

# IMMUNOGENICITY AND REACTOGENICITY OF TWO REGIMENS OF DIPHTHERIA-TETANUS-ACELLULAR PERTUSSIS-HEPATITIS B-INACTIVATED POLIO AND *HAEMOPHILUS INFLUENZAE* TYPE B VACCINES ADMINISTERED TO INFANTS PRIMED AT BIRTH WITH HEPATITIS B VACCINE

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**Abstract.** An open, randomized study evaluated the immune response and safety of two different regimens of diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus-*Haemophilus influenzae* type b (DTPa-HBV-IPV-Hib) immunization in infants primed at birth with hepatitis B vaccine. One-half of the 150 healthy, full-term infants received a DTPa HBV-IPV-Hib vaccine at 1 <sup>1</sup>/<sub>2</sub>, 3 and 5 months of age; the other received a DTPa-IPV-Hib vaccine at 1 <sup>1</sup>/<sub>2</sub>, 3 and 5 months of age with separate HBV vaccine at 1 and 5 months of age. Immune response was similar following the two regimens with 100% of the vaccinees seroprotected for HBV, diphtheria, tetanus, Hib and poliovirus types 2 and 3 diseases after the full vaccination course. One vaccinee in the DTPa HBV-IPV-Hib group failed to respond to the poliovirus type 1 antigen. Response to the three pertussis antigens ranged from 92-97% in the DTPa-IPV-Hib plus separate HBV group and 100% in the DTPa HBV-IPV-Hib group. The most frequently reported post-vaccination symptoms were irritability in the DTPa-IPV-Hib plus separate HBV group (49% of vaccinees) and fever, defined as axillary temperature  $\geq 37.5^{\circ}\text{C}$ , in the DTPa HBV-IPV-Hib group (50% of vaccinees).

## INTRODUCTION

According to current recommendations (Avis du Conseil Superieur d'Hygiene Publique de France, 1998; Helwig *et al*, 1998; American Academy of Pediatrics, 1999; World Health Organization, 1999), children should be routinely vaccinated against diphtheria, tetanus, pertussis, polio and *Haemophilus influenzae* type b (Hib). Implementation of hepatitis B vaccination at or near birth in countries of high endemicity of the disease has proven to be a reliable strategy to ensure the elimination of hepatitis B (HBV) and its chronic sequelae (Da Villa *et al*, 1995; Chen *et al*, 1996; Chang *et al*, 1997; Kane, 1997). The combined trivalent diphtheria, tetanus, pertussis (DTP) vaccines have been available since the 1940s and have become the cornerstone for vaccination of infants worldwide. More

recently, an acellular pertussis-containing vaccine (DTPa) has been developed as an effective and less reactogenic replacement for the traditional whole cell pertussis vaccine (DTPw) (Brown *et al*, 1997; Richie *et al*, 1999).

The development of multivalent combination vaccines would appear to be the most effective way to provide protection against these major childhood diseases offering the advantages of convenience, cost-effectiveness and better compliance. Such combined vaccines must be studied thoroughly in terms of safety and clinical tolerability and to ensure that there is no immunologic interference between the different components (Decker *et al*, 1995; Eskola *et al*, 1996).

This study assessed the immunogenicity and safety of two vaccination regimens that employed either a combined pentavalent DTPa-HBV-inactivated poliovirus (DTPa-HBV-IPV) vaccine or a combined quadrivalent DTPa-IPV vaccine extemporaneously mixed with Hib conjugate vaccine administered to infants primed with a birth dose of

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HBV vaccine. The group receiving the DTPa-IPV/Hib vaccine regimen received the HBV vaccine according to the locally recommended immunization schedule.

## MATERIALS AND METHODS

This open clinical study was conducted at the KK Women's and Children Hospital, Singapore, in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines in force at the time. The study was approved by the respective Ethics Review Committee and written informed consent was obtained from all parents/guardians prior to infant enrolment.

A total of 150 full-term infants were enrolled and equally randomized to receive either the DTPa-IPV/Hib+HBV regimen or the DTPa-IPV/Hib regimen. Eligible infants were born to mothers seronegative for hepatitis B surface antigen (HBsAg) and were declared medically fit after a clinical examination. Exclusion criteria included history of allergic disease or reactions likely to be exacerbated by any vaccine component, receipt of immunoglobulins and/or any blood products since birth, and history of any neurologic disorders or seizures. Concomitant administration of any other vaccine (with the exception of Bacille Calmette-Guérin) was not permitted.

