was given. Concomitantly, one dose of MMRV was offered and given to the majority of subjects (93,3 - 99,3%).

This was the only study were the same vaccine has been used for priming and the booster immunisation. Group 2, which had been primed with CombActHib + Engerix, were vaccinated in the second year of life with CombActHib only, no Engerix booster has been given.

Objectives

Secondary and observational endpoints define this subpart of study A3L15.

Secondary objectives are to describe in each group:

- The Ab persistence for each primary series vaccine component prior to a booster vaccination at 15 to 18 months of age
- The immunogenicity parameters to each primary series vaccine component 1 month after a booster vaccination at 15 to 18 months of age
- The immunogenicity parameters to measles, mumps, and rubella (MMR) and varicella 1 month after a booster vaccination at 15 to 18 months of age

Observational objective:

To describe in each group the immunogenicity parameters to Mumps, Measles and Varicella, assessed with the functional test assay, one month after a booster vaccination at 15 to 18 months of age.

Outcomes/endpoints

Secondary endpoints:

Ab persistence (for all valences) ***before the booster dose*** at V07 (M15-M18):

- Ab titres for each valence
- Ab titres above the following cut-off:
    - Anti-T Ab titres ≥0.01 IU/ml and ≥0.1 IU/ml
    - Anti-D Ab titres ≥0.01 IU/ml and ≥0.1 IU/ml
    - Anti-Hep Bs Ab titres ≥10 mIU/ml and ≥100 mIU/ml
    - Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
    - Anti-polio titres ≥8 (1/dil)

The following endpoints were used ***to assess the booster responses*** at V08:

- Ab titres for each valence
- Ab titres above a cut-off:
    - Anti-T Ab titres ≥0.01 IU/ml, ≥0.1 IU/ml, and ≥1.0 IU/ml
    - Anti-D Ab titres ≥0.01 IU/ml, ≥0.1 IU/ml, and ≥1.0 IU/ml
    - Anti-Hep Bs Ab titres ≥10 mIU/ml and ≥100 mIU/ml
    - Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
    - Anti-polio titres ≥8 (1/dil)
    - Anti-measles (≥300 mIU/ml by enzyme-linked immunosorbent assay [ELISA])
    - Anti-mumps (≥500 EU/ml by ELISA)
    - Anti-rubella (≥10 IU/ml by ELISA)
    - Anti-varicella (≥300 mIU/ml by ELISA)
- Individual titer ratio for anti-T, anti-D, anti-Hep B, anti-PRP and anti-polio (V08/V07)
- Seroconversion for anti-PT and anti-FHA, defined as:
    - Anti-PT and anti-FHA ≥four-fold Ab titres increase from V07 to V08
- Booster response to pertussis (PT and FHA), defined as:
    - Subjects whose pre-vaccination Ab concentrations were less than the<LLOQ demonstrated the booster response if they had post-vaccination levels ≥four times LLOQ
    - Subjects whose pre-vaccination Ab concentrations are ≥LLOQ but<four times the LLOQ demonstrated a booster response if they had a four-fold response (i.e. post/pre-vaccination ≥four)

- Subjects whose pre-vaccination Ab concentrations are ≥four times the LLOQ demonstrated a booster response if they had a two-fold response (i.e. post /pre vaccination ≥two)

Observational endpoints:

- Ab titres
- Ab titres above a cut-off:
  - Anti-measles (neutralizing Ab titer ≥120 mIU/ml)
  - Anti-mumps (neutralizing Ab titer ≥60 1/dil)
  - Anti-varicella (FAMA ≥4 1/dil)
- Seroresponse is defined as:
  - Anti-measles ELISA titer ≥300 mIU/ml or Anti-measles Neutralizing Ab titer ≥120 mIU/ml
  - Anti-mumps ELISA titer ≥500 EU/ml or Anti-mumps Neutralizing Ab titer ≥60 (1/dil)
  - Anti-varicella ELISA titer ≥300 mIU/ml or Anti-varicella FAMA titer ≥4 (1/dil)

Results of A3L15bo

Participant flow

565 of 567 subjects finished the study, the drop-out between the two study phases (primary and booster vaccination parts) is very low and not considered an issue.

Conduct of the study

The following amendments were made and approved of by IECs and MCC:

- The addition of MMR and varicella vaccinations at 15 to 18 months of age, and a change in the timing of the booster dose to 15 to 18 months
- Amendment to the ICF, and addition of inclusion criteria for booster phase (namely, signing of ICF addendum, plus subject's age)
- The collection of information on injection site events / reactions for the MMR and varicella vaccines during the booster phase, and addition of extensive limb swelling after the booster vaccination as a solicited AE
- Clarification of the relevant vaccine for each immunogenicity endpoint, and the analyses to be performed
- The addition of a secondary endpoint to allow the optimal analysis of immunogenicity results from aP components constituting the investigational vaccine
- Anti-polio Ab titres assay changed from Hep2 cell culture to mammalian cell culture
- Anti-PRP Ab titres assay changed from enzyme immunoassay (EIA) to RIA, and the LOQ changed from 0.065 µg/ml to 0.06 µg/ml
- The addition of five further protocol violation criteria for the PP Analysis Set (three for the primary series and two for the booster series): "no definite contraindication present at the time of vaccination

with any dose and no development of a relevant exclusion criterion that may affect immunogenicity assessment during the entire trial period; and "BL2-V05 (D570) drawn or with any measurement available" and "no contraindications to the study vaccine Nos. 3 to 7, no contraindications to MMR Nos. 2 to 5, and no contraindications to varicella Nos. 2 to 5" for the booster phase. The following violation criterion: "Use of vaccine declared not usable due to cold chain break" was also used for the booster phase.

- Update on the assessment method for testing Haemophilus influenzae antigen (PRP). The ELISA technique was replaced by RIA.
- Confirmation of which MMRV assessment methods were performed, their LLOQs, and addition of an additional functional testing.

Baseline data

In the ITT Analysis Set, the mean age was similar in all groups and there was a similar distribution of males and females in each group. The same results were observed in the PP Analysis Set. The study groups are comparable.

Numbers analysed

Table 14 Subjects Disposition for Immunogenicity Analyses During Booster Phase – ITT Analysis Set and PP Analysis Set; A3L15bo

	Group 1: DTaP-IPV-Hep B-PRP-T * (N=218)		Group 2: CombAct-Hib™ + OPV * (N=219)		Group 3: DTaP-IPV-Hep B-PRP-T (Engerix B™ at birth)* (N=130)		Total (N=567)	
	n	%	n	%	n	%	n	%
ITT Analysis Set	218		219		130		567	
Per Protocol Analysis Set	204	93.6	202	92.2	116	89.2	522	92.1
Subjects excluded from PP Analysis Set	14	6.4	17	7.8	14	10.8	45	7.9

Primary vaccination: Group 1: DTaP-IPV-Hep B-PRP-T; Group 2: CombAct-Hib +Engerix B + OPV; Group 3: DTaP-IPV-Hep B-PRP-T and Engerix B at birth; * All subjects were proposed to receive Trimovax and Havrix in addition to the booster vaccination with investigational or control vaccines; N: number of subjects analyzed according to ITT Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in ITT Analysis Set for ITT Analysis Set part and Reason for exclusion from PP Analysis Set, and percentages are calculated according to the subjects in PP Analysis Set,for PP Analysis Set part;

Outcomes and estimation A3L15bo

GMTs and seroconversion rates for the Hexaxim antigens after the booster vaccination were similar between the groups (for tabulated results, please see the respective table in section "Summary of main studies" below). Persistence of antibodies is significantly better for anti-D but significantly worse for anti-T in the Hexaxim groups. Of note, the significant difference for anti-T vanishes after the booster.

One month after vaccination (group 1: Hexaxim + MMRV vs. group 2: CombAct Hib + OPV+MMRV) immune responses to the MMR and varicella were assessed, in terms of seroprotection rates at predefined thresholds.

Seroresponses to MMV were assessed using two methods: ELISA or functional (Neutralization/FAMA: Florescent antibody to membrane antigen) tests.

In general, GMTs and seroconversions are very similar for both vaccines. Anti-PRP GMTs that had been slightly lower in the Hexaxim group after primary vaccination are now at the same level as in the

CombActHib-group. Antibody persistence is also very similar between the groups and within known bounds of other combination vaccines for this indication.

RCDCs show only a marginal effect of the birth HepB dose on antibody titres against D, T, PRP and PT, FHA concerning persistence and booster effect.

Regarding Hep B, the lowest pre-booster GMTs were observed in Group 1 (Hexaxim, without Hep B at birth) when compared with Groups 2 and 3 (51.3, 103 and 228 mIU/ml, respectively). Similarly, the lowest seroprotection rate (78.9%) was found in Group 1. However, after the booster dose, the seroprotection rate (≥ 10 mIU/ml) was 98.5% for Hexaxim.

Concerning polio, no clinically significant differences in seroprotection rates and GMTs comparing Hexaxim with or without a Hep B-at-birth-dose (group 1 vs. group 3) have been observed. Of note, in group 2, in which OPV has been used for primary vaccination, lower immune responses have been measured post booster.

However, post-booster seroprotection rates were similar for all valences tested (Hep B, Polio, Tetanus, Diphtheria and Pertussis).

Concomitant use with measles, mumps, rubella, varicella (MMRV) vaccine:

Concomitant use of Trimovax (Schwarz strain, Urabe AM9 strain and Wistar RA 27/3M) and Varilix (Oka strain) investigated in study A3L15bo demonstrated that subjects were sufficiently protected against all valences included in Hexaxim.

Comparing GMTs and seroprotection rates of measles, mumps, rubella and varicella components no clinically relevant differences have been found between group 1 (Hexaxim +MMRV) and group 2 (CombActHib + OPV+MMRV).

For measles and rubella acceptable protection levels have been reached by the majority of subjects (100% and 97.4%, respectively).

Regarding the mumps component a correlate for protection is not established. 96.9% of vaccinees acquired an antibody titer ≥60 l/dil (used as a cut-off set for the neutralisation assay).

Regarding the varicella component only 81.8% of subjects acquired minimum titres corresponding to the accepted surrogate parameter of ≥ 4l/dil. This finding is particularly important as some countries do not recommend a second dose of varicella vaccine. Following administration of a single dose of currently marketed varicella vaccines seroconversion is usually observed in about 95% of healthy children. No comparison of concomitant use versus administration at different time points has been performed. Considering the historic comparison low varicella seroprotection rate of only 82% must be interpreted as an immunological interference phenomenon. Therefore it was reflected in the SmPC that

- data on concomitant administration of a booster dose of Hexaxim with measles-mumps-rubella vaccines have shown no clinically relevant interference in the antibody response to each of the antigens, and
- that there may be a clinically relevant interference in the antibody response of Hexaxim and Varilrix and these vaccines should not be administered at the same time.

Study A3L22

This study evaluated whether a booster with Hexaxim is immunogenic even if the priming has been done with Pentaxim plus Engerix:

"Immunogenicity and Safety Study of a Booster Dose of DTaP-IPV-Hep B-PRP-T Combined Vaccine at 15 to 18 Months of Age Following a Primary Series at 2, 3 and 4 Months of Age in Healthy Turkish Infants"

Methods

Study Participants

This was the booster study for study A3L10. The same (still healthy) subjects were enrolled if consent was given.

Inclusion and exclusion criteria were appropriate for a booster study setting.

Safety follow-up time was again 6 months after the vaccination.

Treatments

The participants received one dose of Hexaxim, no control.

Objectives

The objectives were to describe antibody persistence against all antigens in either Hexaxim or Pentaxim +Engerix B and to describe the immunogenicity of the booster dose of Hexaxim.

Outcomes/endpoints

The following endpoints were used to assess the Ab persistence (for all valences) before the booster dose at Day 0 (Visit [V01]):

- Ab titres for each valence
- Ab titres above a cut-off:
- Anti-T Ab titres ≥0.01 IU/ml and ≥0.1 IU/ml
- Anti-D Ab titres ≥0.01 IU/ml and ≥0.1 IU/ml
- Anti-Hep Bs Ab titres ≥10 mIU/ml and ≥100 mIU/ml
- Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
- Anti-polio titres ≥8 (1/dil)
- Anti-pertussis toxoid (PT) Ab titres ≥4 EU/ml
- Anti-filamentous haemagglutinin (FHA) Ab titres ≥4 EU/ml

The following endpoints were used to assess the booster responses at D30 (V02):

- Ab titres for each valence
- Ab titres above a cut-off:

- Anti-T Ab titres ≥0.01 IU/ml, ≥0.1 IU/ml, and ≥1.0 IU/ml
- Anti-D Ab titres ≥0.01 IU/ml, ≥0.1 IU/ml, and ≥1.0 IU/ml
- Anti-Hep Bs Ab titres ≥10 mIU/ml and ≥100 mIU/ml
- Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
- Anti-polio titres ≥8 (1/dil)
- Anti-PT Ab titres ≥4 EU/ml
- Anti-FHA Ab titres ≥4 EU/ml
- Individual titer ratio for each valence (V02/V01)
- Seroconversion for anti-PT and anti-FHA, defined as:
    - Anti-PT and anti-FHA ≥four-fold Ab titres increase from V01 to V02
- Booster response to pertussis (PT and FHA), defined as:
    - Subjects whose pre-vaccination Ab concentrations were less than the Lower Limit Of Quantitation (<LLOQ) would demonstrate the booster response if they had post-vaccination levels ≥4 x LLOQ
    - Subjects whose pre-vaccination Ab concentrations were ≥LLOQ but <4 x LLOQ would demonstrate the booster response if they had a four-fold response (i.e. post-/pre-vaccination ≥4)
    - Subjects whose pre-vaccination Ab concentrations were ≥4 x LLOQ would demonstrate the booster response if they had a two-fold response (i.e. post-/pre-vaccination ≥2)

Results of A3L22

Participant flow

254 of the 302 subjects who completed the primary vaccination study were enrolled in this study. Of those all but two completed this booster study. Those two subjects did not receive Hexaxim as a booster but Pentaxim as no consent was given for Hexaxim. This possibility was included in the trial outline.

