



## REVIEW MEMORANDUM-TOXICOLOGY OF MPL

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Subject: BLA STN 125259 from GSK (Glaxo Group Ltd., d/b/a GlaxoSmithKline) for Human Papillomavirus Vaccine, AS04 Adjuvant-Adsorbed: Review of toxicological information relevant to 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)

Date: September 29, 2009

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2, 3, 4, 5. Repeat-dose toxicity of MPL for up to 8 days in -(b)(4)- rats, 7 days in -(b)(4)- rats, and 14 days in -(b)(4)- dogs plus repeat-dose toxicity of MPL--(b)(4)- for up to 14 days in dogs (Sections 2.6.6.3 and 2.6.7.7)	
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2. Ref. Sec. 4.2.3.2.1 - -(b)(4)- Study No. 3262.2: 8-Day Intravenous Toxicity Study of MPL in Rats (Test article: MPL from RIBI Immunochem Research, Inc. [summarized in Sections 2.6.6.3 and 2.6.7.7 in Nonclinical Summary (Module 2)]	
3. Ref. Sec. 4.2.3.2.1 - -(b)(4)- Study No. 3262.4: 7-Day Intravenous Dose Range- Finding Toxicity Study in -(b)(4)- Rats with MPL (Test article: MPL (Lot No. -(b)(4)-) [summarized in Sections 2.6.6.3 and 2.6.7.7 in Nonclinical Summary (Module 2)]	
4. Ref. Sec. 4.2.3.2.3 - -(b)(4)- 3262.1: 14-Day Intravenous Toxicity Study of MPL in Dogs (Test article: MPL (Lot No. -(b)(4)-) [summarized in Sections 2.6.6.3 and 2.6.7.7 in Nonclinical Summary (Module 2)]	

### **FDA Reviewer's Overall Summary and Conclusions:**

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Results from nonclinical toxicology studies conducted to detect potential toxicities associated with administration of MPL alone were submitted by GSK as part of the nonclinical safety assessment program conducted to support the evaluation and licensing requirements for Human Papillomavirus Vaccine, AS04 Adjuvant-Adsorbed (Cervarix). This new vaccine, a prophylactic, bivalent recombinant protein vaccine, which consists of HPV-16/18 L1 proteins assembled as virus-like particles (VLPs) and adsorbed onto aluminum hydroxide prior to mixing with AS04 adjuvant, consisting of aluminum-adsorbed 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL), is proposed for use in the prevention of cervical cancer (squamous cell cancer and adenocarcinoma) by protecting against certain precursor lesions and infections caused by oncogenic Human Papillomavirus (HPV) types 16 and 18.

Toxicology studies carried out to detect potential toxicities associated with administration of MPL alone included a single-dose toxicity study, repeated-dose toxicity studies, genotoxicity studies, embryofetal toxicity studies, and a pre/post-natal reproductive toxicity study. The final study reports from single- and repeat-dose toxicity studies conducted with MPL (alone) are reviewed in this document. The single-dose and repeated-dose MPL toxicity studies reviewed herein, which were carried out (1988 - 1992) very early in or prior to the product and nonclinical development stages of Cervarix, provided an adequate evaluation of the nonclinical safety of intravenous administration of the MPL component of the AS04 adjuvant, and thus, provided additional supportive information about the MPL component of the new vaccine.

The repeat-dose toxicity studies were conducted for up to 8 days in rats and 14 days in -(b)(4)-dogs, and the MPL was given once daily by the intravenous route, maximizing the exposure. When administered by this route, the effects seen were generally dose-related, ranging from 6 mcg/kg/day being considered a no-observed effect level in -(b)(4)- dogs and 40 mcg/kg/day being considered well-tolerated in rats, to 5,000 mcg/kg/day resulting in mortality in rats. In conclusion, the data reported here showed findings expected to be associated with strong stimulation of the immune system (expressed primarily as increased spleen weight and white blood cell count).

While the repeat-dose toxicity studies of MPL were performed following intravenous administrations of MPL, they can be viewed as providing limited supportive toxicity information for administration of MPL via the intramuscular (IM) route of administration intended for Cervarix. The 50 µg of MPL contained in one dose of the AS04-containing vaccine, which would represent 1.7-0.7 µg/kg dose for 30-70 kg-weighting individuals, lies far below the dose considered well-tolerated, and well below the no-observed effect level, in the toxicity studies mentioned above. In addition, the systemic exposure expected after intramuscular vaccination with an AS04-containing vaccine (in which the MPL is adsorbed to aluminum), when administered on a 0, 1, 6 month immunization schedule, is expected to be reduced as compared to that expected with the daily intravenous MPL injections used in the repeated-dose toxicity studies. These multiple differences taken together lead to the conclusion that the results from the

toxicology studies conducted with MPL alone can be interpreted as providing an increased safety margin.

Overall, these data indicate that MPL adsorbed to aluminum hydroxide, as it exists in *Cervarix*, the product described in Biologics License Application (BLA) STN 125259, is suitable as a human vaccine adjuvant at the proposed dosage and formulation, as it appears to have an acceptable safety margin, especially when administered by the proposed route of administration and dosing regimen.

**List of Submissions Reviewed Herein:**

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**Type/Application ID/Amendment #/ Receipt Date:**

Original Biologic License Application (BLA)/STN 125259/Original Application/29-MAR-2007:

- Module 2 Narrative and Tabular Toxicology Summaries: Sections 2.6.7.5; 2.6.7.6; 2.6.7.7
- Module 4 Toxicology Study Reports:  
Ref. Sec. 4.2.3.1.1 - -----(b)(4)-----: The Acute Intraperitoneal Toxicity of Monophosphoryl Lipid A (MPL) in Rats (Test article: GMP-MPL (lot -(b)(4)-) [summarized in Sections 2.6.6.2 and 2.6.7.5 in Nonclinical Summary (Module 2)]

Ref. Sec. 4.2.3.2.2 - -(b)(4)- Study No. 3262.2: 8-Day Intravenous Toxicity Study of MPL in Rats (Test article: MPL from RIBI Immunochem Research, Inc. [summarized in Sections 2.6.6.3 and 2.6.7.7 in Nonclinical Summary (Module 2)]

Ref. Sec. 4.2.3.2.1 - -(b)(4)- Study No. 3262.4: 7-Day Intravenous Dose Range-Finding Toxicity Study in -(b)(4)- Rats with MPL (Test article: MPL (Lot No. -(b)(4)-) [summarized in Sections 2.6.6.3 and 2.6.7.7 in Nonclinical Summary (Module 2)]

Ref. Sec. 4.2.3.2.3 - -(b)(4)- 3262.1: 14-Day Intravenous Toxicity Study of MPL in Dogs (Test article: MPL (Lot No. -(b)(4)-) [summarized in Sections 2.6.6.3 and 2.6.7.7 in Nonclinical Summary (Module 2)]