All vaccines used in this study were developed and manufactured by GlaxoSmithKline Biologicals (Rixensart, Belgium). One dose (0.5 ml) of the DTPa-IPV vaccine contained  $\geq 30$  IU (25 Lf) of diphtheria toxoid,  $\geq 40$  IU (10 Lf) of tetanus toxoid, 25  $\mu$ g of pertussis toxoid (PT), 25  $\mu$ g of filamentous hemagglutinin (FHA), 8  $\mu$ g of pertactin (PRN), 10  $\mu$ g of hepatitis B surface antigen (HBsAg, recombinant), 40 D antigen units of poliovirus type 1 (Mahoney), 8 D antigen units of poliovirus type 2 (MEF-1), 32 D antigen units of poliovirus type 3 (Saukett), 0.7 mg aluminium as salts and 2.5 mg of 2-phenoxyethanol.

One dose (0.5 ml) of commercial DTPa-IPV was composed of  $\geq 30$  IU (25 Lf) of diphtheria toxoid,  $\geq 40$  IU (10 Lf) of tetanus toxoid, 25  $\mu$ g of PT, 25  $\mu$ g of FRA, 8  $\mu$ g of PRN, 40 D antigen units of poliovirus type 1 (Mahoney), 8 D antigen units of poliovirus type 2 (MEF-1), 32 D antigen units of poliovirus type 3 (Saukett), 0.5 mg aluminium as

salts and 2.5 mg of 2-phenoxyethanol.

In both groups, the liquid DTPa combination vaccine (either DTPa-HBV-IPV or DTPa-IPV) was used to reconstitute the lyophilized Hib vaccine containing 10  $\mu$ g polyribosylribitol phosphate (PRP) antigen conjugated to 20 to 40  $\mu$ g of tetanus toxoid.

The commercial recombinant hepatitis B vaccine contained, in one dose (0.5 ml), 10  $\mu$ g of recombinant HBsAg and 0.5 mg aluminium as salts.

Subjects received DTPa-IPV/Hib or DTPa-HBV-IPV/Hib vaccines administered as a single injection in the left anterolateral thigh at 1<sup>1/2</sup>, 3 and 5 months of age, following one dose of monovalent HBV vaccine in the right anterolateral thigh at birth. Subjects receiving the DTPa-IPV/Hib vaccine completed the hepatitis B primary vaccination course with monovalent HBV vaccine intramuscularly in the right anterolateral thigh at 1 and 5 months of age.

Blood samples were obtained at birth and at 3 and 6 months of age. Serological analysis was conducted at GlaxoSmithKline Biologicals in a blinded fashion. Antibodies against diphtheria, tetanus, PRP, pertussis antigens (PT, FHA and PRN) and HBsAg were measured by an ELISA assay. The assay cut-off for anti-diphtheria and anti-tetanus antibodies was set at 0.1 IU/ml. This higher threshold level (0.1 IU/ml) was selected because of the poorer correlation between ELISA and functional neutralization test at lower antibody concentrations. The cut-off for the test for antibodies to PRP was 0.15  $\mu$ g/ml. The cut-off for all three pertussis antibodies was 5 EL. U/ml. The assay cut-off for antibodies to HBsAg was 10 mIU/ml. Antibodies against poliovirus types 1, 2, and 3, were assayed only at study end and were determined by a virus micro-neutralization test. The lowest dilution at which serum samples were tested was 1/8. The following definitions were used for the purpose of assessing responders. A seropositive subject was one whose antibody titers were greater than or equal to ( $\geq$ ) the assay cut-off value.

Seroprotection for anti-HBs antibodies was defined as an antibody titer  $\geq 10$  mIU/ml. An anti-PRP titer  $\geq 0.15$   $\mu$ g/ml was indicative of short term protection and a titer  $\geq 1$   $\mu$ g/ml indicated long-term protection. Anti-tetanus and anti-diphtheria titers

$\geq 0.1$  IU/ml and anti-poliovirus titers  $\geq 1/8$  were also considered seroprotective. Vaccine responses for anti-pertussis antigens were defined as the appearance of seropositive antibody titer  $\geq$  the assay cut-off in initially seronegative subjects, or by the presence of post-vaccination antibody titers at least twice as high as the initial pre-vaccination titers.