Conduct of the study

GCI laboratory method for PRP was changes via an amendment of the protocol.

During the study 2 subjects received apparently frozen vaccine, the follow-up (safety and immunogenicity) showed no concerns, and the subjects were protected.

Baseline data

The mean age was the same in both groups. In each primary vaccine group, there were more males than females. The same results were observed in the PP Analysis Set. The two groups were comparable.

Numbers analysed

Table 15 Subject Disposition for Immunogenicity Analysis According to Randomization - FAS and PP Analysis Sets; A3L22

	Booster vaccination with DTaP-IPV-Hep B-PRP-T					
	Vaccine group at primary series					
	Group1: DTaP-IPV-Hep B-PRP-T (N=130)		Group2: Pentaxim™ + Engerix™ B (N=124)		Pooled group (N=254)	
	n	%	n	%	n	%
Full Analysis Set*†	130	100.0	124*	100.0	254	100.0
Per Protocol Analysis Set	114	87.7	103	83.1	217	85.4

N: number of subjects analyzed according to Full Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in Full Analysis Set for Full Analysis Set part and Reason for,exclusion from Per Protocol Analysis Set, and percentages are calculated according to the subjects in Per Protocol Analysis Set for Per Protocol Analysis Set part; * Includes Subject 001-00002 and Subject 001-00015 who received Pentaxim + Engerix B as a booster vaccination. Both subjects were analyzed in Group 2, in accordance with their primary series vaccination; † The FAS for Ab persistence (as specified in the SAP) is not presented, however the population was identical to the FAS;

Outcomes and estimation of A3L22

In view of GMTs and individual GMT ratios for selected valences that pronounced differences between the two groups were shown. For anti-T and anti-D GMTs after the booster dose were significantly lower in the Hexaxim primed group than for the Pentaxim primed group

Concerning the persistence of antibodies the two groups (Hexaxim versus Pentaxim+Engerix B primed) are similar. The booster effect is also very similar for most antigens. Although the GMT individual ratio for PRP shows a pronounced difference between Hexaxim (being lower) and Pentaxim primed toddlers this effect is not considered of clinical relevance.

The pronounced difference for anti-D and anti-T between booster effect of Hexaxim and Pentaxim primed toddlers with the Hexaxim primed group reaching significantly lower (halved for anti-D) the GMT of the Pentaxim primed group as well as the difference in the individual ratio might be a concern when it comes to the timing of a next booster. Nevertheless, concerning seroprotection (long and short-term levels) this criterion was fulfilled in both groups for nearly all but one subject (long-term level).

Anti-FHA GMTs were significantly lower for Hexaxim primed subjects in the inter-individual comparison, too. Again, the surrogate for protection (4-fold increase of titres) was similar to Pentaxim primed individuals.

Overall, although seroprotection levels were reached in all cases there are significant differences in the immunogenicity for some antigens.

Pre-booster GMTs for HepB in Group 2 (priming with Pentaxim +Engerix) were higher than in Group 1 (priming with Hexaxim) and the percentage of subjects with seroprotection titres was only 80.7% for the Hexaxim group versus 99% for the Engerix group (threshold criterion ≥10mIU/ml). As stated previously, in case no booster vaccination would be given in the second year of live, this could have a negative effect on the persistence of protection. However, regardless which HepB containing vaccine was used for the primary series (Pentaxim plus Engerix or Hexaxim) following booster vaccination with Hexaxim all groups experienced an effective anamnestic anti HepB immune response.

Following primary vaccination with Hexaxim, but before booster vaccination sufficient percentages of subjects were still seroprotected against polio types 1 and 2 . However, regarding polio type 3 only 85% of subjects had sufficiently high anti-polio type 3 titer ≥ 81/dil. Nevertheless, this effect is not considered to be of clinical relevance as after booster vaccination with Hexaxim a substantial increase of GMTs has been measured for all polio types and 100% of subjects were seroprotected.

Study A3L21

This study aims to show whether a booster with Hexaxim is immunogenic regardless if the priming has been done with Infanrix hexa or Hexaxim (3 batch consistency study A3L11):

"Immunogenicity Study of the Antibody Persistence and Booster Effect of the DTaP-IPV-Hep B-PRP-T Combined Vaccine at 15 to 18 Months of Age Following a Primary Series of DTaP-IPV-Hep B-PRP-T or Infanrix hexa Administered at 2, 4, and 6 Months of Age in Healthy Mexican Infants"

Methods

Study Participants

This is the booster study for study A3L11. The same (still healthy) subjects were enrolled if consent was given, one centre from the primary study did not participate in the booster study, thus, those children are missing here.

Inclusion and exclusion criteria were appropriate for a booster study setting.

Safety follow-up time was again 6 months after the vaccination.

Treatments

One dose of Hexaxim for all participants

Objectives

Immunogenicity was assessed in a subset of 310 subjects.

The objective was the persistence of antibodies for all antigens and the description if the immunogenicity of the booster dose Hexaxim.

Outcomes/endpoints

1. At D0 (pre-booster) and D30 (post booster):
    - Ab titres for each valence
    - Ab titres above a cut-off:
    - Anti-T and anti-D Ab titres ≥0.01 IU/ml and ≥0.1 IU/ml
    - Anti-Hep B Ab titres ≥10 mIU/ml and ≥100 mIU/ml
    - Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
    - Anti-polio titres ≥8 (1/dil)
2. Only at D30:
    - Individual titer ratio for each valence (V02/V01)
    - Seroconversion for pertussis Ab (anti-acellular pertussis toxoid [PT] and anti-filamentous haemagglutinin [FHA]) defined as:
        - Anti-PT and anti-FHA ≥4-fold Ab titres increase from V01 to V02
    - Booster response to pertussis (PT and FHA) was defined as:

- Subjects whose pre-vaccination Ab concentrations were less than the Lower Limit of Quantitation (LLOQ) demonstrated a booster response if they have post-vaccination levels ≥4 x LLOQ.
- Subjects whose pre-vaccination Ab concentrations were ≥LLOQ but <4 x LLOQ demonstrated a booster response if they had a four-fold response (i.e. post-/pre-vaccination ≥4).
- Subjects whose pre-vaccination Ab concentrations were ≥4 x LLOQ demonstrated a booster response if they had a two-fold response (i.e. post-/pre-vaccination ≥2).

Results of A3L21

Participant flow

881 out of the 1056 subjects who completed the primary vaccination study were enrolled in this study.

Of these 881 subjects, 768 had received Hexaxim and 113 Infanrix hexa in the previous study.

875 of 881 toddlers completed the trial; all drop-outs are accounted for.

Conduct of the study

No relevant changes were made to the protocol.

Baseline data

In the ITT Analysis Set, the mean age was similar in both groups, and there was a similar distribution of males and females in each group. The same results were observed in the PP Analysis Set. The groups were comparable.

Numbers analysed

Table 16 Subject Disposition for Immunogenicity Analyses - ITT for Immunogenicity Analysis Set- A3L21

	Booster vaccination with DTaP-IPV-Hep B-PRP-T Vaccine group assigned for primary series									
	DTaP-IPV-Hep B-PRP-T Batch A (N=72)		DTaP-IPV-Hep B-PRP-T Batch B (N=75)		DTaP-IPV-Hep B-PRP-T Batch C (N=76)		Infanrix hexa™ (N=87)		Overall (N=310)	
	n	%	N	%	n	%	n	%	N	%
ITT for Immunogenicity Analysis Set	72	100	75	100	76	100	87	100	310	100
ITT for Ab persistence	68	94.4	65	86.7	74	97.4	81	93.1	288	92.9
Per Protocol Analysis Set	58	80.6	61	81.3	58	76.3	65	74.7	242	78.1

N: number of subjects analyzed according to ITT for Immunogenicity Analysis Set; n: number of subjects; %: percentages are calculated according to the subjects in ITT for Immunogenicity Analysis Set;

The number of subjects per group was comparable in both ITT and the PP analysis sets.

Outcomes and estimation of A3L21

The immunogenicity analysis subset consisted of 310 subjects.

For all antigens the booster dose of Hexaxim produced similar results regardless of the priming vaccine. Persistence of antibodies was similar in the two groups as well.

Antibody persistence and booster effect were similar between the two groups (three individual batches of Hexaxim or Infanrix hexa primed) for most antigens.

Prior to the booster 89.8 % of subjects primed with Hexaxim were still seroprotected (≥10mIU/ml threshold); in the control group primed with Infanrix hexa even 95.4 % reached this threshold. As similar (or even higher) differences in the pre-boost seroprotection rates have been found in the majority of booster studies (A3L15s, A3L22, A3L16 and A3L21) this could be a signal for reduced persistence of protection and should be followed up carefully on a long term basis. However, at an age of 15 to 18 months after a 4th dose of Hexaxim 99.4% of subjects were seroprotected.

Similar to study A3L22, prior to booster vaccination significantly lower GMTs have been found for poliovirus type 3 in the group primed with Hexaxim compared to the group primed with Infanrix hexa (GMT: 339 vs. 896, respectively) For poliovirus types 1 and 2 no such statistically significant differences have been observed.

Nevertheless, seroprotection rates have been sufficient at that timepoint (96.5% for anti-poliovirus type 3 and 100 % for the other poliovirus types).

Following booster vaccination with Hexaxim a substantial increase of poliovirus-antibodies (all types) was measured, and all subjects were seroprotected against all poliovirus types.

Altogether, taking into consideration the high level of antibodies and the sufficient seroprotection rates, these differences do not have clinical relevance.

Study A3L01

This Phase I study assessed the effect of one dose of Hexaxim versus Hexavac on toddlers that had been primed according to local standard:

"Phase-I Safety of a Booster Dose of Either the Investigational DTaP-IPV-HB-PRP~T Combined Vaccine or HEXAVAC in Healthy Argentinean 16- to 19-Month-Old Toddlers"

Methods

Study Participants

In this phase I mono-centre study the 60 healthy subjects had been primed with 3 doses of standard infant T, D, wP, OPV or IPV, Hib and HepB vaccines for Argentina.

Inclusion and exclusion criteria are similar to other studies. Additionally, blood chemistry was tested prior to vaccination and compared to the second blood-draw for safety reasons (Phase I).

Treatments

One dose of either Hexaxim or Hexavac

Objectives

The primary objective was the safety of one dose of Hexaxim as this was the phase I in the clinical development.

Immunogenicity of the booster dose was documented as the secondary objective for all components.

This conduct was considered common for very early (Phase I) vaccine trials.

Outcomes/endpoints

- Anti-tetanus and anti-diphtheria antibody titres
- Anti-PT and anti-FHA Ab titres
- Anti-HBsAg Ab titres
- Anti-PRP Ab titres
- Anti-Polio 1, 2, and 3 Ab titres

The following cut-offs were used:

Table 17 Cut-offs for titres (underlined cut-offs = primary seroprotective levels)

Titer		Cutoffs
Anti-tetanus and anti-diphtheria Ab titers		*≥ 0.01 IU/mL, ≥ 0.1 IU/mL, ≥ 1 IU/mL*
Anti-HBs Ab titers		*≥ 1 mIU/mL, ≥ 10 mIU/mL*
Anti-PRP Ab titers		*≥ 0.15 µg/mL, ≥ 1.0 µg/mL*
Anti-Polio 1, 2 and 3 Ab titers		*≥ 8 (1/dil)*

- Seroprotection and seroconversion rates, defined as the percentage of subjects seroprotected above the primary seroprotection level and seroconverted.
- Percentage of subjects with Ab titres above the defined non-primary cut-offs
- Geometric mean of antibody titres (GMT).
- Geometric mean of individual titres ratio (GMTR) (V03/SC), for each criterion except anti poliomyelitis 1, 2 and 3 Ab titres.
- For anti-PT and anti-FHA Ab titres, the 4-fold increase was to be determined:
    - Percentage of subjects with ≥ 4-fold increase in titres from SC to V03 (D30 to D37)

Statistics were calculated among toddlers assessed for immunogenicity at the considered time point. The 95% confidence intervals (95% CIs) were calculated.

Of note, the endpoints and parameters measured are those used in later studies.

Results of A3L01

Participant flow

All 60 subjects enrolled in the study (30 per group) completed the trial.