Amendment to BLA Original Application Submitted in Response to item 13 in FDA's Complete Review Letter dated December 14, 2007:

- "Resubmission" to BLA: STN 125259/Ser. 28/06-FEB-2008 regarding one of the toxicology study narrative summaries lacking adequate details

Amendment to BLA Original Application Submitted in response to Item 3 of August 3, 2007 letter from FDA to GSK regarding various MPL-containing vaccines:

- BLA/STN 125259/Am. 12/27-AUG-2007: Summary of all animal data available to GSK that may reflect neurological and autoimmune-based adverse reactions to MPL-containing investigational products

**List of Abbreviations**

BLA	Biologics license application
GSK	Glaxo Group Ltd., d/b/a GlaxoSmithKline
-(b)(4)-	------(b)(4)-----
HPV	Human papillomavirus
VLP	Virus-like particle
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
Al(OH) <sub>3</sub>	Aluminum hydroxide
L1	Major capsid protein of HPV

**MPL Formulations Used in Toxicology Studies:**

GMP-MPL (lot -(b)(4)-)      MPL produced under GMP conditions by RIBI Immunochem Research, Inc., Hamilton, Montana, fill date 11-24-87

MPL (Lot No. -(b)(4)-)      MPL produced under GMP conditions by RIBI Immunochem Research, Inc., Hamilton, Montana, fill date 11-24-87

3D-MPL (Lot No. -(b)(4)-)      MPL produced under GMP conditions by RIBI Immunochem Research, Inc., Hamilton, Montana, receipt date 9-25-91

3D-MPL (Lot Nos. ------(b)(4)-----  
-----) refers to MPL produced under GMP conditions by RIBI Immunochem Research, Inc., Hamilton, Montana, fill date 6/19/95

## **Introduction**

This original BLA is for GlaxoSmithKline Biologicals' HPV-16/18 L1 VLP AS04 vaccine, Cervarix™, a new recombinant adjuvanted preservative-free sterile vaccine turbid liquid suspension for intramuscular injection.

The proposed use is for active immunization of females 10 years of age and older for the prevention of cervical cancer (squamous cell cancer and adenocarcinoma) by protecting against the following precursor lesions and infections caused by oncogenic Human Papillomavirus (HPV) types 16 and 18:

- Cervical intraepithelial neoplasia (CIN) grade 2 and 3
- Cervical intraepithelial neoplasia (CIN) grade 1
- Abnormal cytology (i.e., atypical squamous cells of undetermined significance, low-and high-grade squamous intraepithelial lesions)
- Persistent infection
- Incident infection

Please refer to the package insert for the final indication.

GSK states that the HPV vaccine composition was determined based on the data obtained from nonclinical challenge animal models and from clinical epidemiological and natural history studies, which showed that in order to be efficacious in preventing HPV infection and related clinical lesions an HPV vaccine would need to induce strong antibody responses against the L1 capsid protein (assembled as VLPs), B-cell memory and T-cell responses. The HPV vaccine formulation was therefore selected to be a combination of L1 proteins assembled as VLPs, to insure the induction of protective anti-HPV L1 VLP antibodies, and the proprietary adjuvant AS04 developed by GSK Biologicals, to insure the induction of sustained high levels of antibodies as well as the induction of a specific cell-mediated immunity [Garcon, 2006; Giannini et al, 2006].

The vaccine Adjuvant System, AS04, consists of aluminum hydroxide-adsorbed MPL. The MPL, which GSK refers to as an immunostimulant, is a detoxified derivative of the lipopolysaccharide (LPS) of the gram negative bacterium *Salmonella minnesota* R595 strain that is manufactured under GMP and supplied by Corixa Corporation, doing business as GSK Biologicals North America, Hamilton, Montana, USA. The AS04 adjuvanted HPV Vaccine is manufactured under GMP by GlaxoSmithKline Biologicals, Rue de l'Institut, 89, B-1330 Rixensart, Belgium.

Lipopolysaccharide (LPS) is obtained by extracting *S. minnesota* R595 harvested and concentrated cells with -----(b)(4)-----  
----- MPL is prepared by subjecting the LPS from *S. minnesota* R595 to sequential acid and base hydrolyses, -----(b)(4)-----  
-----  
----- (b)(4) -----

Nonclinical pharmacological, pharmacokinetic and toxicology studies were conducted on HPV-16/18 L1 AS04, AS04, and MPL. The single-dose toxicity study and repeated-dose toxicity studies conducted with MPL (alone) were carried out very early in or even prior to (1988 - 1992) the Cervarix product and non-clinical development stages. In fact, many of the toxicology studies were done to evaluate the safety of IV administration of MPL in animals to support the safety of conducting clinical trials in -(b)(4)- patients.



### **Product Summary**

GlaxoSmithKline Biologicals' HPV-16/18 L1 VLP AS04 vaccine, Cervarix™, is presented as a 0.5 ml monodose in 1.25 ml glass syringes (fill volume = -(b)(4)-) and as a 0.5 ml monodose in 3 ml glass vials (fill volume = -(b)(4)-).

The HPV-16/18 L1 VLP AS04 vaccine, proposed in this application, contains the following ingredients:

- The HPV-16/18 L1 VLP AS04 vaccine contains recombinant C-terminally truncated L1 proteins of human papillomavirus Type 16 and Type 18 each assembled separately as virus-like particles (VLP) and produced on -----(b)(4)----- cells;
- GlaxoSmithKline Biologicals' proprietary AS04 adjuvant system composed of aluminium hydroxide ( $\text{Al}(\text{OH})_3$ ) and 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL);
- Sodium Chloride (NaCl);
- Sodium dihydrogen phosphate dihydrate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ); and
- water for injections (WFI).

The composition of the HPV-16/18 L1 VLP AS04 vaccine per 0.5 mL dose is as follows.

#### **Composition of GSK's HPV Vaccine**

<b>Ingredients</b>	<b>Quantity (per 0.5ml dose)</b>
HPV-16 L1 VLP	20 µg
HPV-18 L1 VLP	20 µg
MPL	50 µg
Aluminium (hydroxide salt)	500 µg
Sodium chloride (NaCl)	4.4 mg (150 mM)
Sodium dihydrogen phosphate dihydrate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ )	0.624 mg (8 mM)
Water for injection	q.s. ad 0.5 ml

### **Overview of Toxicology Data for Candidate Vaccine**

Each section (i.e., Pharmacology, Pharmacokinetics, and Toxicology) of Module 2.6 and Module 4 is composed of two separate parts: the first part is the Cervarix part, which includes the nonclinical documentation regarding the HPV-16/18 L1 AS04 vaccine and AS04 adjuvant system, and the second part is the MPL part, which includes the nonclinical documentation regarding MPL. Table 1 below lists the studies summarized in Module 2.6.