Diary cards were supplied to parents or guardians to record solicited local reactions (pain, redness, and swelling at the injection site) and general symptoms (drowsiness, irritability/fussiness, loss of appetite, fever) on the day of each vaccination and for three subsequent days. Actual diameters of redness and swelling at each injection site and actual values of axillary body temperature were recorded and their intensity subsequently graded 1 to 3 prior to data analysis as defined in the study protocol. Redness and swelling with a diameter  $\geq 20$  mm around the injection site was defined as grade 3. Fever was defined as an axillary body temperature  $\geq 37.5^{\circ}\text{C}$  and grade 3 fever as a temperature  $> 39^{\circ}\text{C}$ . For all other symptoms, intensity grading 1 to 3 was performed by the parents or guardians. Grade 3 pain was defined as crying when limb was moved or spontaneously painful. Grade 3 irritability/fussiness was defined as inconsolable crying or preventing normal activity. Grade 3 drowsiness was defined as preventing normal activity and loss of appetite, as not eating at all. Unsolicited symptoms occurring during a 30-day follow-up period after each vaccination and serious adverse events (SAEs) occurring during the entire study period were also recorded.

### Statistical methods

All statistical analyses were descriptive in nature. The seroprotection rates (for anti-diphtheria, anti-tetanus, anti-HBs, anti-PRP, and anti-poliovirus types 1, 2, and 3), vaccine response rates (for anti-PT, anti-FHA, and anti-PRN antibodies), and geometric mean antibody titers (GMTs) for all antibodies with 95% confidence intervals (CIs) were calculated. GMTs were calculated by taking the log-transformation of individual titers and calculating the anti-log of the mean of these transformed values. The percentages of subjects with solicited local and general symptoms and unsolicited adverse events were tabulated with exact 95% CI.

## RESULTS

The total study population was comprised of 60 male and 90 female infants who were enrolled in the study within a maximum of 4 days after birth. The two study groups were equivalent with respect to gender distribution.

### Immunogenicity

Table 1 provides the seroprotection/vaccine response rates and Table 2 provides the GMTs of antibodies against the different vaccine antigens for subjects included in the according-to-protocol (ATP) analysis cohort. Subjects eligible for inclusion in the ATP immunogenicity analysis met all eligibility criteria, complied with the procedures defined in the protocol, and had assay results for antibodies against at least one study vaccine antigen component for at least one post-vaccination time point. Prior to the first dose of the combination study vaccines, 29-30% of the infants in both groups had seroprotective levels of anti-HBs subsequent to the birth dose of HBV vaccine. One month after the full vaccination course (*ie*, at month 6), all subjects in both groups were seroprotected for HBV. At both post-vaccination time points (ages 3 and 6 months), antibody response to the recombinant HBsAg with respect to GMT of anti-HBs antibodies was within the same range in the two groups.

Prior to vaccination, the majority of infants had seroprotective levels of anti-diphtheria and anti-tetanus antibodies ( $\geq 58.5\%$ ). All subjects were seroprotected for anti-diphtheria and anti-tetanus antibodies and GMTs for both antibodies had increased at least four-fold in both groups at 6 months of age.

Overall, vaccine response to the three pertussis (PT, FHA and PRN) antigens was 93.8, 92.2 and 96.9%, respectively, in the DTPa-IPV/Hib + HBV group and 100, 98.4, and 100%, respectively, in the DTPa- HBV-IPV/Hib group. All subjects in both groups who were seronegative pre-vaccination responded to study vaccination. While GMTs tended to be higher in the DTPa-HBV-IPV/Hib group than in the DTPa-IPV/Hib + HBV group, all vaccinees were seropositive for antibodies to all three pertussis antigens after the vaccination course.

One month after the third dose, all subjects in both groups were seroprotected against all three poliovirus types with the exception of one subject

Table 1  
Seropositivity/seroprotection rates with 95% CIs for all vaccine antigens.