Conduct of the study

The following changes in the protocol and the procedures occurred

1. Two database locks were held. Following the Argentinean Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT)'s request for the safety results, a database lock (without immunology data) was held on 06 June 2004 with the safety data of all the 60 subjects. Unblinding of subject codes was done on 15 June 2004 for the statistical analysis of safety. It was ensured that the laboratory personnel performing the serological analysis were kept blinded before final database lock. The final database lock complete with immunology data was held on 03 September 2004."
2. "The protocol defined seropositivity to PT and FHA as an antibody titer ≥10 EU/ml. The number of subjects with titres above this value was not calculated at the time of analysis since some of the results were still not available, however it can easily be estimated from the reverse cumulative distribution curve for anti-PT and anti-FHA (see Appendix 15). The protocol also defined the limit of quantification of the ELISA method to assayed anti-FHA and anti PT titres at 6 EU/ml and 8 EU/ml, respectively. Those limits are the limit of the assays performed in the GCI laboratory in the US. However, anti-FHA and anti-PT assays were performed at the Clinical Immunology Platform in Val de Reuil, France, due to the temporary unavailability of the GCI laboratory in the US at the time of the analysis. The limit of quantification of the anti-FHA and anti-PT were both at 2 EU/ml. The other parameters evaluating the seropositivity to PT and FHA were unchanged between the protocol and the final analysis, i.e. four-fold increase and GMTR (post-vaccination/baseline)."

Baseline data

The Hexavac group had 2/3 male subjects, the ratio in the Hexaxim group was 50/50. Otherwise (weight, BMI, age) the groups were comparable.

As this study's main purpose is the generation of safety data in a small scale the sex imbalance was not considered of importance.

Numbers analysed

Although there were protocol deviations in 10 subjects (6 for Hexaxim and 4 for Hexavac) data are presented for all subjects with available results (6 subjects are missing specific titrations).

Outcomes and estimation of A3L01

Sufficient GMTs were reached after the booster regardless of the vaccine used. Baseline titres show that seroprotection against Tetanus, Polio and Hepatitis B was still given in the majority of subjects.

These "first" GMTs show a similar reaction for both vaccines for most antigens. Anti-D and Anti-FHA are somewhat lower for Hexaxim but ranges overlap. Anti-PRP for Hexaxim is higher than for Hexavac, again, ranges overlap.

Nearly all subjects were still seroprotected before the booster. Anti-D and Anti-PRP show the lowest rates here (40 and 60% respectively); all reached sufficient seroprotection levels after the booster regardless of the vaccine used.

In summary, booster vaccination with Hexaxim induces higher antibody-titres regarding Hep B compared to Hexavac. Generally, all antibody-titres measured were very high and seroprotection rates against both diseases (Polio and Hepatitis B) were nearly 100% post-booster.

Study A3L16

A booster with HepB in the second year of life is not a current practice in all countries. The aim of this study was to evaluate a booster with a **pentavalent** combined vaccine following Hexaxim primary series:

"Immunogenicity Study of the Antibody Persistence and Booster Effect of PENTAXIM at 18 Months of Age Following a Primary Series of DTacP-IPV-HepB-PRP-T Combined Vaccine or of PENTAXIM and ENGERIX B PEDIATRICO at 2, 4, and 6 Months of Age in Healthy Argentinean Infants"

Methods

Study Participants

This study assessed the effect of a booster dose of Pentaxim + Engerix B on healthy toddlers who had been primed with Hexaxim in study A3L02.

Inclusion and exclusion criteria are similar to other studies.

Treatments

One dose of Pentaxim

Objectives

The primary objective of this study is to describe the persistence of antibodies and seroprotection induced by the primary vaccination with Hexaxim and the effect of the booster vaccination with Pentaxim.

The booster response for PT and FHA (Pertussis) are described as observational objective.

Outcomes/endpoints

Antibody persistence:

- Anti-T and anti-D Ab titres ≥0.01 international unit (IU)/ml, ≥0.1 IU/ml, and ≥1 IU/ml
- Anti-HBsAg Ab titres ≥10 mIU/ml
- Anti-PRP Ab titres ≥0.15 µg/ml and ≥1.0 µg/ml
- Anti-PT and anti-FHA Ab titres ≥4 enzyme-linked immunosorbent assay (ELISA) units (EU/ml)
- Anti-polio 1, 2, and 3 Ab titres ≥8 (1/dil).

Booster dose effect:

- Anti-T and anti-D Ab titres ≥0.01 IU/ml, ≥0.1 IU/ml, ≥1.0 IU/ml, and individual titres ratio (V02/V01)
- Anti-PRP Ab titres ≥0.15 µg/ml, ≥1.0 µg/ml, and individual titres ratio (V02/V01)
- Anti-PT and anti-FHA Ab titres ≥4 EU/ml, 4-fold increase, individual titres ratio (V02/V01)
- Anti-polio 1, 2, and 3 Ab titres ≥8 (1/dil), and individual titres ratio (V02/V01)

The booster response to P (PT and FHA) was defined in the SAP as follows:

1) Subjects whose pre-vaccination Ab concentrations were less than the lower limit of quantitation (<LLOQ) demonstrated a booster response if they had post-vaccination levels ≥4 x LLOQ

2) Subjects whose pre-vaccination Ab concentrations were ≥LLOQ but <4 x LLOQ demonstrated a booster response if they had a four-fold response (i.e. post-/pre-vaccination ≥4)

3) Subjects whose pre-vaccination Ab concentrations were ≥4 x LLOQ, demonstrated a booster response if they had a two-fold response (i.e. post-/pre-vaccination ≥2)

Results of A3L16

Participant flow

458 of the original 604 subjects who had completed study A3L02 were enrolled in this study. Of those 453 completed this study. All drop-outs are accounted for.

Conduct of the study

No relevant changes were made to the protocol.

Baseline data

In the ITT population, the mean age in both groups was similar, and there were similar proportions of males and females in each group. The two groups were still comparable.

Numbers analysed

All 458 subjects were included in the ITT population.

Outcomes and estimation of A3L16

Persistence of antibodies was similar in both groups for all antigens. Seroprotection was still given in the majority of subjects for most antigens and again in similar in both groups.

Seroprotection levels were achieved for all antigens in all subjects after the booster vaccination.

Individual titre ratios show significantly lower titres for Anti-PRP, Anti-T and Anti-FHA in Hexaxim primed subjects. The clinical relevance of this difference is unclear and should be explained by the applicant.

As in the other studies, proportion of subjects with anti-Hep Bs pre-boost seroprotection titres (≥10 mIU/ml) was higher in subjects primed with Pentaxim and Engerix B compared to those primed with Hexaxim. A Hep B booster has not been evaluated in this study.

Comparison of Hepatitis B results of all booster vaccination studies

In summary, for all booster studies (AL315, A3L22, A3L16 and A3L21) lower pre-boost GMTs and lower seroprotection rates have been found for the Hexaxim primary series when compared with Engerix, Tritanrix or Infanrix hexa (Table 18 below, marked in green).

In one arm of study A3L15 (group 2, primed with Engerix B) no booster vaccination has been administered. Nevertheless, at months 15 to 19, the Engerix group in this study still had a seroprotection rate of 92% (threshold: ≥10IU/ml), which was significantly higher compared to the primary series performed with Hexaxim (78.9%).

Following administration of a booster dose of Hexaxim (4^{th} dose), which has been done for all groups in all booster studies (apart from study A3L01 where Hexavac has been administered in a control group), a typical anamnestic antibody response resulting in high anti-HBs concentrations (ranging from 1379 to

44893) have been measured one month later. This effective response observed in all groups of healthy vaccinees confirms the presence of immunologic memory. Almost all subjects (97.3% to 100% of subjects) were seroprotected one month after booster vaccination with Hexaxim.

Table 18 Comparison of all GMTs and seroprotection rates regarding Hep B for all booster studies (PP Analyses)

Study	A3L15			A3L22 (Follow-up of A3L10)		A3L16 (Follow-up of A3L02)		A3L21 (Follow-up of A3L011)		A3L01	
priming	H	E (Group 2; no HepB-boost)	H (Group 3; with boost)	H	E	H	E	H (All batches)	I	IPV, Hib and HepB	
booster	H	-	H	H	H	Pentaxim		H	H	H	Hexavac
Preboost- GMT	51.3	103	228	44.2	223	87.6	197	93.3	127	231	157
Postboost -GMT	4630	-	44893	1379	26189	-	-	2553	4757	7890	2629
% ≥10mIU/ml Pre boost	78.9	92.0	94.7	80.7	99.0	85.5	99.5	89.8	95.4	100.0	97.0
% ≥10mIU/ml Post boost	98.5	-	100.0	97.3	100.0	-	-	99.4	100.0	100.0	100.0
% ≥100mIU/ml Pre boost	39.7	54.3	78.8	33.9	76.7	-	-	52.8	58.5	-	-
% ≥100mIU/ml Post boost	98.5	-	100.0	86.5	100.0	-	-	93.2	96.9	-	-
Assay	Ortho-ECi			Ortho-ECi		Ortho-ECi		Ortho-ECi		RIA	
Vaccination-schedule in month (priming)	1,5 - 2,5 - 3,5 (most condensed)			2 – 3 – 4 (most condensed)		2 – 4 - 6		2 – 4 - 6		2 – 4- 6	

A3L16: ITT Analyse Set used; A3L01: Full Analyse Set used; H= Hexaxim; E= EngerixB; I= Infanrix hexa

Summary of Main Efficacy Results

The established correlates and surrogates have been reached with Hexaxim regardless of vaccination scheme, concomitantly used vaccines, or vaccine used for priming. The end of shelf-life did not lead to significant differences in the immunogenicity of Hexaxim. Batch-to-Batch consistency was adequately

shown in two different studies. The majority of the clinical studies were made using the same formulation and scale of Hexaxim.

Differences between GMTs beyond those thresholds were originally been found between Hexaxim and the used control vaccines or if priming/booster had been done with other vaccines:

1. The EPI scheme with vaccinations at 6, 10, 14 weeks (A3L15ps) showed significantly higher GMTs for anti-D. After the booster with Hexaxim (A3L15bo) anti-T and anti-PRP were significantly lower than for the children primed with CombActHib. Lower pre-boost seroprotection rates regarding Hep B at month 15-18 for Hexaxim compared to Engerix (78.9 vs. 92.0%, respectively).
2. Condensed primary vaccination scheme with 2, 3 4 months (A3L10) showed significantly higher GMTs for FHA than Pentaxim vaccinated infants. After the booster with Hexaxim (A3L22) GMTs for anti-D and anti-T were significantly lower, anti-PRP somewhat lower with overlapping CIs. Pre-booster GMTs for Hep B were higher in the group primed with Pentaxim +Engerix than in the group primed with Hexaxim and the percentage of subjects with seroprotection was only 80.7% for the Hexaxim group versus 99% for the Engerix group. Especially, if no booster would follow in the second year of live, this could have an influence on the duration of protection. However, independent from the priming (Pentaxim plus Engerix or with Hexaxim) following booster vaccination with Hexaxim both groups showed a considerable anamnestic response.
3. The vaccination scheme 2, 4, 6 months has been evaluated in several studies using different comparators or Hexaxim only for priming:
    - Comparator Pentaxim+ Engerix:
        - significantly lower PT GMTs in the Hexaxim group versus Pentaxim (A3L02) with significantly lower GMT ratios for anti-T, anti-PRP and anti-FHA after boostering with Pentaxim (A3L16), anti-D was somewhat lower with overlapping CIs.
        - Lower pre-boost GMTs in study A3L16 regarding Hep B in the Hexaxim group compared to the Engerix group (85.5 vs. 99.5%, respectively)
    - Comparator Infanrix hexa:
        - significantly higher GMTs for anti-FHA and anti-PRP in the Hexaxim groups (A3L11 and A3L12)
        - significantly lower GMTs for anti-T and anti-PT in the Hexaxim group (A3L12).
        - Seroprotection rate for Hepatitis B based on the ≥100 mIU/ml threshold criterion one month after the third dose is higher in the Infanrix hexa group (99.2%) compared to the Hexaxim group (91.7%). Likewise, anti-Hep B GMTs were higher in the Infanrix hexa group compared to the Hexaxim group (ITT: 1545 vs. 1044, respectively). Moreover, lower Hep B-GMTs and lower rate of seroprotection at month 15 to 18 (pre-boost) were observed. However, following booster vaccination seroprotection rates against Hep B were sufficiently high and comparable between the two groups (A3L11).
        - Although in the majority of studies lower anti Poliovirus-GMTs were measured in the Hexaxim groups compared to the control vaccines given, this is not indicative for inferior clinical performance. GMTs exceeded by far the threshold of ≥ 8 (1/dil). Consequently, these differences are clinically not relevant.
    - Comparator Tritanrix:

- Following vaccination with Tritanrix threefold higher anti Hep B-GMTs were found compared to Hexaxim (3376 vs. 1075, respectively). However, based on the anti-Hep Bs thresholds of 10 and 100 mIU/ml, sufficiently high seroprotection rates in both groups one month after the third vaccination were measured (A3L04).

All other studies that described GMTs showed similar immune responses for Hexaxim and its comparator. Also, the clinical relevance of the differing results described above is estimated only to possibly affect the timing for next booster vaccinations. The applicant was asked to explain the significantly differing results and their possible effect on the timing of consecutive booster vaccinations. In response to this request it was seen that the unusual differences of GMTs seen in some studies here cannot be attributed to intrinsic or extrinsic factors. As also no trend is seen, the clinical relevance is judged negligible. The data provided by the applicant from ongoing study A3L26 seem conclusive in terms of comparability of the antibody responses with comparator vaccines. It can be assumed that duration of protection and following booster intervals will be similar across the studies.