**Table 1 Studies Summarized in Module 2.6**

<b>Study name</b>	<b>Study type</b>	<b>Test article</b>	<b>Location</b>
-(b)(4)- 1513	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
-(b)(4)- 1758	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
--(b)(4)-- 58678	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
--(b)(4)-- 62369	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
--(b)(4)-- 62370	Repeat-Dose Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.7
-(b)(4)- 249/033160	Reproductive & Developmental Toxicity	HPV-16/18 L1 VLP AS04 and AS04	CERVARIX Module 2.6, Section 2.6.7, Item 2.6.7.13
-(b)(4)-	Single-Dose Toxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.5
-(b)(4)- 3262.2	Repeat-Dose Toxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.7
-(b)(4)- 3262.4	Repeat-Dose Toxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.7
-(b)(4)- 3262.1	Repeat-Dose Toxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.7

Study name	Study type	Test article	Location
-(b)(4)- 1729/3	Genotoxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.8
-(b)(4)- 1729/4	Genotoxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.8
-(b)(4)- 730/052198	Genotoxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.9
-(b)(4)- 1729/8	Reproductive & Developmental Toxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.13
-(b)(4)- 1729/7	Reproductive & Developmental Toxicity	MPL	MPL Module 2.6., Section 2.6.7, Item 2.6.7.13

As indicated in the table above, GSK has focused the majority of the HPV vaccine preclinical safety testing program on formulations of its candidate adjuvanted vaccine and on the AS04 adjuvant. The primary pharmacodynamic properties of the vaccine were evaluated by immunogenicity studies. The specific humoral and cellular properties of the AS04 adjuvanted vaccine formulation were assessed in comparison to the Al(OH)<sub>3</sub> adjuvanted formulation. Most of the studies were performed in mice and thereafter confirmed in a monkey experiment. In parallel, MPL and AS04 specific primary pharmacodynamics *in vitro* (human or mice cells) and *in vivo* (mice) studies were performed providing data about the mode of action and the immunostimulant properties of MPL and AS04. The data related to the evaluation of the immune properties of Cervarix, AS04, and MPL, i.e., the Primary Pharmacodynamics studies (in section 4.2.1.1) have been reviewed by Dr. Robin Levis in a separate document. In addition, Dr. Levis reviewed the Pharmacokinetics data for MPL (in section 4.2.2).

Dr. Steven Kunder reviewed the following data in a separate document: the Safety Pharmacology data for Cervarix and MPL (in section 4.2.1.3); the repeat dose toxicology data submitted for Cervarix and AS04 (IM studies conducted in rats and rabbits in section 4.2.3.2); and the genotoxicity data for Cervarix and MPL (in section 4.2.3.3).

Dr. Marion Gruber reviewed the Reproductive and Developmental study data (section 4.2.3.5) in a separate document.

In addition, as listed in Table 1, toxicology studies were carried out to detect potential toxicities associated with MPL administration. These studies included a single-dose toxicity study, repeated-dose toxicity studies, genotoxicity studies, embryofetal toxicity studies, and pre/post-

natal reproductive toxicity study. The single-dose toxicity study and repeated-dose toxicity studies conducted with MPL were carried out between 1988 – 1992, prior to or very early in the Cervarix product and non-clinical development stages. In fact, many of the toxicology studies were done to evaluate the safety of IV administration of MPL in animals to support the safety of conducting clinical trials with MPL in cancer patients.

GSK states that the materials used in the toxicological studies overall had comparable purity levels as the materials used for clinical studies and the materials proposed for marketing. Table 2, as provided in the BLA by GSK, shows the lots of MPL used in the various toxicology studies included in the BLA and provides information about some of the later lots in terms of overall purity and specific impurities.

**GSK's Table 2 Test Article Summary: MPL**

**[**  
--(b)(4)--  
**]**

### **MPL TOXICOLOGY SUMMARY:**

A summary of the four final study reports reviewed herein from studies conducted with MPL (alone), all of which were performed in compliance with GLP, follows.

**Table 3 Toxicology Studies Conducted with MPL Formulations (Alone)**

<b>Study Number</b>	<b>Study Type</b>	<b>Test article</b>	<b>Location in BLA</b>
-(b)(4)-	Single-Dose Toxicity Species/strain: -----(b)(4)----- rat ROA: Intraperitoneal Duration of Dosing: Acute Doses (mcg/kg): 0, 10, 40, 400, 4000	MPL- -(b)(4)-	Summary in MPL Module 2.6., Sections 2.6.6.2 and 2.6.7.5  Final Study Report in MPL Module 4.2.3.1.1
-(b)(4)- 3262.2	Repeat-Dose Toxicity Species/strain: -(b)(4)- rat ROA: Intravenous Duration of Dosing: 8 days Doses (mcg/kg/day): 0, 100, 1000, 2500, 5000	MPL- -(b)(4)-	Summary in MPL Module 2.6., Sections 2.6.6.3 and 2.6.7.7  Final Study Report in MPL Module 4.2.3.2.2
-(b)(4)- 3262.4	Repeat-Dose Toxicity Species/strain: -(b)(4)- rat ROA: Intravenous Duration of Dosing: 7 days Doses (mcg/kg/day): 0, 40, 200, 1000	MPL- -(b)(4)-	Summary in MPL Module 2.6., Sections 2.6.6.3 and 2.6.7.7  Final Study Report in MPL Module 4.2.3.2.1
-(b)(4)- 3262.1	Repeat-Dose Toxicity Species/strain: -(b)(4)- dog ROA: Intravenous Duration of Dosing: 14 days Doses (mcg/kg/day): 0, 6, 120, 1200	MPL- -(b)(4)-	Summary in MPL Module 2.6., Sections 2.6.6.3 and 2.6.7.7  Final Study Report in MPL Module 4.2.3.2.3

#### **2.6.6.2 Single-dose Toxicity Study**

##### **2.6.6.2 Single-dose Intraperitoneal Toxicity Study in -(b)(4)----- Rats (-(b)(4)-)**

An acute intraperitoneal (ip) toxicity study was performed in adult -----(b)(4)----- rats. This study was performed in 1988 by the -----(b)(4)-----, and it is titled, “The Acute Intraperitoneal Toxicity of Monophosphoryl A (MPLA) in Rats (DT127).” The study report is located in the MPL part of Module 4, Section 4.2.3.1, Item 2.4.3.1.1. In this study, single MPL dosages of approximately 0, 10, 40, 400 and 4000 µg/kg body weight were administered ip in rats (6 rats/sex/group), followed by a 14-day observation period. Potential toxicity was assessed on the basis of overt clinical signs, animal survival, body weight change, ophthalmic examination, urinalysis, hematology and clinical chemistry profiles, absolute and

relative organ weights, and gross and microscopic pathological changes. Treatment-related findings included a slight increase in the incidence and relative severity of interstitial infiltrations of mononuclear inflammatory cells in the omentum and mesentery. This effect was not apparent at dosages below 400 µg/kg nor was it correlated with any other anatomical or clinical changes.