Antibody	DTPa-IPV/Hib + HB (N=65)			DTPa-HB-IPV/Hib + HB (N=65)		
	PRE	3 months	6 months	PRE	3 months	6 months
Anti-HBs	30.8 (19.9, 43.4)	73.4 (60.9, 73.7)	100.0 (94.4, 100.0)	29.2 (18.6, 41.8)	73.8 (61.5, 84.0)	100.0 (94.4, 100.0)
Anti-PT	47.7 (35.1, 60.5)	85.7 (74.6, 93.3)	100.0 (94.4, 100.0)	46.9 (34.3, 59.8)	90.8 (81.0, 96.5)	100.0 (94.4, 100.0)
Anti-FHA	95.4 (87.1, 99.0)	92.2 (82.7, 97.4)	100.0 (94.4, 100.0)	93.8 (85.0, 98.3)	96.9 (89.2, 99.6)	100.0 (94.4, 100.0)
Anti-PRN	47.7 (35.1, 60.5)	93.8 (84.8, 98.3)	100.0 (94.4, 100.0)	36.9 (25.3, 49.8)	90.8 (81.0, 96.5)	100.0 (94.4, 100.0)
Anti-diphtheria	70.8 (58.2, 81.4)	48.4 (35.8, 61.3)	100.0 (94.4, 100.0)	58.5 (45.6, 70.6)	38.5 (26.7, 51.4)	100.0 (94.4, 100.0)
Anti-tetanus	81.5 (70.0, 90.1)	87.5 (76.8, 94.4)	100.0 (94.4, 100.0)	89.2 (79.1, 95.6)	81.5 (70.0, 90.1)	100.0 (94.4, 100.0)
Anti-poliovirus type 1	***	***	100.0 (94.1, 100.0)	***	***	98.4 (91.5, 100.0)
Anti-poliovirus type 2	***	***	100.0 (93.8, 100.0)	***	***	100.0 (94.2, 100.0)
Anti-poliovirus type 3	***	***	100.0 (94.0, 100.0)	***	***	100.0 (92.6, 100.0)
Anti-PRP ( $\geq 0.15$ $\mu\text{g/ml}$ )	35.4 (23.9, 48.2)	39.1 (27.1, 52.1)	100.0 (94.4, 100.0)	43.1 (30.8, 56.0)	47.7 (35.1, 60.5)	100.0 (94.4, 100.0)
Anti-PRP ( $\geq 1.0$ $\mu\text{g/ml}$ )	16.9 (8.8, 28.3)	10.9 (4.5, 21.2)	89.1 (78.8, 95.5)	13.8 (6.5, 24.7)	7.7 (2.5, 17.0)	85.9 (75.0, 93.4)

N: total number of vaccinees in ATP cohort.

The number of subjects actually included in the ATP analyses varies from one antibody to another as not all subjects had results available for each time-point.

PRE: prior to the first dose of combination vaccines (blood sampling obtained at the time of the first dose).

3 Months, 6 Months: 3 months of age (one month after the second dose of combination vaccines), 6 months of age (one month after the last dose).

\*\*\*Results not tabulated subsequent to abnormal chemical toxicity levels found in serum samples.

in the DTPa -HBV- IPV/Hib group who failed to seroconvert for poliovirus type 1. Anti-poliovirus types 1, 2, and 3 antibody GMTs were comparable in the two groups. The laboratory found abnormal serum toxicity levels that affected the result of the anti-poliovirus antibody assay. Potential bacterial toxicity (as a measure of endotoxin) and viral toxicity were evaluated with the remaining volume of sera and both tests were negative, implying that the toxicity observed could only be chemical. However, sufficient quantity of sera were not available to assess the origin of chemical toxicity. This serum toxicity could have affected the results of the assay for poliovirus antibodies at the month 0 and

month 3 time points. Hence, the immunogenicity results for anti-poliovirus antibodies at month 0 and month 3 were not tabulated.

All subjects in both groups had anti-PRP antibody titers  $\geq 0.15$   $\mu\text{g/ml}$  and a total of 89.1% of subjects in the DTPa- IPV/Hib+ HBV group and 85.9% of subjects in the DTP-HBV-IPV/Hib group had anti-PRP antibody titers  $\geq 1$   $\mu\text{g/ml}$ . Anti-PRP GMTs elicited in both groups were similar.

### Reactogenicity

The birth dose of HBV elicited few symptoms with mild local redness/swelling reported in 1.4% (0.0; 7.6) of subjects who would subsequently re-

Table 2  
GMTs with 95% CIs of antibodies against diphtheria, tetanus, pertussis, poliovirus types 1, 2, 3 and Hib vaccine antigens.