In conclusion a full set of three primary vaccinations plus a booster dose are needed to achieve reliable protection.

The final data of study A3L24 and the planned study A3L28 should be supplied as soon as possible and will show the persistence of antibodies three years after the booster dose.

Summary of main studies

The following tables summarise the immunogenicity results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 19 Summary of Efficacy for trial A3L15 (primary series and booster)

Title: Immunogenicity Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to CombAct-Hib Concomitantly Administered with Engerix B Paediatric and OPV at 6, 10, and 14 weeks of Age in South African Infants			
Study identifier	A3L15		
Design	Randomized open-label, controlled 3-arm trial.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		24 months not applicable 6-month follow-up
Hypothesis	Non-inferiority		
Treatments groups	Treatment group		DTaP-IPV-HepB-PRP-T at 6, 10, and 14 weeks of age and booster dose at 15-18 months. In all children: measles vaccination at 40 weeks of age. Trimovax at 15 to 18 months of age.
	Control group		CombAct-Hib + OPV + Engerix B Pediatric at 6, 10, and 14 weeks of age and booster dose at 15-18 months.
Endpoints and definitions	Primary endpoint		Percentage of subjects with antibody titres above predefined cut-off.
	Secondary endpoint		Immunogenicity and safety
Database lock	19 August 2009		
Results and Analysis			

Analysis description		Primary Analysis								
Analysis population and time point description		Per protocol Following Primary Series Vaccination								
		Group1: Hexaxim			Group 2: CombAct-Hib + Engerix B + OPV			Hexaxim (Engerix B at birth)		
Antigen	Criteria	N	% or Mean	(95% CI)	N	% or Mean	(95% CI)	N	% or Mean	(95% CI)
Diphtheria	**≥ 0.01 IU/ml**	206	97.6	(94.4; 99.2)	206	96.1	(92.5; 98.3)	122	95.1	(89.6; 98.2)
	≥ 0.1 IU/ml	206	39.8	(33.1; 46.8)	206	13.6	(9.23; 19.0)	122	39.3	(30.6; 48.6)
	GMT	206	0.074	(0.062; 0.088)	206	0.040	(0.035; 0.046)	122	0.074	(0.059; 0.094)
Tetanus	**≥ 0.01 IU/ml**	213	100	(98.3; 100)	210	100	(98.3; 100)	122	100	(97.0; 100)
	≥ 0.1 IU/ml	213	100	(98.3; 100)	210	100	(98.3; 100)	122	100	(97.0; 100)
	GMT	213	1.51	(1.37; 1.65)	210	1.88	(1.70; 2.07)	122	1.33	(1.17; 1.51)
PT	**≥ 4-fold rise**	172	93.6	(88.8; 96.8)	137	83.2	(75.9; 89.0)	103	95.1	(89.0; 98.4)
	Vaccine response	172	100	(97.9; 100)	137	89.1	(82.6; 93.7)	103	100	(96.5; 100)
	GMT	192	332	(304; 362)	156	191	(147; 249)	108	288	(256; 323)
FHA	**≥ 4-fold rise**	160	93.1	(88.0; 96.5)	130	57.7	(48.7; 66.3)	90	90.0	(81.9; 95.3)
	Vaccine response	160	100	(97.7; 100)	130	93.8	(88.2; 97.3)	90	100	(96.0; 100)
	GMT	178	207	(190; 226)	153	37.4	(33.4; 41.9)	99	188	(166; 212)
Poliovirus 1	**≥ 8 (1/dil)**	186	100	(98.0; 100)	187	93.0	(88.4; 96.2)	104	99.0	(94.8; 100)
	GMT	186	579	(478; 702)	187	198	(153; 256)	104	557	(410; 756)
Poliovirus 2	**≥ 8 (1/dil)**	196	98.5	(95.6; 99.7)	192	100	(98.1; 100)	113	98.2	(93.8; 99.8)
	GMT	196	620	(512; 750)	192	446	(374; 533)	113	371	(281; 489)
Poliovirus 3	**≥ 8 (1/dil)**	182	100	(98.0; 100)	179	98.3	(95.2; 99.7)	98	100	(96.3; 100)
	GMT	182	975	(812; 1170)	179	228	(185; 280)	98	811	(645; 1020)
Hep B	**≥ 10 mIU/ml**	184	95.7	(91.6; 98.1)	194	95.4	(91.4; 97.9)	98	99.0	(94.4; 100)
	GMT	184	330	(259; 420)	194	148	(120; 181)	98	1913	(1457; 2513)
PRP	**≥ 0.15 µg/ml**	219	95.4	(91.8; 97.8)	212	100	(98.3; 100)	122	97.5	(93.0; 99.5)
	GMT	219	3.31	(2.69; 4.08)	212	5.18	(4.47; 6.00)	122	3.83	(2.92; 5.02)

N: number of subjects analyzed according to the PP Analysis Set
%: percentage and 95% CI are calculated according to the number of subjects with available data for the relevant endpoint

Notes		Non-inferiority for tested antigen(s) was demonstrated.								
Analysis population and time point description		Per protocol Following Booster Vaccination								
		Booster vaccination								
		Hexaxim			CombAct-Hib + OPV			Hexaxim		
		Vaccines assigned at primary series								
Antigen	Criteria	Hexaxim			CombAct-Hib + Engerix B OPV			Hexaxim with Engerix B at birth		
		N	% or Mean	(95% CI)	N	% or Mean	(95% CI)	N	% or Mean	(95% CI)
Diphtheria	**≥ 0.1 IU/ml**	195	100	(98.1; 100)	200	99.0	(96.4; 99.9)	111	100	(96.7; 100)

	≥ 1.0 IU/ml	195	97.9	(94.8; 99.4)	200	93.0	(88.5; 96.1)	111	93.7	(87.4; 97.4)
	GMT	195	9.37	(8.05; 10.9)	200	3.33	(2.92; 3.80)	111	7.00	(5.61; 8.72)
Tetanus	**≥ 0.1 IU/ml**	200	100	(98.2; 100)	199	100	(98.2; 100)	114	100	(96.8; 100)
	≥ 1.0 IU/ml	200	98.0	(95.0; 99.5)	199	99.5	(97.2; 100)	114	96.5	(91.3; 99.0)
	GMT	200	10.0	(8.65; 11.7)	199	8.23	(7.49; 9.04)	114	8.13	(6.68; 9.89)
PT	**≥ 4-fold rise**	153	94.8	(90.0; 97.7)	133	83.5	(76.0; 89.3)	99	93.9	(87.3; 97.7)
	Booster response	153	97.4	(93.4; 99.3)	133	91.7	(85.7; 95.8)	99	96.0	(90.0; 98.9)
	GMT	187	288	(260; 318)	184	110	(88.7; 137)	109	235	(206; 268)
FHA	**≥ 4-fold rise**	159	91.2	(85.7; 95.1)	143	96.5	(92.0; 98.9)	94	94.7	(88.0; 98.3)
	Booster response	159	94.3	(89.5; 97.4)	143	99.3	(96.2; 100)	94	97.9	(92.5; 99.7)
	GMT	184	570	(514; 630)	190	211	(193; 231)	105	472	(419; 533)
Poliovirus 1	**≥ 8 (1/dil)**	189	100	(98.1; 100)	191	97.4	(94.0; 99.1)	108	100	(96.6; 100)
	GMT	189	7298	(6202; 8588)	191	329	(260; 417)	108	5346	(4309; 6633)
Poliovirus 2	**≥ 8 (1/dil)**	191	100	(98.1; 100)	190	100	(98.1; 100)	107	100	(96.6; 100)
	GMT	191	6637	(5745; 7668)	190	863	(665; 1118)	107	4190	(3460; 5074)
Poliovirus 3	**≥ 8 (1/dil)**	188	100	(98.1; 100)	187	98.9	(96.2; 99.9)	108	100	(96.6; 100)
	GMT	188	6411	(5525; 7439)	187	315	(245; 404)	108	5144	(4156; 6367)
Hep B	**≥ 10 mIU/ml**	197	98.5	(95.6; 99.7)	196	90.3	(85.3; 94.1)	113	100	(96.8; 100)
	GMT	197	4630	(3402; 6302)	196	86.2	(69.2; 107)	113	44893	(33652; 59890)
PRP	**≥ 1.0 µg/ml**	203	98.5	(95.7; 99.7)	201	98.5	(95.7; 99.7)	115	100	(96.8; 100)
	GMT	203	68.5	(55.7; 84.2)	201	52.2	(43.9; 62.2)	115	63.1	(47.6; 83.8)

N: number of subjects analyzed according to the PP Analysis Set
%: percentage and 95% CI are calculated according to the subjects with available data for the relevant endpoint

Table 20 Summary of Efficacy for trial A3L04

Title: Large Scale Safety Study of a DTaP-IPV-Hep B-PRP-T Combined Vaccine, in Comparison to Tritanrix-Hep B/Hib and OPV Administered at 2, 4, and 6 Months of Age in Latin American Infants			
Study identifier	A3L04		
Design	Randomized, controlled, observer-blind, 4-arm, parallel groups trial		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		300 days not applicable 6-month follow-up
Hypothesis	Non-superiority		
Treatments groups	Treatment group		Hexaxim + placebo Oral Poliovirus Vaccine (OPV) at 2, 4, and 6 months of age.
	Control group		Tritanrix-Hep B/Hib injection + Oral Poliomyelitis Vaccine (OPV) at 2, 4, and 6 months of age
Endpoints and definitions	Primary endpoint		Occurrence of at least one high fever episode (greater or equal to 39.6"C rectal temperature equivalent) within 7 days after any of the 3 injections to each subject
	Secondary endpoint		Immunogenicity and safety
Database lock	19 February 2008		

Results and Analysis

Analysis description	**Secondary analysis**
Analysis population and time point description	Per protocol Following Primary Series Vaccination

Antigen	**Criteria**	**Hexaxim**			**Tritanrix-HepB/Hib+OPV**		
		N	**% or Mean**	**(95% CI)**	**N**	**% or Mean**	**(95% CI)**
Hep B	**≥ 10 mIU/ml**	183	100	(98.0; 100)	94	100	(96.2; 100)
	GMT	183	1075	(890; 1300)	94	3364	(2611; 4334)

N: number of subjects analyzed according to the PP Analysis Set

%: percentage and 95% CI are calculated according to the number of subjects with available data for the relevant endpoint

Table 21 Summary of Efficacy for trial A3L11

Title: Lot-to-Lot Consistency Study of DTaP-IPV-Hep B-PRP-T Vaccine Administered at 2-4-6 Months of Age in Healthy Mexican Infants			
Study identifier	A3L11		
Design	Randomized, observer-blinded, controlled, 4-arm, lot-to-lot consistency trial.		
	Duration of main phase:		10 months
	Duration of Run-in phase:		not applicable
	Duration of Extension phase:		6-month follow-up
Hypothesis	Equivalence		
Treatments groups	Treatment group		Hexaxim at 2, 4, and 6 months of age.
	Control group		Infanrix hexa at 2, 4, and 6 months of age.
Endpoints and definitions	Primary endpoint		To demonstrate the equivalence of 3 batches of Hexaxim in terms of seroprotection rates for D, T, Hep B, PRP, and polio and seroconversion rates for PT and FHA 1 month after the 3rd dose according to predefined cut-off.
	Secondary endpoint		Immunogenicity and safety
Database lock	31 July 2008		

Results and Analysis

Analysis description	**Primary Analysis - Equivalence**
Analysis population and time point description	Per protocol Following Primary Series Vaccination