#### 2.6.6.3 Repeat-dose Toxicity Studies

##### 2.6.6.3.1 An 8-day Intravenous Toxicity Study of MPL in -(b)(4)- Rats (-(b)(4)-3262.2)

This study was performed in 1992 by -----(b)(4)-----, and it is titled, “A 8 Day Intravenous Toxicity Study of MPL in Rats (SLS 3262.2).” The study report is located in the MPL part of Module 4.2. Section 4.2.3.2, Item 4.2.3.2.2. This 8-day intravenous toxicity study consisted of four groups of -(b)(4)- rats with 10 rats/sex/group. Initially, MPL was administered by daily intravenous injection of 0, 100, 1000, 5000 µg/kg/day. On study day 2, the high dose level was decreased from 5000 to 2500 µg/kg/day due to excessive treatment-related toxicity (mortality).

The rats were observed daily and weighed on days 1, 2, and 8. Individual food consumption was measured daily. Clinical pathology determinations were performed on all study animals on the day of scheduled sacrifice. All study animals were subjected to a complete gross necropsy at the time of death or sacrifice. A per protocol set of tissues and organs was preserved from each rat and selected tissues were processed for microscopic examination.

Treatment-related mortality occurred in the high dosage level group (5000 µg/kg/day then lowered to 2500 µg/kg/day) where 3/20 rats died or were sacrificed moribund during the study.

Clinical signs of toxicity, decreased body weight gain, and decreased food consumption were observed in all three MPL treatment groups. The body weight and food consumption decreases were readily reversible in surviving animals.

Clinical pathology and organ weight effects were observed in all three MPL treatment groups. The clinical pathology changes generally followed a dose-related pattern and included decreased RBCs, decreased platelets, increased leukocytes (with a shift towards neutrophilia), increased fibrinogen, increased BUN, and decreased albumin. Organ-weight changes also occurred in a dose-related fashion in most cases and included increased spleen, liver, kidney, and heart weights.

Test article-related microscopic changes were observed in the eyes, heart, kidneys, liver, lung, and spleen of rats from each MPL treatment group sacrificed at study termination. These changes were generally characterized by infiltration of mononuclear inflammatory cells.

Since treatment-related effects were observed at all MPL treatment levels tested, a no-

observed-effect level for MPL was not established in this study.

2.6.6.3.2 A 7-day Intravenous Dose Range-finding Toxicity Study of MPL in -(b)(4)- Rats (-(b)(4)- 3262.4)

This study was performed in 1992-1993 by -----(b)(4)-----, and it is titled, “A 7-Day Intravenous Dose Range-Finding Toxicity Study in -(b)(4)- Rats with MPL (-(b)(4)- 3262.4).” The study report is located in the MPL part of Module 4.2. Section 4.2.3.2., Item 4.2.3.2.1. This intravenous toxicity study consisted of groups of 3 male and 3 female -(b)(4)- rats given an injection of either control vehicle or MPL at dosage levels of 40, 200 and 1000 µg/kg/day, once daily for 7 days.

The animals were observed daily for clinical signs of toxicity. Body weights and food consumption were measured on days 1, 2, 4 and 7. Blood samples were collected on Day 8 for evaluation of clinical pathology parameters. A gross necropsy examination was performed and fresh organ weights were recorded.

No mortality was observed. Dose-dependent reductions in body weight gain and food consumption were observed in both sexes of the 40, 200 and 1000 µg/kg/day groups during study days 1-2. Subsequent weight gain was comparable to or exceeded control values. Additional reductions in food consumption were noted in the 200 µg/kg/day males and 1000 µg/kg/day males and females.

A possible slight decrease in platelets was observed in the MPL treated males at the 1000 µg/kg/day level. In the MPL treated females, erythrocytes, hemoglobin and hematocrit were slightly decreased in a dose-dependent manner, at the 200 and 1000 µg/kg/day levels. A slight but dose-related increase in segmented neutrophils was also noted in the 40, 200, and 1000 µg/kg/day females. There were no apparent treatment-related changes in clinical chemistry parameters among the groups. Dose-dependent increases in absolute and relative spleen weights were observed in the MPL treated animals at all dosage levels.

The 40 µg/kg/day iv dose was considered as well-tolerated in rats as it only produced minor signs of toxicity consistent with an immunostimulant effect of MPL.

2.6.6.3.3 A 14-day Intravenous Toxicity Study of MPL in Dogs (-(b)(4)- 3262.1)

This study was performed in 1992 by -----(b)(4)-----, and it is titled, “Fourteen (14) Day Intravenous Toxicity Study of MPL in Dogs (-(b)(4)- 3262.1).” The study report is located in the MPL part of Module 4.2. Section 4.2.3.2, Item 4.2.3.2.2. This 14-day intravenous toxicity study consisted of four groups of -(b)(4)- dogs with 3 dogs/sex in each group. MPL was administered by iv injection at levels of 6, 120 or 1200 µg/kg/day. Control dogs received the vehicle iv (10 % ethanol and 90 % of a 5 % dextrose in water for injection solution).

The dogs were observed daily and weighed on days 1, 2, 8 and 14. Individual food consumption was measured daily. ECG, blood pressure measurements and clinical pathology were performed prior to and at specified intervals during the study. At study termination, dogs were sacrificed and subjected to a gross necropsy examination followed by microscopic examination.

There was no mortality and no statistical differences in mean body weights for either sex. There were no treatment-related changes in ECG, blood pressure measurements or microscopic examination of the tissues.

Food consumption of the 1200 µg/kg/day males was moderately decreased during the first few days of treatment. In females, occasional statistical reductions in food consumption were noted at the 120 and 1200 µg/kg/day levels. No treatment-related differences in food consumption were observed for the 6 and 120 µg/kg/day males or 6 µg/kg/day females. Slight to moderate reduction in platelets were observed in the 120 and 1200 µg/kg/day males and females on days 2, 8 and 15/16. Marked increases in serum fibrinogen levels were observed in the 1200 µg/kg/day males (day 2) and females (days 2 and 8). Marked increases in leukocytes were noted in the 1200 µg/kg/day males and females on day 2. It was speculated that the leukocytosis may have been due to an increase in segmented neutrophils.

Increased absolute and relative spleen weights were also observed in the 1200 µg/kg/day animals. Leukocytosis and splenomegaly represent pharmacological effects of MPL. Six micrograms/kg/day were considered a no-observed-effect level.