Antibody	DTPa-IPV/Hib + HBV (N=65)			DTPa-HBV-IPV/Hib (N=65)		
	PRE	3 months	6 months	PRE	3 months	6 months
Anti-PT (EL.U/ml)	6.1 (4.6, 8.0)	9.1 (7.6, 10.9)	58.4 (50.8, 67.1)	5.4 (4.3, 6.9)	11.1 (9.4, 13.1)	77.1 (66.2, 89.7)
Anti-FHA (EL.U/ml)	31.4 (23.8, 41.5)	13.8 (11.6, 16.5)	213.4 (188.6, 241.4)	25.0 (19.4, 32.2)	14.5 (12.5, 16.8)	294.1 (259.4, 333.4)
Anti-PRN (EL.U/ml)	6.3 (4.7, 8.4)	17.5 (13.8, 22.2)	154.6 (129.3, 184.9)	4.6 (3.6, 5.8)	16.0 (12.7, 20.2)	199.3 (169.7, 233.9)
Anti-diphtheria (IU/ml)	0.232 (0.173, 0.311)	0.099 (0.082, 0.121)	1.159 (0.925, 1.452)	0.177 (0.129, 0.243)	0.088 (0.072, 0.108)	1.292 (1.073, 1.556)
Anti-tetanus (IU/ml)	0.418 (0.302, 0.578)	0.245 (0.190, 0.316)	2.402 (1.975, 2.922)	0.574 (0.406, 0.811)	0.226 (0.177, 0.290)	2.796 (2.386, 3.275)
Anti-poliovirus type 1	***	***	414.9 (311.1, 553.3)	***	***	367.9 (265.2, 510.4)
Anti-poliovirus type 2	***	***	476.4 (343.8, 660.1)	***	***	288.0 (218.7, 379.3)
Anti-poliovirus type 3	***	***	1,011.5 (804.3, 1,272.0)	***	***	836.1 (635.7, 1,099.8)
Anti-PRP (µg/ml)	0.2 (0.1, 0.3)	0.2 (0.1, 0.2)	4.3 (3.3, 5.6)	0.2 (0.1, 0.3)	0.2 (0.1, 0.2)	5.3 (3.8, 7.2)

N: total number of vaccinees in ATP cohort.

PRE: prior to the first dose of combination vaccines (blood sampling obtained at the time of the first dose).

3 Months, 6 Months: 3 months of age (one month after the second dose of combination vaccines), 6 months of age (one month after the last dose).

\*\*\*Results not tabulated subsequent to abnormal chemical toxicity levels found in serum samples.

ceive the DTPa-IPV/Hib+HBV regimen and in 4.4% (0.9; 12.4) of subjects who would subsequently receive the DTPa-HBV-IPV/Hib regimen. Fever (axillary temperature  $\geq 37.5^\circ\text{C}$ ) was reported in 5.6% (1.6; 13.8) and 14.7% (7.3; 25.4) of subject, respectively.

The percentage of subjects reporting any symptom, solicited or unsolicited, tended to be lower in the group receiving the single vaccine than in the group receiving HBV as a separate vaccine. Pain at the injection site was the most frequently reported solicited local symptom in subjects receiving DTPa-IPV/Hib + HBV versus redness in subjects receiving DTPa-HBV-IPV/Hib (Table 3). Only one solicited local symptom was accorded grade 3 intensity: pain at the DTPa-IPV/Hib injection site.

Irritability and fever were the two most frequently reported solicited general symptoms in both

groups. Few grade 3 general symptoms were reported and the incidence of these symptoms was similar in the two groups. The majority of the solicited general symptoms were considered by the investigators to have no causal relationship with study vaccination.

Unsolicited symptoms were reported in 45 subjects (29 in the DTPa-IPV/Hib+HBV group and 16 in the DTPa-HBV-IPV/Hib group) following 57 doses (36 in the DTPa-IPV/Hib+HBV group and 21 in the DTPa- HBV-IPV/Hib group) during the 30-day follow-up period after each dose.