Criteria	Batch 1 Hexaxim			Batch 2 Hexaxim			Batch 3 Hexaxim			Equivalence analysis		
	n/M	%	(95%CI)	n/M	%	(95%CI)	n/M	%	(95%CI)	Batches	(90%CI)	EQ Y/ N
Anti-D ≥ 0.01 IU/ml	220/ 231	95.2	(91.6; 97.6)	228/ 236	96.6	(93.4; 98.5)	222/ 228	97.4	(94.4; 99.0)	1 vs. 2 1 vs. 3 2 vs. 3	(-4.60;1.75) (-5.27; 0.87) (-3.58; 2.04)	Y
Anti-T ≥ 0.01 IU/ml	231/ 231	100	(98.4; 100)	236/ 236	100	(98.4; 100)	227/ 227	100	(98.4; 100)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.16; 1.13) (-1.16; 1.18) (-1.13; 1.18)	Y
Anti-PT ≥ 4-fold rise	223/ 228	97.8	(95.1; 99.3)	226/ 234	96.6	(93.4; 98.5)	218/ 233	97.8	(94.8; 99.3)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.46; 4.01) (-2.47; 2.60) (-3.97; 1.55)	Y
Anti-FHA ≥ 4-fold rise	225/ 227	99.1	(96.9; 99.9)	229/ 233	98.3	(95.7; 99.5)	216/ 221	97.7	(94.8; 99.3)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.15; 2.97) (-0.71; 3.77) (-1.81; 3.04)	Y
Anti-polio 1 ≥ 8 l/dil	230/ 230	99.6	(97.6; 100)	236/ 236	100	(98.4; 100)	225/ 225	100	(98.4; 100)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.92; 0.75) (-1.92; 0.80) (-1.13; 1.19)	Y
Anti-poliovirus 2 ≥ 8 l/dil	230/ 230	100	(98.4; 100)	236/ 236	100	(98.4; 100)	226/ 226	100	(98.4; 100)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.16; 1.13) (-1.16; 1.18) (-1.13; 1.18)	Y
Anti-poliovirus 3 ≥ 8 l/dil	229/ 230	99.6	(97.6; 100)	235/ 235	100	(98.4; 100)	226/ 226	100	(98.4; 100)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.93; 0.75) (-1.93; 0.79) (-1.14; 1.18)	Y
Anti-Hep B ≥ 10 mIU/ml	226/ 230	98.3	(95.6; 99.5)	231/ 234	98.7	(96.3; 99.7)	221/ 226	97.8	(94.9; 99.3)	1 vs. 2 1 vs. 3 2 vs. 3	(-2.67; 1.65) (-1.89; 2.93) (-1.27; 3.32)	Y
Anti-PRP ≥ 0.15 µg/ml	229/ 231	99.1	(96.9; 99.9)	232/ 236	98.3	(95.7; 99.5)	226/ 228	99.1	(96.9; 99.9)	1 vs. 2 1 vs. 3 2 vs. 3	(-1.12; 2.94) (-1.80; 1.84) (-2.93; 1.15)	Y

EQ: equivalence

n: number of subjects
M: number of subjects available for the endpoint

Results and Analysis

Analysis description	**Primary Analysis – Non-inferiority**
Analysis population and time point description	Per protocol Following Primary Series Vaccination

		Hexaxim*			**Infanrix hexa**		
Antigen	**Criteria**	**N**	**% or Mean**	**(95% CI)**	**N**	**% or Mean**	**(95% CI)**
Diphtheria	**≥ 0.01 IU/ml**	695	96.4	(94.7; 97.7)	119	99.2	(95.4; 100)
	≥ 0.1 IU/ml	695	62.7	(59.0; 66.3)	119	55.5	(46.1; 64.6)
	GMT	695	0.196	(0.173; 0.222)	119	0.173	(0.132; 0.226)
Tetanus	**≥ 0.01 IU/ml**	694	100	(99.5; 100)	119	100	(96.9; 100)
	≥ 0.1 IU/ml	694	99.3	(98.3; 99.8)	119	100	(96.9; 100)
	GMT	694	1.84	(1.72; 1.98)	119	2.20	(1.93; 2.52)
PT	**≥ 4-fold rise**	685	97.4	(95.9; 98.4)	118	95.8	(90.4; 98.6)
	Vaccine response	685	100	(99.5; 100)	118	98.3	(94.0; 99.8)
	GMT	691	240	(230; 251)	119	228	(205; 254)
FHA	**≥ 4-fold rise**	681	98.4	(97.1; 99.2)	115	96.5	(91.3; 99.0)
	Vaccine response	681	100	(99.5; 100)	115	99.1	(95.3; 100)
	GMT	690	239	(229; 250)	118	182	(165; 200)
Poliovirus 1	**≥ 8 (1/dil)**	692	99.9	(99.2; 100)	119	100	(96.9; 100)
	GMT	692	882	(803; 970)	119	1370	(1082; 1736)
Poliovirus 2	**≥ 8 (1/dil)**	692	100	(99.5; 100)	118	100	(96.9; 100)
	GMT	692	1655	(1507; 1818)	118	2337	(1878; 2909)
Poliovirus 3	**≥8 (1/dil)**	691	99.9	(99.2; 100)	117	100	(96.9; 100)
	GMT	691	1106	(1005; 1218)	117	2186	(1752; 2727)
Hep B	**≥ 10 mIU/ml**	690	98.3	(97.0; 99.1)	119	100	(96.9; 100)
	GMT	690	1142	(1012; 1289)	119	1576	(1283; 1934)
PRP	**≥ 0.15 µg/ml**	695	98.8	(97.7; 99.5)	119	99.2	(95.4; 100)
	GMT	695	12.2	(10.8; 13.7)	119	6.68	(5.10; 8.74)

N: number of subjects analyzed according to the PP Analysis Set
%: percentage and 95% CI are calculated according to the number of subjects with available data for the relevant endpoint
*: 3 lots pooled of Hexaxim

Notes	- Equivalence for consistency batches was demonstrated. - Non-inferiority for tested antigen(s) was demonstrated.

Table 22 Summary of Efficacy for trial A3L17

Title: Immunogenicity Study of DTaP-IPV-Hep B-PRP-T Combined Vaccine in Comparison to Infanrix hexa, at 2-4-6 Months of Age in Healthy Peruvian Infants			
Study identifier	A3L17		
Design	Randomized, observer-blind, controlled, 2-arm trial.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	204 days not applicable 6-month follow-up	
Hypothesis	Non-inferiority		
Treatments groups	Treatment group		DTaP-IPV-HepB-PRP-T at 2, 4, and 6 months of age.
	Control group		Infanrix hexa.
Endpoints and definitions	Primary endpoint		Anti-Hep Bs antibody (Ab) titres 1 month after the 3rd dose of the primary series.
	Secondary endpoint		Immunogenicity and safety
Database lock	24 June 2009		

Results and Analysis

Analysis description	**Primary Analysis**
Analysis population and time point description	Per protocol Following Primary Series Vaccination

Antigen	**Criteria**	**Group 1: Hexaxim**			**Group 2: Infanrix hexa**		
		N	**% or Mean**	**(95% CI)**	**N**	**% or Mean**	**(95% CI)**
Diphtheria	**≥ 0.01 IU/ml**	132	95.5	(90.4; 98.3)	130	100	(97.2; 100)
	≥ 0.1 IU/ml	132	58.3	(49.4; 66.8)	130	65.4	(56.5; 73.5)
	GMT	132	0.156	(0.119; 0.204)	130	0.192	(0.154; 0.239)
Hep B	**≥ 10 mIU/ml**	132	99.2	(95.9; 100)	130	100	(97.2; 100)
	GMT	132	986	(764; 1270)	130	1139	(961; 1350)
PRP	**≥ 0.15 µg/ml**	132	100	(97.2; 100)	130	99.2	(95.8; 100)
	GMT	132	5.22	(4.04; 6.73)	130	3.93	(3.17; 4.89)

N: number of subjects analysed according to PP Analysis Set
%: percentage and 95% CI are calculated according to the subjects available for the endpoint

Notes	Non-inferiority for tested antigen(s) was demonstrated.

Analysis performed across trials (pooled analyses and meta-analysis)

A pooled analysis is provided for the 2, 4, 6 months without hepB at birth using studies in Latin America (A3L02,A3L04, A3L11 and A3L17):

- For the Pertussis antigens PT and FHA 96% and 97% respectively have reached a ≥4 fold increase of GMTs
- 100% achieved a short-term, 99,5% a long-term protection against Tetanus
- 97,1% achieved a short-term, 62,6% a long-term protection against Diphtheria
- 98% achieved a short-term, 90,2% a long-term protection against Haemophilus influenza b after primary vaccination.

- 99.9-100% reached seroprotection against Polio types 1, 2 and 3
- 98.8% achieved seroprotection (≥10mIU/ml) against Hep B (regarding a threshold of ≥100mIU/ml 93.0 % were seroprotected)

These results are satisfactory taking into account that normally the booster vaccination follows well before the long-term protection time-span (usually 5-10 years) for anti-D will be of importance.

Clinical studies in special populations

Specific studies were not carried out. Premature infants were only included if they had ≥2000g at birth. Immunocompromised infants were excluded from studies.

69% of included subjects have been Hispanic. However, Caucasian, Asian and Black participants have been enrolled as well, which is considered important as Hexaxim is planned to be used in different counties in the international area.

Supportive studies

Further supportive studies are not available.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Design and conduct of clinical studies

The overall ethics, conduct, and design of the studies are satisfactory. During the clinical development all major primary vaccination schemes have been tested. Also, the major ethnicities have been subject to the trials, even though a strong focus lies on South and Central America. The studies were conducted on all continents with the exemption of Australia and took place with a wide range of locally used comparators and prior vaccinations (BCG and/or HepB at birth). This is considered obligatory for a childhood vaccine intended for global use under the recommendation of the WHO.

Concerning this overall approach the clinical development programme follows the recommendations laid down in the WHO "Guidelines on clinical evaluation of vaccines: regulatory expectations" and the EMA "Guideline on clinical evaluation of new vaccines".

Nevertheless, there are some points not covered in the studies:

- Immunogenicity of immunosuppressed individuals; here, the applicant is requested to plan and conduct a study in HIV+ or other immune-compromised children to generate real data in this relevant population. The per se exclusion of HIV+ children was considered not acceptable as this vaccine is intended to be used in countries with high HIV burdens and comparatively low use or possibility of medical treatment for this life-long condition due to the heavy burden on the public medical system. It is expected that the actual protection will be of a high relevance for national recommendation boards and regulatory authorities especially in developing countries.
- Possible influence of the concomitant use on the immunogenicity of Prevenar 7 (see study A3L12) and Rotarix; here, the applicant is asked to supply the final data from study A3L24 in a variation procedure. Newer pneumococcal vaccines (and Prevenar 13) will be tested in further studies planned to take place in the EU.

- No concomitant use study for other relevant childhood vaccines (Recommended Routine Immunizations for Children - Summary of WHO Position Papers, [24]) except Pneumococcal and MMRV vaccines

These shortcomings should be bared in mind considering other observations made in the healthy infants studied.

Efficacy data and additional analyses

The specific WHO guidance given in the weekly epidemiological records (WER) was taken into consideration, and the conclusions are summarised below by antigen:

Antigens contained in Hexaxim:

- ***Tetanus:***

Immunological protective threshold has been shown as required using validated assays.

Primary vaccinations follows the recommended age, the timing of the booster is given as between 4-7 years in the recommendations. This is not adhered to in the trials that rather use a booster at the age of 2 years irrespective of the primary schedule used. Despite the lower than expected GMTs seen in some of the booster trials there does not seem to be a necessity for a second booster prior to the then recommended 12-15years by the WHO. The data from study A3L26 will help to estimate further booster timing and should be supplied as soon as available.

- ***Diphtheria:***

Immunological protective threshold has been shown as required using validated assays.

Here primary as well as booster recommendations of the WHO are fully covered in the tested schedules. A further booster is advised for the age of 4-7 years. Given the fact that long-term protection thresholds were achieved in all studies after the booster dose the significantly lower GMTs seen in the condensed schedule study are not considered clinically relevant when taking into account that the next booster for this population should be given within the next 5 years according to the recommendations.

- ***Hib***

Immunological protective threshold has been shown as required using validated assays.

Here primary as well as booster recommendations of the WHO are fully covered in the tested schedules. The necessity of further boosters or the duration of protection is not specifically discussed as the vulnerability against the disease wanes rapidly beyond the second year of life.

The significantly lower GMTs seen in some of the studies might be due to formulation especially in comparison with the used comparator vaccines (CombActHib, Pentaxim and Infanrix hexa) are not expected to give reason for clinical concern as the protective thresholds were achieved in all cases.

- ***Pertussis***

The WHO reports that although 3- and 4-component acellular Pertussis vaccines might have hinted a higher protection in clinical studies one- and two-component acellular Pertussis vaccines have shown the same high-level protection against disease in the long-term large-scale use. This is important as so far no accepted correlate of protection (and thus, antibody titre threshold) exists.

The primary and booster vaccination timing recommendation (3 doses within first year of life and booster in the second year) are covered in the studies provided here. Further boosters are so far not considered

necessary before adulthood (to provide protection of vulnerable persons, e.g. new-borns or in special settings, e.g. care-givers).

Taking all this into account the clinical relevance of significantly lower GMTs for the FHA-component seen in some studies is unknown but not expected to be of concern.

It is acknowledged that the acellular Pertussis vaccines provide a lower protection than whole-cell formulations and need at least 2 doses to be protective. According to the WHO no data suggest that switching between wP- and aP-containing vaccines negatively affects protection rates.

- ***Polio***

According to the WHO position paper a primary series of 3 doses IPV should be administered beginning at 2 months of age. In case the primary series starts earlier (for example when following a 6-week, 10-week and 14-week schedule as in study A3L15) a booster dose should be administered after an interval of ≥6 months.

In all studies sufficiently high GMTs (between 100 and 4100) as well as sufficiently high seroprotection rates (94.7%-100%) have been observed for all 3 poliovirus types following completion of the primary series consisting of three doses. In two studies (A3L15 and A3L02) it was demonstrated that following administration of Hexaxim anti-Poliovirus titres relevant for seroprotection were non-inferior compared to the control vaccines (Tritanrix HepB/Hib +OPV or Pentaxim + Engerix B). The other studies provided descriptive analyses only. Although in the majority of studies lower GMTs were observed in the Hexaxim groups compared to the control vaccines, this is not indicative for clinical inferiority. Routinely, GMTs by far exceeded the threshold of ≥ 8 (1/dil).