#### Local Tolerance

Local tolerance data were obtained in the repeated dose toxicity study in dogs -(b)(4)-3262.1 (see MPL Module 2-6, Section 2.6.6.3., Item 2.6.6.3.3 A 14-day intravenous toxicity study of MPL in dogs -(b)(4)- 3262.1)). No test article-related injection site lesions were observed microscopically in the MPL-treated dogs.

#### ***FDA Reviewer's Comment:***

Narrative and tabular toxicology study summaries were reviewed, and with the exception of the study summary provided in section 2.6.6.3.1, they were found to represent a valid summary of each study and study results as compared to the full study reports. In the summary provided in section 2.6.6.3.1 for the study titled “An 8-day Intravenous Toxicity Study of MPL in -(b)(4)-Rats -(b)(4)- 3262.2),” only those changes observed in rats from each MPL treatment group sacrificed at study termination were discussed. The specific test article-related microscopic changes (i.e., edema or hemorrhage in brain and spinal cord) observed in the 3/20 rats that died or were sacrificed moribund during the study were not discussed in this study summary.

According to Harrison's Principles of Internal Medicine, “A fulminant presentation with sepsis and brain edema occurs in some cases [of neurologic infections, such as bacterial meningitis, with septic shock].” In addition, it is stated, “Much of the pathophysiology of bacterial meningitis is a direct consequence of elevated levels of CSF cytokines and chemokines. TNF and IL-1 act synergistically to increase the permeability of the blood-brain barrier, resulting in



induction of vasogenic edema and the leakage of serum proteins into the subarachnoid space. The subarachnoid exudate of proteinaceous material and leukocytes obstructs the flow of CSF through the ventricular system and diminishes the resorptive capacity of the arachnoid granulations in the dural sinuses, leading to obstructive and communicating hydrocephalus and concomitant interstitial edema.” Although MPL is less potent than LPS, it is reported elsewhere in the BLA that MPL still is capable of interacting with TLR 4 to trigger the release of TNF. Therefore, TNF release may be the possible explanation for the brain edema observed in rats upon MPL overexposure.

**Summary of item 13 in the Complete Response letter which the FDA sent to GSK on December 14, 2007 and GSK's response to this item**

***The following comment was included as item 13 in the Complete Response letter which the FDA sent to GSK on December 14, 2007:***

With respect to Module 2.6.6, Toxicology Written Summary-MPL, Common Technical Document Summaries, it was noted in Module 2.6.6.3.1 (p. 5) that the summary discussion of the 8 day IV toxicity study in -(b)(4)- rats (Study -(b)(4)- 3262.2) included mention of the treatment-related mortality that occurred in the high dose level group (started at 5000 mcg/kg/day and then lowered to 2500 mcg/kg/day). However, only those changes observed in rats from each MPL treatment group sacrificed at study termination were discussed in the summary of the test-article related microscopic changes. Please discuss in Module 2.6.6.3.1 the specific test article-related microscopic changes (i.e., edema or hemorrhage in brain and spinal cord) observed in the 3/20 rats that died or were sacrificed moribund during the study.

***GSK's response:***

In an amendment to the BLA (serial 28 received 2/6/2008), GSK submitted a response to this question posed in item 13 of the CR letter, which consisted of a revision to paragraph 3 of the study summary provided in Module 2.6.6.3.1 to include additional details about the specific test article-related microscopic changes observed in the 3/20 rats that died or were sacrificed moribund during Study -(b)(4)- 3262.2. In addition, the firm provided an additional section in Module 2.6.6, Toxicology Written Summary-MPL, Common Technical Document Summaries (Module 2.6.6.3.4) and an additional final study report in MPL Module 4.2.3.2.1 in Serial 28 titled "A 14-day intravenous toxicity study of MPL--(b)(4)- (-(b)(4)- 67244)." Along with this additional study, in their response to our item 13, GSK concluded that they believe the following: "that death on test following intravenous administration of 5 mg/kg/day of MPL----(b)(4)----- that was used in Study -(b)(4)- 3262.2 and many of the repeat dose toxicity studies included in the BLA) was due to endotoxic shock in the -(b)(4)- rat, which accounts for the edema, hemorrhage and other histopathologic observations in the decedents. The -(b)(4)- in MPL--(b)(4)- formulation allows the hydrophobic MPL molecules -----(b)(4)-----, increasing the toxicity of the compound, due to the ease with which it can disperse and activate the TLR4 receptors throughout the body. This type of toxicity is especially marked following intravenous administration."

**2.6.6.3.4 A 14-day Intravenous Toxicity Study in Dogs Administered MPL--(b)(4)- (-(b)(4)- 67244)**

This study was performed in 1995 by -----(b)(4)-----, and it is titled, "Fourteen (14) Day Intravenous Toxicity Study in Dogs Administered MPL -(b)(4)- (-(b)(4)- 67244)." The additional study report is located in Ser. 28 in the MPL part of Module 4.2. Section 4.2.3.2, Item 4.2.3.2.1. The objective of this study was to evaluate the intravenous toxicity of MPL--(b)(4)- (MPL in -----(b)(4)-----)

---(b)(4)---) to dogs over 14 days. Four groups of three (b)(4)- dogs per sex per group were administered i.v. doses of 0, 12, 60 or 300 µg/kg/day for 14 days. The control group was administered the (b)(4)- vehicle without MPL.

The dogs were observed twice daily and weighed on days -8, -1, 1, 8, 14 and just prior to necropsy. Individual food consumption was measured daily. ECG, blood pressure measurements and clinical pathology were performed prior to and at specified intervals during the study. At study termination, dogs were sacrificed and subjected to a gross necropsy examination followed by microscopic examination.

Repeated intravenous administrations of MPL--(b)(4)- produced inflammatory changes such as decreased platelets and WBCs and increased spleen weights at the two highest doses. No treatment-related histopathological changes in a full panel of tissues and organs were observed following 14 days of intravenous administration of up to 300 mcg/kg/day MPL--(b)(4)-. A dosage level of 12 mcg/kg/day was considered a no-observed adverse-effect-level (NOAEL) for this study.