Thirteen SAEs (eight in the DTPa-IPV/Hib+HBV group and five in the DTPa-HBV-IPV/Hib group) were reported during the study period. One event was considered by the investigator to have a causal relationship to the study vaccination (hospitalization in the DTPa-IPV/Hib+HBV group



Table 3  
Incidence of local and general symptoms reported during the 4-day follow-up period.

			DTPa-IPV/Hib + HBV N = 71 % (95% CI)	DTPa-HBV-IPV/Hib N = 68 % (95% CI)
<b>Any symptom<sup>a</sup></b>			83.3 (72.7, 91.1)	75.0 (63.0, 84.7)
<b>Local systems</b>				
Pain	DTPa-HBV-IPV/Hib	Any	-	16.2 (8.4, 27.1)
		Grade 3	-	0 (0.0, 5.3)
	DTPa-IPV/Hib	Any	31.0 (20.5, 43.1)	-
		Grade 3	1.4 (0.0, 7.6)	-
	HBV	Any	26.8 (16.9, 38.6)	-
		Grade 3	0 (0.0, 5.1)	-
Redness	DTPa-HBV-IPV/Hib	Any	-	17.6 (9.5, 28.8)
		≥20 mm	-	0 (0.0, 5.3)
	DTPa-IPV/Hib	Any	14.1 (7.0, 24.4)	-
		≥20 mm	0 (0.0, 5.1)	-
	HBV	Any	11.3 (5.0, 21.0)	4.4 (0.9, 12.4)
		≥20 mm	0 (0.0, 5.1)	-
Swelling	DTPa-HBV-IPV/Hib	Any	-	11.8 (5.2, 21.9)
		≥20 mm	-	0 (0.0, 5.3)
	DTPa-IPV/Hib	Any	11.3 (5.0, 21.0)	-
		≥20 mm	0 (0.0, 5.1)	-
	HBV	Any	7.0 (2.3, 15.7)	1.5 (0.0, 7.9)
		≥20 mm	0 (0.0, 5.1)	0 (0.0, 5.3)
<b>General symptoms</b>				
Drowsiness	Any		29.6 (19.3, 41.6)	22.1 (12.9, 33.8)
	Grade 3		0 (0.0, 5.1)	0 (0.0, 5.3)
	Related <sup>b</sup>		0 (0.0, 5.1)	0 (0.0, 5.3)
Irritability	Any		49.3 (37.2, 61.4)	33.8 (22.8, 46.3)
	Grade 3		2.8 (0.3, 9.8)	4.4 (0.9, 12.4)
	Related <sup>b</sup>		0 (0.0, 5.1)	0 (0.0, 5.3)
Loss of appetite	Any		36.6 (25.5, 48.9)	19.1 (10.61, 30.5)
	Grade 3		0 (0.0, 5.1)	0 (0.0, 5.3)
	Related <sup>b</sup>		0 (0.0, 5.1)	0 (0.0, 5.3)
Fever <sup>c</sup>	≥37.5°C		40.8 (29.3, 53.2)	50.0 (37.6, 62.4)
	>39.0°C		0 (0.0, 5.1)	1.5 (0.0, 7.9)
	Related <sup>b</sup>		4.2 (0.9, 11.9)	0 (0.0, 5.3)

N: number of vaccinees; %: percentage of vaccinees with a given symptom; <sup>a</sup>Any symptom: solicited or unsolicited  
<sup>b</sup>Related to vaccination as determined by the investigator; <sup>c</sup>axillary temperature

due to fever, reported as an axillary temperature of 37.8°C, one day after the first dose of the combined vaccine). However, this subject received the remaining doses of the study vaccine as per schedule, without any further adverse reaction.

## DISCUSSION

The increasing number of active immunizations recommended as routine pediatric vaccina-

tion practice clearly points to combination vaccines containing multiple antigens as a highly effective approach to simplifying current vaccination procedures, and to facilitate the co-administration of other established vaccines.

This study was designed to compare the immunogenicity and reactogenicity profiles of hexavalent DTPa-HBV-IPV/Hib vaccine (administered at 1 1/2, 3, and 5 months of age) with that of

separate concomitant injections of DTPa-IPV/Hib (administered at 1 1/2, 3, and 5 months of age) and HBV vaccine (given at 1 and 5 months of age) in infants primed at birth with hepatitis B vaccine.