The vaccination schedule for Hexaxim foresees a 4th dose in the second year of life. For all booster studies descriptive analyses of the polio immune response have been provided. GMTs were still sufficiently high at the beginning of the second year of life and further increased following booster vaccination with Hexaxim. Pre-boost seroprotection rates for all three poliovirus types were between 85% and 100%. Following booster vaccination with Hexaxim 100% of subjects were seroprotected indicating effective priming.

- ***Hepatitis B (wer8440)***

In all studies the amount of HepB antigen used in the various vaccines was identical (10µg).

Development of an anti-HBs response exceeding 10 mIU/ml is generally accepted as a correlate for protective immunity against hepatitis B. Such levels of protective immunity have been observed in all clinical trials conducted with Hexaxim following the primary series (seroprotection rates between 94.0 and 100%).

Although children born to HepB infected mothers have been excluded from all clinical trials the effect of a HepB vaccine administered directly after birth has been evaluated (A3L15, A3L4 andA3L12). In these studies a positive effect of a HepB dose given at birth, as recommended in several endemic regions, has been demonstrated. Following the WHO position paper (wer8440) a HepB-at-birth-dose should be followed by 3 Hep B-doses with a minimum interval of 4 weeks in case a multivalent vaccine is used. This recommendation was followed in all clinical trials performed. Starting with the primary vaccination series at an age of 6 to 8 weeks, it has been demonstrated that less condensed schedules (month 2-4-6; in studies: A3L02, A3L04, A3L11, A3L12 and A3L17) resulted in increased anti-HBs titer compared to the more condensed schedules (1.5-2.5-3.5 month; in studies A3L15 and A3L10). However, seroprotection was sufficient in all studies.

Comparing different Hep B vaccines (Engerix, Tritanrix or Infanrix hexa or Hexaxim) in various studies (A3L10, A3L04, A3L11 and A3L17) higher GMTs have been measured for these vaccines compared to the Hexaxim groups. Moreover, these studies demonstrated that for the more conservative threshold for

protection (≥ 100mIU/ml), higher seroprotection rates were generated by the comparator vaccines than with Hexaxim (A3L10: 64.9% vs. 78.1%; AL304: 96.2% vs. 98.9%; A3L11: 91.7% vs. 99.2% and A3L17: 93.9% vs. 99.2%, respectively). Since it is known that higher anti-HBs concentrations will take longer to decline below the minimum protective threshold value of ≤ 10mIU/ml lower GMTs could potentially be interpreted as a signal for reduced persistence of protection. This should be followed carefully on a long-term basis. One on-going study (A3L26) is already considering this fact.

The Applicant further committed to perform two other long-term-protection studies in children 3.5 or 4.5 years of age (A3L26 and A3L28).

According to WHO recommendations there is no compelling evidence for recommending administration of a booster dose of hepatitis B vaccine in routine immunization programs. However, the vaccination schedule of Hexaxim foresees a forth dose in the second year of life. In all booster studies (AL315, A3L22, A3L16 and A3L21) lower pre-boost GMTs and lower seroprotection rates have been found for Hexaxim when compared to Engerix or Infanrix hexa- has been used for primary vaccination.

In one arm of study A3L15 (group 2, primed with Engerix B) no booster dose has been administered. At months 15 to 19, the Engerix group still had a seroprotection rate of 92% (threshold: ≥10IU/ml), which was significantly higher than after priming with Hexaxim (78.9%).

Following booster vaccination with Hexaxim (4th dose), which has been used for all groups in all booster studies (apart from study A3L01 where Hexavac has been administered in a control group), a robust anamnestic antibody response resulting in high anti-HBs titer concentrations (ranging from 1379 to 44893) were measured one month later. This effective response was observed in all groups of healthy vaccinees and confirms the presence of a functional immunologic memory.

Responses to antigens of concomitant vaccines

- ***Pneumococcal conjugate vaccines:***

The WHO recommends the use of conjugated pneumococcal vaccines especially in countries with a high epidemiological burden and in high-risk populations (e.g. HIV positives). Here the vaccine should be given in three doses with optional booster (to be studied at the time of the WER) and ideally concomitantly with other childhood vaccines. The WER does not elaborate on possible immunological interferences. Thus, the study using Prevenar 7 (A3L12) is completely in line with the recommendation. Nevertheless, since then other pneumococcal conjugate vaccines have been licensed in the EU and globally (Synflorix and Prevenar13) and studies showed that not only antibody titres might be lowered by increasing the number of serotypes in the vaccines (compared to the 7-valent Prevenar) but also that concomitantly given childhood vaccines can additionally lower immunogenicity of some serotypes. The clinical relevance of these findings is still under discussion and will finally only be resolved by extensive epidemiological surveillance. It should be taken into account that this effect might be needed to be studied further in the future especially if other conjugated pneumococcal vaccines are given.

Given the lack of data in study A3L12 in regard to the effect of concomitant use on the serotypes of Prevenar 7 a concomitant use cannot yet be recommended. The data from study A3L24 should be submitted as soon as the study is completed. Also, as further studies are planned with the newer pneumococcal vaccines in the EU (A3L38, A3L39 and A3L40) those data should be taken into account when available as well.

- ***MMRV***

Routine immunizations with MMRV vaccines are usually scheduled for the second year of life. A second dose after a minimum interval of 1 month is standard for some national immunization programmes. For the time being WHO does not recommend routine varicella vaccination for developing countries.

In study A3L015s concomitant use of MMR vaccine Trimovax (Schwarz strain, Urabe AM9 strain and Wistar RA 27/3M) and Varicella vaccine Varilix (Oka strain) together with Hexaxim (group 1) or with ComActHib + OPV (group 2) has been investigated. Administration of MMRV did not impair immunogenicity of Hexaxim components.

When comparing GMTs as well as seroprotection rates for measles, mumps, rubella and varicella components no clinically relevant differences have been found between group 1 (Hexaxim) and group 2 (CombAct Hib + OPV).

For measles and rubella established titres correlating with protection have been exceeded by virtually all study subjects (100% and 97.4%, respectively).

For the mumps component an established correlate is not defined. 96.9% of the vaccines obtained antibody titer ≥60 l/dil (used as a cut-off set for the neutralisation assay).

For the varicella component only 81.8% of subjects developed titres correlating with the minimum correlate for protection of ≥ 4l/dil. This finding is particularly important as some countries do not recommend a second (booster) dose of varicella vaccine.

Historically, a single dose of currently licensed varicella vaccines generates seroconversion rates of about 95% of healthy children (WER 7332).

Since no comparison of a concomitant use (Hexaxim plus MMRV) versus non-concomitant administration (Hexaxim and MMRV given at different time points) was performed differences observed in the concomitant use study and historic experience regarding anti varicella protection rates must currently be interpreted as an immunological interference phenomenon precluding simultaneous administration of both vaccines at the same time.

2.5.4. Conclusions on the clinical efficacy

Overall the clinical efficacy was considered satisfactory regardless of the primary vaccination scheme if a booster dose is given. Minor deviations in the GMTs were not considered clinically relevant.

All populations studied showed similar immunological data.

The CHMP however noted that children at high-risk (e.g. HIV+) were not studied yet. The per se exclusion of HIV+ children was considered to be of concern as this vaccine is intended to be used in countries with high HIV burdens and comparatively low use or possibility of medical treatment for this life-long condition due to the heavy burden on the public medical system. It is expected that the actual protection will be of a high relevance for national recommendation boards and regulatory authorities especially in developing countries. The applicant committed to plan and conduct a study in HIV+ or other immune-compromised children to generate real data in this relevant population.

The CHMP considers the following measures necessary to address issues related to efficacy:

- The applicant already undertakes and planned follow-up studies in which the duration of protection is studied for all valences of Hexaxim to determine the timing and necessity of future booster vaccinations, the data should be supplied as soon as possible.
- The CHMP agreed with the applicant's plans to study Safety and Immunogenicity of Hexaxim in immunosuppressed infants.
- Studies in preterm infants should be considered.
- Further studies for concomitant use with immunogenicity results for all valences of all vaccines concerned in these studies should be envisaged.
- Further studies of concomitant use of rotavirus and pneumococcal vaccines are either already on-going or planned and the data should be provided as soon as possible
- Further studies in non-Hispanic populations and regions would be desirable.

2.6. Clinical safety

In view of the study design, safety was a secondary objective for all clinical studies submitted, except study A3L04 where the safety was a primary objective.

Patient exposure

Overall there were 12057 doses administered in these studies:

- 10546 doses were administered to 3631 infants in the 7 primary series trials. Of them, 3434 subjects received a full 3 doses Hexaxim primary series, and completed the studies.
- 1511 doses were administered to toddlers in 4 booster studies. Of the 1511 subjects who received a booster dose, 1243 had been primed with Hexaxim.

Adverse events

Hexaxim has a slightly higher reactogenicity regarding solicited local and systemic events/reactions as compared to Pentaxim + Engerix, but it is lower in comparison with the preceding product Hexavac.

There was a tendency for higher reactogenicity of Hexaxim as compared to Infanrix hexa, especially regarding injection site reactions. In addition, a higher percentage of injection site reactions and pyrexia in Hexaxim + Prevenar as compared to Infanrix hexa + Prevenar was observed.

Overall, the reactogenicity profile of Hexaxim was shown to be similar to, or better than, that of the Tritanrix-Hep B/Hib + OPV control vaccine.

Serious adverse events and deaths

Overall, within the eleven completed studies, 205 of 3896 subjects (5.3%) reported a total of 247 serious adverse events following Hexaxim administration.

The most frequently reported SAEs were of infectious nature: gastroenteritis (n=51), bronchiolitis (n=30), bronchopneumonia (n=23), pneumonia (n=22). In addition, 13 cases of febrile convulsions and 1 case of convulsion, none of them considered related, were reported. SAEs occurred with a similar frequency in Hexaxim and control groups.

Out of 247 SAEs reported, one SAE was considered related to the administration of Hexaxim.

Subject A3L04-002-01241, a seven-week-old female subject, presented with pallor, hypotonia, hyporesponsiveness and dyspnoea 7 hours after first dose of Hexaxim, and was diagnosed with hypotonic hyporesponsive episode (HHE). Event lasted 3 hours. The subject spontaneously recovered and was discontinued from the study.

Identified risks

One case of HHE and 2 cases of ELS were reported after administration of Hexaxim.

Important potential risks

Convulsions

A total of 14 subjects experienced 2 episodes of convulsions and 13 episodes of febrile convulsions in the Hexaxim or Hexaxim + OPV groups. All cases but one were considered serious; none was considered related by the investigator.

Other convulsive disorders

Two additional subjects were diagnosed with epilepsy and West syndrome (infantile spasms), respectively 17 days and 59 days after vaccination. These events were not considered related by the investigator.

Anaphylactic reactions

No cases of anaphylaxis were identified, with respect to Brighton Collaboration case definition.

Apnoea

Two subjects presented with apnoea episodes in Hexaxim arms. Of these, one subject had not yet received Hexaxim. The second patient developed life-threatening apnoea episodes 19 days after first dose of Hexaxim, in a context of cough and rhinitis, which may explain the occurrence of the event.

A third subject presented with breath holding one day after the second dose of Hexaxim, and was diagnosed with breath holding spells. Breath holding spells are considered as inappropriate psychic reaction to stress and pain and always have a spontaneous favourable outcome.

No cases of apnoea were considered related by the investigator.

Severe neurological conditions

No case of encephalopathy was reported after vaccination with Hexaxim so far.

No cases of ADEM were reported during the clinical trial program.

Two subjects developed encephalitis and viral meningoencephalitis respectively 53 days and 29 days post immunization. Although causal virus was not identified, CSF analyses, context of flavivirus outbreak in encephalitis case, and prompt recovery within 5 to 9 days were consistent with the reported or suspected viral aetiology.

Sudden Infant Death Syndrome / Sudden Unexplained Death

During the clinical trials evaluating Hexaxim, one African subject (A3L15-001 S0430) died at the age of 3 days, after receiving intradermal BCG vaccine, and before being included in a randomized arm. The death certificate indicated natural causes, and no autopsy was performed. No cases of SIDS or SUD were reported after administration of Hexaxim.

Deaths

Eleven subjects died while included in the Hexaxim arms of the completed studies. None was considered related to the study vaccine administered.

Laboratory findings

Study A3L01: Phase-I Safety of a Booster Dose of Either the Investigational DTaP-IPV-HB-PRP~T Combined Vaccine or HEXAVAC in Healthy Argentinean 16-to 19-Month-Old Toddlers:

At the screening visit, biological parameters were in the normal range for both groups, except for one subject in the Hexaxim group with a low haemoglobin level (<10 g/dl) (subject 001-00009 = 8.0 g/dl).

At V03 (D30 to D37) post dose, six subjects had abnormal laboratory values, however, none of these out-of-range values was clinically significant as judged by the Investigator. Hexaxim group: Two subjects had haemoglobin level < 10 g/dl: Subject 001-00054 had 9.4 g/dl and the other one was the subject with haemoglobin level <10 g/dl at screening (subject 001-00009 = 8.9 g/dl). Two other subjects had white blood cells counts >15,000/mm3 (subject 001-00007 = 16,000/mm3 and subject 001-00017 = 22,000/mm3). HEXAVAC group: Two subjects had haemoglobin< 10 g/dl (subject 001-00036 = 9.9 g/dl and subject 001-00053 = 9.6 g/dl).