***FDA reviewer's comment regarding GSK's response:***

GSK's response consisted of a revision to paragraph 3 of the study summary provided in Module 2.6.6.3.1 to include additional discussion regarding the specific test article-related microscopic changes observed in the 3/20 rats that died or were sacrificed moribund during the Study (b)(4)-3262.2; an additional section in Module 2.6.6, Toxicology Written Summary-MPL, Common Technical Document Summaries (Module 2.6.6.3.4); and an additional final study report in MPL Module 4.2.3.2.1 titled "A 14-day intravenous toxicity study of MPL--(b)(4)- ((b)(4)- 67244)." I found this response to be complete and adequate and I concur with the additional discussion provided regarding Study (b)(4)- 3262.2 in the Study summary Section and their summary and description of (b)(4)- Study 67244 as well as the study results. GSK states, "the (b)(4)- in MPL--(b)(4)- formulation allows the -----(b)(4)----- form, increasing the toxicity of the compound, due to the ease with which it can disperse and activate the TLR4 receptors throughout the body. This type of toxicity is especially marked following intravenous administration." While GSK did not provide physicochemical data to demonstrate that MPL in -----(b)(4)-----, it is not unlikely as MPL is -----(b)(4)----- . In addition, they provided contrasting data from another tox study in which the MPL was formulated as an (b)(4)-. In this case, no treatment-related histopathological changes in a full panel of tissues and organs were observed following 14 days of intravenous administration of up to 300 µg/kg/day MPL--(b)(4)-. A dosage level of 12 µg/kg/day was considered a NOAEL for this study. GSK argues that MPL--(b)(4)- (MPL in -----(b)(4)----- -----) is less toxic than MPL--(b)(4)-, because the MPL in the (b)(4)- formulation is somewhat "----- (b)(4)----- structures, and consequently less available for interaction with TLR4 receptors.

## **INDIVIDUAL TOXICOLOGY STUDY SUMMARIES:**

Complete, final study reports were submitted by GSK in Module 4. The Summary section from each report was submitted in Module 2 as a narrative summary. The Methods and Results section from each report is included below followed by the FDA reviewer's comment.

### **Single Dose Toxicity Studies**

#### **Study 1 (of 1): Study No. -(b)(4)- - The Acute Intraperitoneal Toxicity of Monophosphoryl Lipid A (MPL) in Rats**

**Performing Laboratory:** -----(b)(4)-----.

**Study Initiation Date:** December 4, 1987

**Final Report Date:** May 17, 1988

**Animal species and Strain:** Adult -----(b)(4)----- rats

**Breeder supplier:** -----(b)(4)-----

**Animals per sex and group:** 6 males & 6 females per group

**Age:** approximately 8-11 weeks of age at the initiation of dosing

**Body weight range:** 212-286 g

**Route:** Intraperitoneal

**Site of administration:** Intraperitoneum

**Volume of injection:** (4 ml test article/kg followed by 36 ml Dianeal/kg = 40 ml/kg animal

**Method of administration:** Single IP dose of test article or control article followed by Dianeal with 1.5% Dextrose (was used as a delayed, Intraperitoneal (IP) diluent of the reconstituted test/control article and was administered at an IP dosage of 36 ml/kg approximately 10-15 minutes after the test article was administered.

**Dose:** Single dosages of approximately 0, 10, 40, 400, and 4000 mcg per kg body weight

**Test Article:** 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) manufactured by Ribi ImmunoChem Research, Inc. (Hamilton, MT). The test article was identified with Lot No. -(b)(4)-.

**Vehicle/Formulation:** The control article (MPLA diluent) for this study was 0.9% Sodium Chloride Injection, USP (NaCl; -(b)(4)-; Lot No. -(b)(4)-; Code No. 2A1321 containing 0.2% triethylamine (TEA; ---(b)(4)---; Lot No. -(b)(4)-).

**Duration of Postdose:** 14-day observation period

**GLP Compliance:** Yes

**Methods:**

### Laboratory Methods

This study was conducted using five treatment groups, each consisting of six male and six female rats. Animals in the four test article groups received a single 4 ml/kg intraperitoneal dosage of MPLA at 10 (manufacturers recommended safe human dose), 40 (active human dose/clinical dose = IX), 400 (IOX), or 4000 (IOOX) mcg/kg of body weight, respectively, followed 10-15 minutes later by a 36 ml/kg intraperitoneal dosage of Dianeal (combined volume dosage = 40 ml/kg). Rats in the control group received a single 4 ml/kg intraperitoneal dosage of the control article (MPLA diluent) followed 10-15 minutes later by a 6 ml/kg dosage of Dianeal. Clinical observations were recorded for fourteen days post-treatment.

In order to accommodate the necropsy procedure and the hematology laboratory schedule, each group of animals was randomly divided into two equally numbered replicates. Treatment of the two replicates was initiated over successive days.

Ophthalmic examinations were performed on all animals pretreatment and prior to sacrifice.

Each rat was weighed immediately prior to injection, and the calculated dosage was administered intraperitoneally. All rats were observed for signs of toxicity within approximately 60 minutes after treatment and again one to three hours after treatment. Unfasted body weights were recorded on study day 0 (for calculation of dosage volume), and day 13. A fasted body weight was recorded at necropsy (day 14). Clinical observations were conducted daily for a 14 day period. On day 13, subsequent to recording body weight, rats were fasted and placed into metabolism cages for overnight urine collection.

Prior to necropsy, animals were anesthetized with ether and a terminal blood sample was drawn (from the abdominal aorta) for hematologic and clinical chemistry analyses. The animals were then killed by exsanguination. Urine samples were scheduled to be aspirated from the bladder at the time of necropsy if no previous sample was collected. A complete necropsy was performed and major organs and tissues fixed for histopathologic evaluation.

### Measurements and Records

The following measurements were made:

General Health Status

Clinical Observations

Overt signs of toxicity were recorded within approximately 60 minutes after treatment and again 1-3 hours after treatment. In addition, clinical signs of toxicity were recorded once daily throughout the 14 day observation period.

Ophthalmic Examinations

Ophthalmic examinations were performed on each rat prior to the first treatment and again prior to completion of the 14 day observation period. Ophthalmic examinations were performed using a slit-lamp biomicroscope and an indirect ophthalmoscope to assess all ocular structures.

#### Body Weights

Unfasted body weights were measured on days 0 and 13. Fasted body weights were measured at necropsy (day 14). Because of scheduling problems, body weights could not be recorded on day 6 as proposed.

#### Urinalysis

An overnight urine sample was collected from each rat just prior to necropsy. Thymol was placed in each collection vessel as a preservative. Urinalyses were performed. Ames Hultistlx reagent strips were used to semiquantitatively determine pH, protein, glucose, ketones, bilirubin, occult blood, and urobilinogen. The urine sediment was examined microscopically, and the specific gravity of the urine was measured using a refractometer.

#### Blood Sample Collection

A fasted blood sample from the abdominal aorta was collected from each rat at necropsy.

#### Hematology Assays

Blood was collected in tubes containing either sodium citrate (3.8) or potassium ethylenediaminetetraacetate (EDTA). Blood collected in tubes containing sodium citrate was used for the determination of prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen level. Blood collected in tubes containing EDTA was used for determination of all other hematologic parameters. Samples were collected on the day of necropsy.

#### Clinical Chemistry Assays

Blood was collected in tubes containing no anticoagulant. Following centrifugation, the serum was used to perform clinical chemistry assays. Samples were collected on the day of necropsy.