Anti-HBs antibody responses, the parameter of primary interest, were similar in both groups. One month after the full vaccination course (Month 6), 100% of subjects were seroprotected for anti-HBs antibodies. This high seroprotection rate following completion of the primary vaccination course has been previously reported following vaccination with the DTPa- HBV-IPV vaccine either mixed with Hib or injected separately at schedules of 6, 10, and 14 weeks (Gylca *et al*, 2001) and 2, 3, and 4 months (Schmitt *et al*, 2000). The additional HBV antigen dose in the DTPa-HBV-IPV/Hib group was not evidenced in the evolution of anti-HBsAg as demonstrated by the similar seroprotection rates and GMTs elicited by the two vaccine regimens. The immune response in terms of seropositivity rates for antibodies against all other vaccine antigens (PT, FHA, PRN, diphtheria and tetanus toxoids, poliovirus types 1, 2 and 3 and PRP) was similar and high following the complete primary vaccination course for both vaccine regimens. The similar and high number of subjects with anti-PRP antibody levels  $\geq 0.15$  and  $1.0 \mu\text{g/ml}$  in both vaccine groups indicates there was no interference with the response to the PRP antigen due to the HBV component in the single vaccine group. The high seropositivity rates observed are consistent with previous studies involving DTPa-based combinations including Hib, administered as a three dose schedule in the first six months of life, and are within the broad range of GMT values observed with licensed vaccines of established efficacy (Schmitt *et al*, 2001). Similar results have been reported with other DTPa/Hib combination vaccines (Dagan *et al*, 2001; Poolman *et al*, 2001; Halperin *et al*, 1999). Furthermore, clinical trials following a three-dose schedule in the first year of life with DTPa-IPV/Hib and DTPa- HBV-IPV/Hib combinations have also demonstrate evidence for the induction of immunologic memory which indicate that the addition of the IPV or HBV to the DTPa/Hib combinations does not interfere with the induction of immune memory (Zepp *et al*, 1997; Dagan *et al*, 2001).

The percentage of subjects reporting any

symptom (solicited/unsolicited, local/general) tended to be lower in the single vaccine group (DTPa-HBV-IPV/Hib) than in subjects receiving separate vaccines (DTPa-IPV/Hib+HBV). This could be explained by the higher number of injections and the additional vaccine dose in the separate vaccines group. Although the difference was not clinically significant, the incidence of solicited fever tended to be higher in the DTPa- HBV-IPV/Hib group than in the DTPa- IPV/Hib+HBV group. It cannot, however, be excluded that this difference was only observed by chance as fever following the birth dose of HBV was reported in 14.7% of subjects in the DTPa-HBV- IPV/Hib group compared to only 5.6% of subjects in the DTPa- IPV/Hib+HBV group. Few symptoms were described as grade 3 in intensity, the incidence being similar in both groups. The low incidence of grade 3 symptoms compares favorably with previous reports for DTPa-containing vaccines (Decker *et al*, 1995; Brown *et al*, 1997; Schmitt *et al*, 1996; 2000). The single SAE for which the investigator attributed a causal relationship to study vaccination did not impact the uptake or the reactogenicity of subsequent doses for that subject. The reactogenicity profiles of both vaccine regimens were similar and generally comparable to those previously reported for DTPa combination vaccines (Schmitt *et al*, 2000; Gylca *et al*, 2001).

It is concluded that, following a birth dose of HBV vaccine, the vaccine regimen in which the combined DTPa- HBV-IPV/Hib vaccine was given as a single injection at 1 1/2, 3 and 5 months of age was as safe and immunogenic as the vaccine regimen in which DTPa-IPV/Hib vaccine was given as a single injection at 1 1/2, 3 and 5 months of age concomitantly with HBV vaccine given at 1 and 5 months of age. It is generally acknowledged that routine immunization of infants is the only effective way to prevent circulation of hepatitis B and to meet the WHO's goal of reducing the incidence of childhood infection by 80% (World Health Organization, 1995). A combination vaccine with HBV would be a cost-effective way of delivering HBV vaccines to infants without increasing the number of injections with the inherent logistical and compliance complications. Results of this study indicate that the hexavalent DTPa- HBV-IPV/Hib vaccine can be incorporated into the immunization pro-

grams in regions with high perinatal transmission in which a HBV monovalent vaccine dose should be given at birth as was done in this study.

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