Although six subjects showed abnormal laboratory values none of these out-of-range values was clinically significant as judged by the Investigator. The CHMP concurs with this judgment.

Immunological events

No anaphylactic reaction was identified using the Brighton Collaboration case definition.

A total of 14 subjects presented with 15 related allergic type events. Of these, 13 events were reported within 3 days post immunization and 2 more than 3 days post immunization (2 injection site rash occurred at 5 days and 11 days post immunization, respectively). All reactions were not serious and are detailed below.

Nine subjects presented with injection site allergic reactions: injection site dermatitis (n=1), injection site pruritus (n=1), injection site rash (n=4), injection site urticaria (n=2), injection site vesicle (n=1).

Five subjects experienced systemic allergic reaction: rash (n=1), rash generalized (n=1), rash maculopapular (3 subjects, 4 events).

No difference was observed in the occurrence of these allergic reactions between males and females. Intensity for each reaction was assessed as Grade 1 for 10 reactions, Grade 2 for 2 reactions, Grade 3 for 2 reactions and the recorded intensity was missing for 1 reaction. Duration of events varied from 1 to 8 days, 66% of subjects (10/15) recovered within 4 days.

The frequency of hypersensitivity reaction was 3.6 per 1000 subjects, and 12.4 per 10,000 doses. Nature and intensity of hypersensitivity reactions are consistent with expected safety profile of similar combined vaccines.

Safety related to concomitant use

Study A3L12: Concomitant use of Hexaxim or Infanrix hexa with Prevenar 7 (Thailand):

There was a higher rate of injection site pain in the Hexaxim group (78.5% post-dose 1, with 95% CI: 72.3; 84.0) than in the Infanrix hexa group (65.5% post-dose 1, with 95% CI: 58.6; 72.0). Grade 2

injection site swelling was significantly more frequent in the Hexaxim group. The grade 3 reactions are similar in both groups for all solicited local and systemic reactions.

Pyrexia after the first dose was more frequent in the Hexaxim group (53.2% with 95% CI: 46.1; 60.2) than in the Infanrix hexa group (33.0% with 95% CI: 26.6; 39.9). All other solicited systemic events occurred in the same frequency and all solicited systemic events including pyrexia showed the same grading in both vaccine groups. Unsolicited events were seen in both vaccine groups in similar frequencies.

All 31 SAEs in the study with 412 subjects are covered in detailed and conclusive narratives. None of these cases are judged related to either vaccine by the applicant. The CHMP concurs with that judgment.

No deaths occurred in this study up to 6 months after the last vaccination (follow-up time). No anaphylaxis was seen immediately (up to 30 minutes) after the vaccination.

Case of special interest:

There is one case of Kawasaki disease (confirmed, Subject #003-00004) after the third dose of Hexaxim + Prevenar that is rated "unrelated to the vaccination" by the applicant. This judgement is shared by the CHMP. As the definite causality of Kawasaki disease is unknown but relations are often made up to 30 days after an infection or other immunological event the on-set time seen here - 173 days after vaccination but only 18 days after pyrexia of unknown origin – it is highly unlikely that the KD can be attributed to the vaccination. The case resolved after application of IV immunoglobulin and did not occur again; the subject remained in the trial.

Study A3L15 (safety of Hexaxim or Hexaxim + one dose of Engerix-B at birth in comparison with CombAct-Hib + Engerix + OPV, and concomitant use with Trimovax and Varilrix (South Africa))

The descriptive analysis of safety showed no important differences between the three groups. Notably, Hep B vaccine (Engerix B) injection at birth had no observed impact on the reactogenicity of Hexaxim.

In the primary series, Hexaxim vaccine group showed slightly higher incidence of fever (approx. 11% more) than did the CombAct-Hib + Engerix b + OPV control group, but it was not considered of significance based on the overlapping of the 95% CI and that fact that the majority of the event was of Grade 1. Grade 3 fever was reported in maximum of 1.7% of subjects in the primary series and booster phase, and lasted less than one day. The overall incidence of Grade 3 solicited reactions in Hexaxim group was similar to or lower than the CombAct-Hib + Engerix b + OPV control group.

Unsolicited adverse events considered related to the vaccine were reported slightly lower in Hexaxim group than in CombAct-Hib + Engerix b + OPV control group (3.4% vs. 5.0% respectively). Of note, these data were collected within 7 days after each injection.

Booster vaccination with Hexaxim or CombAct-Hib + OPV control vaccine also showed overall similar safety and reactogenicity profiles in terms of solicited reactions, unsolicited AEs and ARs. There were no reports of extensive swelling of the vaccinated limb.

Concomitant use with Trimovax or Varilrix at the time of booster vaccination was associated with similar incidences of solicited injection site reactions in Hexaxim and CombAct-Hib + OPV boosted subjects. Concomitant use of these vaccines did not significantly increase reactogenicity of Hexaxim and CombAct-Hib + OPV booster vaccine. These data also confirm the published finding that co-administration of combined DTP vaccines (Hexaxim, CombAct-Hib in this study) with MMRV can be safe.

Safety in special populations

Clinical studies in special populations were not performed.

Discontinuation due to AES

Four children discontinued the prophylactic vaccination with Hexaxim due to adverse events (2 AEs, 2 SAEs).

Post marketing experience

No post marketing experience has been gathered, as Hexaxim has not been marketed anywhere else.

2.6.1. Discussion on clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

The applicant provided safety analyses of 12 clinical studies and an integrated safety analysis including 11 clinical studies. Important findings were:

- Hexaxim has a slightly higher reactogenicity regarding solicited local and systemic events/reactions as compared to Pentaxim + Engerix.
- The incidence of solicited local and systemic events/reactions was slightly higher in children administered Hexavac as compared to Hexaxim.
- Tendency for higher reactogenicity of Hexaxim as compared to Infanrix hexa, especially regarding injection site reactions.
- Higher percentage of injection site reactions and pyrexia in Hexaxim + Prevenar as compared to Infanrix hexa + Prevenar. Further data provided by the applicant can only be taken into account after this procedure as the data are not final (6 month safety data still missing) and should be filed as a variation (see also efficacy assessment of concomitant use).
- One case of hypotonic hyporesponsive episode (HHE) was observed 7 hours after first dose of Hexaxim, and two related cases of extensive limb swelling. These events have been reported for other childhood vaccines with a similar composition. Therefore, HHE and ELS can be considered as identified risks.

It was observed that Hexaxim has a slightly higher reactogenicity regarding solicited local and systemic events/reactions as compared to Pentaxim + Engerix, but has a lower reactogenicity in comparison with Hexavac. This finding suggests that the higher reactogenicity of Hexaxim might not be associated with the higher Al content. The applicant attributes the necessity of the doubled dose of aluminium as adjuvant to the good immune response to the HepB component. As the reactogenicity was only marginally higher versus the comparators it can be accepted but should be mentioned in the SmPC.

In view of ethnicity, it was highlighted that 75.7% of the study subjects are of Hispanic, 10.6 % are of Black, 7.9% of Caucasian and 5.8% of Asian origin, which is no equal distribution. Furthermore, the only studies including Caucasian subjects were conducted in Turkey.

The safety cohort is relatively small (<4000 subjects) so that the safety analyses performed so far only control for very common, common and uncommon adverse events, but not for rare and very rare adverse events. The CHMP acknowledges that a safety cohort of 4000 subjects is in accordance with the current guidelines.

In addition, clinical studies do not cover specific populations (premature infants, immunocompromised individuals, subjects suffering from acute or chronic illness including cardiac or renal insufficiency, subjects

with a history of seizures, population with genetic polymorphism has not been studied nor excluded). This fact was reflected in the SmPC during the procedure, in addition to the below standard sentences:

"The immunogenicity of the vaccine may be reduced by immunosuppressive treatment or immunodeficiency. It is recommended to postpone vaccination until the end of such treatment or disease. Nevertheless, vaccination of subjects with chronic immunodeficiency such as HIV infection is recommended even if the antibody response may be limited."

"In chronic renal failure subjects, an impaired hepatitis B response is observed and administration of additional doses of hepatitis B vaccine should be considered according to the antibody level against hepatitis B virus surface antigen (anti-HBsAg).

If any of the following events are known to have occurred in temporal relation to receipt of pertussis-containing vaccine, the decision to give further doses of pertussis-containing vaccine should be carefully considered:

- Temperature of ≥ 40°C within 48 hours not due to another identifiable cause,
- Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours of vaccination,
- Persistent, inconsolable crying lasting ≥ 3 hours, occurring within 48 hours of vaccination,
- Convulsions with or without fever, occurring within 3 days of vaccination.."

Apart from immune-compromised and polymorphisms these sentences are sufficient, and the applicant agreed to perform a study in immunocompromised subjects (preferably HIV positive infants) infants to generate real data in this relevant population.

Regarding genetic polymorphisms the following sentence was included in the SmPC under paragraph 4.4 Special warnings and precautions for use:

"Immune responses to the vaccine have not been studied in the context of genetic polymorphism."

In view of non-clinical safety data the SmPC section 5.3 'Preclinical safety data' reflects now that "At the injection sites, chronic histological inflammatory changes were observed, that are expected to have a slow recovery."

2.6.2. Conclusions on the clinical safety

Despite the tendency to a higher reactogenicity of Hexaxim as compared to the standard of care pentavalent vaccine Pentaxim + Engerix B or compared to the hexavalent vaccine Infanrix hexa, especially when administered concomitantly with the pneumococcal vaccine Prevenar, the safety profile of Hexaxim overall resembles those of other penta- or hexavalent vaccines.

Two safety concerns have been identified in the integrated safety analysis: HHE and ELS. These events are included in the section 4.8 "adverse events" in the SmPC. The measures taken to monitor these events are adequate.

The CHMP considers the measures committed in the Risk Management Plan described further below necessary to address issues related to safety. Therefore, the following pharmacovigilance activities (routine and additional) shall be performed for important identified and important potential risks:

Routine pharmacovigilance activities:

- Spontaneous reports

- Periodic Safety Update Reports
- Signal detection process
- Events identified as Adverse Event of Special Interest

Clinical trial program:

- planned studies in Europe and Latin America
- local studies to be conducted for registration purpose

Post licensure safety studies required by national regulation in place and upon Health Authority requirement

Regarding SIDS/SUD/ALTE an additional commitment was made:

The Applicant is obliged to present a cumulative assessment of these events in each PSUR using Observed versus Expected analysis on SIDS/SUD and ALTE when possible, depending on availability of epidemiologic data on SIDS and ALTE in the concerned countries.

With respect to important missing information, besides routine pharmacovigilance activities a study in immuno-compromised population (preferably HIV infected subjects) will be performed to generate new data.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements, by analogy.

In addition, the CHMP considered that the applicant should take the following minor points into consideration when an update of the Pharmacovigilance system is submitted:

The Applicant presented a contact list of affiliates/offices or partners in EU and non EU countries including the following information: country, company, address of the PV representative. Of note, several countries will be managed by an affiliate/office located in another country, e.g. Belarus is coordinated by the Russian Federation and Burkina Faso by Ivory Coast, a fact that may cause difficulties. The applicant agreed to implement appropriate incentives for AE reporting in countries without established pharmacovigilance system in close collaboration with the national authorities.

Risk Management Plan

The applicant submitted a risk management plan.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Important identified risks		
Hypotonic Hyporesponsive Episode Extensive Limb Swelling	Routine pharmacovigilance activities: - Spontaneous reports - Periodic Safety Update Reports - Signal detection process - Events identified as Adverse Event of Special Interest Clinical trial program: -planned studies in Europe and Latin America -local studies to be conducted for registration purpose Post licensure safety studies required by national regulation in place and upon Health Authority requirement	**Summary of Product Characteristics:** **Section 4.8: Undesirable effects** Nervous system disorders Very rare: Hypotonic reactions or hypotonic-hyporesponsive episodes General disorders and administration site conditions Rare: Extensive limb swelling. Large injection site reactions (>50 mm), including extensive limb swelling from the injection site beyond one or both joints, have been reported in children. These reactions start within 24-72 hours after vaccination, may be associated with erythema, warmth, tenderness or pain at the injection site and resolve spontaneously within 3-5 days. The risk appears to be dependent on the number of prior doses of acellular pertussis containing vaccine, with a greater risk following the 4th and 5th doses.
Important potential risks		
Anaphylaxis Convulsions Apnoea Encephalopathy, encephalitis Sudden Infant Death Syndrome/ Sudden Unexplained Death/ Apparent life Threatening Event (events under close monitoring without evidence of causal relationship with vaccination)	Routine pharmacovigilance activities: - Spontaneous reports - Periodic Safety Update Reports - Signal detection process - Events identified as Adverse Event of Special Interest Clinical trial program: -planned studies in Europe and Latin America -local studies to be conducted for registration purpose Post licensure safety studies required by national regulation in place and upon Health Authority requirement	Summary of Product Characteristics: **Section 4.3 Contraindications**: • Hypersensitivity to the active substances, to any of the excipients listed in section 6.1, to any pertussis vaccine, or after previous administration of the vaccine or a vaccine containing the same components or constituents • The vaccination with Hexaxim is contraindicated if the infant has experienced an encephalopathy of unknown aetiology, occurring within 7 days following previous vaccination with pertussis containing vaccine (whole cell or acellular pertussis vaccines). In these circumstances pertussis vaccination should be discontinued and the vaccination course should be continued with diphtheria-tetanus, hepatitis B, polio and Haemophilus influenza b vaccines. • Uncontrolled neurologic disorder or uncontrolled epilepsy. Pertussis vaccine should not be administered to individuals with these conditions until the treatment regimen has been established, the condition has stabilized and the benefit clearly outweighs the risk. **Section 4.4 Special warnings and precautions for use:** • As each dose may contain undetectable traces of glutaraldehyde, formaldehyde, neomycin, streptomycin and polymyxin B, caution should be exercised when the vaccine is administered to subjects with hypersensitivity to these substances. • As with all injectable vaccines, appropriate medical treatment and supervision should always be readily available in case of an anaphylactic event following administration of the vaccine. • If any of the following events are known to have occurred in temporal relation to receipt of pertussis-containing vaccine, the decision to give further doses of pertussis-containing vaccine should be carefully