#### Necropsy Procedures

At the time of necropsy, each rat was weighed, anesthetized, and subsequently killed by exsanguination. A necropsy was performed immediately thereafter.

#### Organ Weights

The brain, heart, lungs, spleen, liver, kidneys (right and left), and adrenals (right and left) were weighed at the time of necropsy.

Gross Pathology. Macroscopic lesions detected at necropsy were recorded on Necropsy Worksheets.

#### Histopathology

Tissues and organs taken at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The following tissues were examined microscopically: brain, heart, spleen, liver (two portions from opposing lobes), lungs, kidneys (right cross section and left longitudinal section), eyes (right and left), adrenals (right and left), diaphragm, abdominal aorta, thymus, urinary bladder, gonads (right and left), stomach, duodenum, jejunum, ileum, cecum, omentum, mesentery, ventral abdominal wall, lymph nodes (mesenteric and bronchial) and gross pathological lesions.

All histopathologic specimens were submitted, received, and archived according to standard procedures.

## **Results:**

### **Survival and Clinical Signs**

None of the test article animals died during the course of the study and there were no adverse clinical signs associated with the intraperitoneal administration of MPLA.

### **Ophthalmic Examinations**

No lesions were detected at the pretreatment or post-treatment examination periods.

### **Urinalysis**

Objective analysis of post-treatment urinalysis profiles indicated that no toxicologically significant changes resulted from administration of the test or control articles.

### **Body Weight**

Absolute body weight and cumulative body weight change were not affected by the acute intraperitoneal administration of MPLA.

### **Hematology**

No toxicologically significant differences were apparent for any of the hematology parameters.

Random group differences were exhibited with respect to fibrinogen, Burr cells and mean corpuscular hemoglobin (MCH), but no dosage-response effects were apparent.

An increasing dosage-response effect and higher group means were exhibited for hemoglobin and hematocrit (both sexes) of the test article groups relative to controls. However, since the magnitude of change was small and not correlated with other biochemical or histopathologic effects, no meaningful toxicological significance was attached to this observation.

### ***FDA Reviewer's comment on Study -(b)(4)-:***

Complete study reports were reviewed. I concur with the sponsor's conclusion that the only test-article related observations were small changes in terms of the animal's hemoglobin and hematocrit. All animals gained weight over the test period and there were no deaths.

## Repeat Dose Toxicity Studies

### **Study 1 (of 3): Study No. -(b)(4)- 3262.2 - 8-Day Intravenous Toxicity Study of MPL in Rats**

**Test Article:** 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)

**Species/Strain:** -(b)(4)- Rat

**Duration of Dosing:** 8 days

**Initial Age:** 7 weeks

**Duration of Postdose:** N/A

**Date of First Dose:** 16 October 1991

**Method of Administration:** intravenous

**Vehicle/Formulation:** MPL in -----(b)(4)----- in water for injection

**GLP Compliance:** Yes

**Performing Laboratory:** -----(b)(4)-----

**In-life Portion of Study Initiation Date:** October 16, 1991

**Final Report Date:** July 23, 1992

### **Methods:**

This 8-day intravenous toxicity study consisted of four groups of -(b)(4)- rats with 10 rats/sex/group. Initially, MPL was administered by daily intravenous injection of 0, 100, 1000, 5000 µg/kg/day. On study day 2, the high dose level was decreased from 5000 to 2500 µg/kg/day due to excessive treatment-related toxicity (mortality). The rats were observed daily and weighed on days 1, 2, and 8. Individual food consumption was measured daily. Clinical pathology determinations were performed on all study animals on the day of scheduled sacrifice. All study animals were subjected to a per protocol gross necropsy at the time of death or sacrifice. A complete set of tissues and organs was preserved from each rat and selected tissues were processed for microscopic examination.

### **Results:**

#### **Survival:**

Two high-dose rats (#83/F and #91/F) died after receiving a single 5.0 mg/kg dose of MPL. A third high-dose rat (#61 /M) was sacrificed moribund on day 4 due to severe debility. These deaths were considered treatment related. One control rat (#91/F) died shortly after dosing on day 8. The specific cause of this death was not determined; however, it is suspected to be unrelated to vehicle treatment since similar mortality was not observed in other control rats.

#### **Clinical Observations:**

Clinical signs of toxicity were observed in the MPL treated rats at each level. The most severe changes were recognized at the 2.5 mg/kg/day level and included decreased activity, prostration, soft stools, few feces, mucoid stools, rough coat, unkempt appearance, piloerection, fecal and urine staining, dehydration, hypothermia, reddened pinna(e), partially closed eye lids, corneal



opacity, and dark material around the eyes, nose and/or mouth. As compared to the 2.5 mg/kg/day level, the overall incidence of clinical signs was lower in the 1.0 and 0.1 mg/kg/day rats. At the 1.0 mg/kg/day level, MPL-related clinical signs included rough coat, urine staining, decreased activity, tail discoloration, and dark material around the eyes. MPL-related clinical signs at the 0.1 mg/kg/day level consisted of fecal staining, corneal opacity, decreased activity and lacrimation. Both control and MPL-treated rats exhibited a high incidence of wobbly gait following dosing. This change was thought to be associated with intravenous administration of the vehicle, -----(b)(4)----- in water for injection, USP.

#### Body Weights and Weight Gain:

Statistically significant, dose-dependent reductions in mean body weight gain were observed in the 0.1, 1.0 and 2.5 mg/kg/day males and females on study day 2. All groups exhibited a net loss in body weight between days 1 and 2, with the exception of the 0.1 mg/kg/day males that showed a slight but statistically lower net weight gain. The reductions in body weight gain for days 1-2 led to statistically decreased mean body weights in the 2.5 mg/kg/day males and females, and 1.0 mg/kg/day males on day 2. Mean body weight of the 2.5 mg/kg/day males remained statistically lower than controls on day 8. For all groups, mean weight gain returned to normal levels or exceeded control values during days 2-8 indicating that a recovery had occurred.

#### Food Consumption:

Statistically significant decreases in food consumption (g/animal/day and g/kg/day) were observed in the 0.1, 1.0 and 2.5 mg/kg/day males and females. These reductions were first observed in all groups for days 1-2 and followed a dose-related pattern in both magnitude and duration. In males, the persistence of statistically reduced food consumption (g/animal/day) ranged from 3 days (0.1 mg/kg/day group) to 5 days (2.5 mg/kg/day group). In females, the persistence of statistically reduced food consumption (g/animal/day) ranged from 1 day (0.1 mg/kg/day group) to 3 days (2.5 mg/kg/day group). After these periods, daily food consumption returned to normal levels or exceeded control values indicating that a recovery had occurred.