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
		considered: • Temperature of ≥ 40°C within 48 hours not due to another identifiable cause; • Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours of vaccination; • Persistent, inconsolable crying lasting ≥ 3 hours, occurring within 48 hours of vaccination; • Convulsions with or without fever, occurring within 3 days of vaccination. • The potential risk of apnoea and the need for respiratory monitoring for 48-72 h should be considered when administering the primary immunization series to very premature infants (born ≤ 28 weeks of gestation) and particularly for those with a previous history of respiratory immaturity. As the benefit of vaccination is high in this group of infants, vaccination should never be withheld or delayed. • A history of febrile convulsions, a family history of convulsions or Sudden Infant Death Syndrome do not constitute a contraindication for the use of Hexaxim. Vaccinees with a history of febrile convulsions should be closely followed up as such adverse events may occur within 2 to 3 days post vaccination. **Section 4.8: Undesirable effects** Skin and subcutaneous tissue disorders Rare: Rash Potential adverse events Convulsion with or without fever. Apnoea in very premature infants (≤ 28 weeks of gestation) (see section 4.4) Encephalopathy, encephalitis
Important missing information		
Immunocompromised patients Patients with a history of convulsions Severe disease condition such as cardiac, hepatic or renal insufficiency Premature infants Sub-populations with genetic polymorphism	Routine pharmacovigilance activities Study in immuno-compromised population	**Summary of Product Characteristics:** **Section 4.4 Special warnings and precautions for use:** • The immunogenicity of the vaccine may be reduced by immunosuppressive treatment or immunodeficiency. It is recommended to postpone vaccination until the end of such treatment or disease. Nevertheless, vaccination of subjects with chronic immunodeficiency such as HIV infection is recommended even if the antibody response may be limited. • If any of the following events are known to have occurred in temporal relation to receipt of pertussis-containing vaccine, the decision to give further doses of pertussis-containing vaccine should be carefully considered: • Temperature of ≥ 40°C within 48 hours not due to another identifiable cause; • Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours of vaccination; • Persistent, inconsolable crying lasting ≥ 3 hours, occurring within 48 hours of vaccination; • Convulsions with or without fever, occurring within 3 days of vaccination. • A history of febrile convulsions, a family history of convulsions or Sudden Infant Death Syndrome do not constitute a contraindication for the use of Hexaxim. Vaccinees with a history of febrile convulsions should be closely followed up as such adverse events may occur within 2 to 3 days post vaccination

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
		• In chronic renal failure subjects, an impaired hepatitis B response is observed and administration of additional doses of hepatitis B vaccine should be considered according to the antibody level against hepatitis B virus surface antigen (anti-HBsAg). • No data are available for premature infants. However, a lower immune response may be observed and the level of clinical protection is unknown. • Potential variability of immune responses to the antigen components of Hexaxim due to genetic polymorphism has not been assessed

The CHMP considers that the Pharmacovigilance plan as described by the applicant on the whole fulfils the requirements. The applicant agreed to closely monitor ALTE/SIDS/SUD and to perform Observed versus Expected ratio analyses as requested by the CHMP for SIDS/SUD, and if feasible for ALTE. The results will be presented in each PSUR or earlier, if a signal emerges. Important missing information includes immunocompromised patients, patients with a history of convulsions, patients with severe disease condition such as cardiac, hepatic or renal impairment as well as premature infants. The applicant agreed to add a study in immunocompromised subjects (if feasible HIV positive subjects) to the clinical trial program as well as a monitoring of pertussis break through cases. This shall be implemented in the RMP. In addition, the close monitoring of break through cases of the other 5 diseases (diphtheria, poliomyelitis, haemophilus influenzae type B, tetanus, and hepatitis B) shall be included in the RMP, although very few cases are expected to occur.

The CHMP, having considered the data submitted, was of the opinion that the below pharmacovigilance activities in addition to the use of routine pharmacovigilance are needed to investigate further some of the safety concerns:

Description	Due date
1. Final study report of ongoing study A3L24 should be submitted when finalized (Immunogenicity and safety data on concomitant use with Prevenar and Rotarix)*	Q3 2012
2. Final study report of ongoing study A3L26 should be submitted when finalized (Antibody persistence after study A3L15)	December 2012
3. Final study report of ongoing study A3L27 should be submitted when finalized (Immunogenicity and safety of booster vaccination after study A3L24)	December 2013
4. Final study report of planned study A3L28 should be submitted when finalized (4.5 years follow-up on Hep B long-term immunogenicity)	Q1 2016
5. Final study report of planned studies A3L38 should be submitted when finalized (Immunogenicity and safety of concomitant use of Hexaxim with Prevenar 13 after a 2+1-dose schedule)*	Q4 2014
6.Final study report of planned studies A3L39 and A3L40 should be submitted when finalized (Immunogenicity and safety of primary and booster vaccination scheme of concomitant use of Hexaxim with Prevenar 13 after a 3-dose primary series (2, 3, 4 months))*	Q2 2016
7. Outline and synopsis of study in immune compromised infants (Immunogenicity and safety of primary and booster vaccination scheme in immune compromised infants)	Q4 2013

*The measures concerning concomitant administration (e.g. Prevenar 13, Rotarix) should only be included in the RMPs for those countries in which the concomitant vaccines are actually licensed. The measures shall, however, be included in the RMPs as soon as vaccines intended for concomitant use are approved in those countries

No additional risk minimisation activities were required beyond those included in the product information.

2.8. User consultation

Not applicable, as the medicinal product will not be distributed in the European Union.

3. Benefit-Risk Balance

Benefits

Beneficial effects

The PDT, PTxd, FHA, PRP-T, IPV and HBsAg manufacturing process is well controlled. In-process controls, release and shelf life specifications indicate the high quality of the drug substance.

The proposed formulation of Hexaxim has been shown to elicit immune response above the predefined and accepted thresholds of protection for each antigen. The clinical data show that the vaccine can be used for both primary and booster vaccination regardless of vaccination scheme (EPI, 2-3-4 or 2-4-6 months with a booster in the second year of life). The clinical data are derived from different developing and developed countries and cover all major ethnicities although these were not equally represented in the hitherto studied subjects.

Uncertainty in the knowledge about the beneficial effects

There are no data with Hexaxim in immunosuppressed infants or in infants with underlying infectious diseases yet but an immunocompromised population is planned to be studied.

There are also no data concerning use in premature infants with a birth weight < 2000g and Sub-populations with genetic polymorphism.

Risks

Unfavourable effects

Hexaxim has a slightly higher reactogenicity as compared to standard of care products (Pentaxim + Engerix or Infanrix hexa). This increased reactogenicity is even more pronounced when being administered concomitantly with a pneumococcal polysaccharide vaccine (such as Prevenar). The extent and clinical relevance of these findings will be addressed by on-going and newly planned studies.

Two important risks have been identified:

hypotonic hyporesponsive episode, and extensive limb swelling.

Uncertainty in the knowledge about the unfavourable effects

Concomitant use has only been tested with two other vaccines: MMRV and Prevenar 7 (Pneumococcal conjugate vaccine against 7 serotypes). The possible immunological interference of Hexaxim with the pneumococcal vaccine in view of diminished antibodies against the pneumococcal serotypes has not been assessed. At least for pneumococcal vaccines (Prevenar 7 and Prevenar 13 and Synflorix) and Rotarix one study is still on-going and others are already planned. The data from these studies should be awaited to

decide on granting the claim of concomitant use. Regarding concomitant use of Hexaxim with Varilrix, an immunological interference phenomenon cannot be excluded for the time being. There are no data on the concomitant use with other vaccines recommended for that age (e.g. meningococcal etc.).

Antibody GMTs against various antigens of Hexaxim have shown to be some times inferior to that of the comparator vaccines although the thresholds of protection were always met. The clinical consequence is unknown; on-going persistence studies might show the earlier need for the next booster vaccination.

Benefit-risk balance

Importance of favourable and unfavourable effects

The primary goal of a new vaccine is to induce antibody levels above an established threshold should one exist. This goal has been reached for all antigens included in Hexaxim. Considering the fact that this vaccine is intended for a global use under the recommendation of the WHO it is another prerequisite to prove suitability for all vaccination schemes, booster ability and use regardless of ethnicity. This has also been shown.

The differences seen in some Hexaxim versus comparator induced GMTs in a few studies are - though in some cases statistically significant - relatively small. A clinical relevance might be found in the timing of the next booster vaccination. But as even those inferior GMTs are still well beyond long-term protection thresholds this possible need also is considered of minor importance as long as the primary vaccination is followed by a booster in the second year of life.

Concomitant use studies have shown that there can be immunological interference between different vaccines. The data presented here only show that there is no interference for Hexaxim antigens regardless of concomitant use with Prevenar 7 or MMRV. The antibodies against the serotypes of Prevenar 7 have not been measured and might be affected. Given the fact that some serotypes showed rather low function antibody titres in other studies alone or in concomitant use with other vaccines this risk should not be underestimated and further studies are needed to recommend concomitant use of Hexaxim with Prevenar 7. Varicella antibody titres were diminished in the concomitant use of MMRV with Hexaxim as well as with Infanrix Hexa. This again leads to a discouragement of concomitant use for Hexaxim with a Varicella-containing vaccine. MMR vaccines can be used concomitantly.

Other concomitant use studies have not been performed. Given the already full vaccination recommendations and the need for rather condensed application schemes in some developing countries this is an important shortcoming. Further studies could remove this issue.

Another shortcoming is the missing information about immunosuppressed, premature and "chronically" ill infants. This data should be generated post-licensure of the vaccine. It is not expected that the immunogenicity or safety will be profoundly different from other inactivated vaccines containing similar antigens in these populations but considered important, as the countries of intended use are often also highly burdened with HIV and/or chronic infectious diseases. As the applicant has already agreed to perform a study in immunocompromised infants these data will become available.

Benefit-risk balance

Considering favourable and unfavourable effects based on the available non-clinical and clinical data presented for this submission, the CHMP is of the opinion that the benefits clearly outweigh the risks.

Discussion on the benefit-risk balance

The only component of Hexaxim which has not been used before as component of other approved vaccines is the hepatitis B antigen which demonstrated non-inferiority as compared to the standard of care in the studies provided within the scope of this dossier. The Applicant has successfully eliminated this shortcoming. Despite the fact that the reactogenicity of Hexaxim appears to be slightly higher in comparison with Infanrix hexa, its safety profile is similar to the profiles of the standard of care pentavalent or hexavalent vaccines.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Hexaxim in the indication

for primary and booster vaccination of infants and toddlers from six weeks to 24 months of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis and invasive diseases caused by Haemophilus influenzae type b

is favourable.

This opinion is based upon the risk-benefit scenarios on the populations and conditions of use as documented with clinical data by the applicant.

This medicinal product, Hexaxim (suspension for injection in pre-filled syringe and suspension for injection), is exclusively intended for markets outside the European Union.

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription

Official batch release

The CHMP recommends that batch compliance control of individual batches be performed by an independent control laboratory before release on to the market in third countries.

Conditions and requirements of the Marketing Authorisation

Risk Management System and PSUR cycle

Pharmacovigilance system

The Scientific Opinion Holder must ensure that the system of pharmacovigilance presented in Module 1.8.1. of the Scientific Opinion Application is in place and functioning before and whilst the medicinal product is on the market.

Risk Management Plan (RMP)

The Scientific Opinion Holder shall perform the pharmacovigilance activities detailed in the Pharmacovigilance Plan as agreed in the Risk Management Plan presented in Module 1.8.2. of the Scientific Opinion Application and any subsequent updates of the RMP agreed by the Committee for Medicinal Products for Human Use (CHMP).

As per the CHMP Guideline on Risk Management Systems for medicinal products for human use, the updated RMP should be submitted at the same time as the next Periodic Safety Update Report (PSUR).

In addition, an updated RMP should be submitted

- When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan or risk minimisation activities
- Within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached
- At the request of the European Medicines Agency.

PSURs

The PSUR cycle for the medicinal product should follow the standard requirements* until otherwise agreed by the CHMP.

* in analogy with Volume 9A of The Rules Governing Medicinal Products in the European Union – Guidelines on Pharmacovigilance for Medicinal Products for Human Use

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Not applicable

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable

New Active Substance Status

Not applicable