#### Clinical Pathology:

##### 1. Hematology -

**RBC Parameters:** RBC count, hemoglobin and hematocrit were statistically decreased in the 0.1, 1.0 and 2.5 mg/kg/day males and females. These reductions followed a dose-related pattern and were most severe in the 2.5 mg/kg/day males and females. Other statistically significant RBC parameters consisted of increased reticulocytes in 2.5 mg/kg/day males and 1.0 and 2.5 mg/kg/day females, decreased MCV in the 1.0 mg/kg/day females, and increased mean corpuscular hemoglobin concentration (MCHC) in the 2.5 mg/kg/day females.

**Platelets:** Statistically significant decreases in platelets were observed in the 1.0 and 2.5 mg/kg/day males. Although these decreases were relatively minor, they did occur in a dose-related pattern. The mean platelet level of the 2.5 mg/kg/day females also appeared to be decreased slightly, but was not statistically different from the controls.

**Total and Differential Leukocytes:** Total leukocytes, segmented neutrophils, lymphocytes and monocytes were statistically increased in the 2.5 mg/kg/day males and segmented neutrophils were statistically increased in the 1.0 mg/kg/day males. Total leukocytes, lymphocytes and monocytes also appeared to be increased in the 2.5 mg/kg/day females, but were not statistically different from the controls. Segmented neutrophils were statistically increased in the 1.0 and 2.5 mg/kg/day females. With regard to red cell morphology, apparent increases in slight to moderate polychromasia and anisocytosis were observed in the 2.5 mg/kg/day males and females. Similar changes in red cell morphology were also noted in the 1.0 mg/kg/day females.

## 2. Coagulation

**PT and APTT:** No statistically significant or biologically meaningful differences in PT or APTT were observed among the groups.

**Fibrinogen:** Fibrinogen levels were slightly but statistically increased in the 0.1, 1.0 and 2.5 mg/kg/day males and females. The biological significance of these increases was not clear. The increased fibrinogen values were generally similar for MPL-treated males and females and followed no apparent dose response pattern.

## 3. Clinical Chemistry

**BUN and Creatinine:** BUN was slightly but significantly increased in the 2.5 mg/kg/day males and females. In the 2.5 mg/kg/day males, serum creatinine was slightly but statistically decreased as compared to controls. This decrease was minor and appeared to be incidental.

**Alkaline Phosphatase:** Alkaline phosphatase was statistically decreased in the 0.1, 1.0 and 2.5 mg/kg/day males. The significance of this difference was not determined. Similar decreases in alkaline phosphatase were not observed in the female treatment groups.

**Total Protein, Albumin and Globulin:** There were no statistical differences in total protein among the groups, however, in both males and females, an apparent dose-related trend toward decreased total protein was observed. This trend correlated with statistically significant, dose-dependent decreases in serum albumin in the 0.1, 1.0 and 2.5 mg/kg/day females. A similar pattern of reduced albumin was observed in MPL-treated males, however, only the albumin level of the 2.5 mg/kg/day males was statistically different from the controls. This latter decrease led to a slight but statistically significant decrease in A/G ratio of the 2.5 mg/kg/day males. A/G ratios were not statistically different in the other study groups and no statistical differences in globulin levels were observed.

AST and ALT: Serum ALT levels were slightly but statistically decreased in the 1.0 and 2.5 mg/kg/day females. These decreases were very minor and appeared to be unrelated to MPL treatment. There were no apparent differences in serum AST levels among the groups.

Glucose: Glucose levels were statistically increased in the 0.1, 1.0 and 2.5 mg/kg/day females as compared to controls. The biological significance of this change was not clear since high fasting glucose levels were also observed in the control, 0.1, 1.0 and 2.5 mg/kg/day males.

Amylase: Amylase was statistically decreased in the 2.5 mg/kg/day males. This difference was not considered to be biologically significant since the reduced amylase level (1493.6 IU/L) remained similar to the pretest amylase level for males (1557.8 IU/L).

#### Gross Necropsy

At necropsy, enlarged spleens were observed in 2 rats of the 0.1 mg/kg/day group (2 males), 10 rats of the 1.0 mg/kg/day group (6 males and 4 females), and 15 rats of the 2.5 mg/kg/day group (9 males and 6 females). Necropsy findings in the remaining animals were generally unremarkable.

#### Organ Weights

Statistically significant and apparent changes in absolute and relative organ weight data are described below.

Spleen: Dose-dependent increases in absolute and relative spleen weights were observed in the 0.1, 1.0 and 2.5 mg/kg/day males and females. As compared to controls, the magnitude of the spleen weight increases ranged from approximately two-fold (0.1 mg/kg/day level) to approximately three-fold (2.5 mg/kg/day). All spleen weights were statistically increased as compared to controls, with the exception of the absolute spleen weights of the 0.1 mg/kg/day females.

Liver: Absolute and relative liver weights were increased in a dose-related fashion in the 0.1, 1.0 and 2.5 mg/kg/day females. In males, absolute and relative liver weights also appeared to be increased at all three MPL treatment levels; however, a clear dose response relationship was not observed and only the liver weight (absolute and relative) of the 2.5 mg/kg/day males was statistically increased.

Adrenal Glands: Absolute and relative adrenal gland weights were statistically increased in the 2.5 mg/kg/day males. In 2.5 mg/kg/day females, absolute and relative adrenal weights also appeared to be increased slightly; however, they were not statistically different from the control group.

Thymus Gland: Absolute and relative thymus gland weights of the 2.5 mg/kg/day females were slightly, but statistically decreased as compared to controls. The biological significance of this change was not determined.

Kidney: Relative kidney weights of the 0.1, 1.0 and 2.5 mg/kg/day males and females were slightly but statistically increased. These differences followed no apparent dose-response pattern.

Heart: Relative heart weight was statistically increased in the 1.0 and 2.5 mg/kg/day males, and 0.1, 1.0 and 2.5 mg/kg/day females. Relative heart weight of the 0.1 mg/kg/day males also appeared to be increased, although it was not statistically different from the controls.

#### Histopathology

Test article-related microscopic changes were observed in the eyes, heart, kidneys, liver, lung and spleen of rats from each of the MPL treated groups which were sacrificed at study termination. The changes were generally characterized by minimal to mild infiltrations of mononuclear inflammatory cells and are probably related to the pharmacologic action of the test article. Similar changes were observed in rats which died or were sacrificed moribund during the study. However, the extents of the changes were less in these rats because of their brief treatment. In addition, either edema or hemorrhage was observed in the brain and spinal cord in the three 2.5 mg/kg/day rats which died or were sacrificed moribund. This change was also considered to be test article related. No cause of death could be established for the control group female which was found dead on day 8.

#### ***FDA Reviewer's comment on -(b)(4)- 3262.2:***

Complete study reports were reviewed. I concur with the sponsor's conclusion that since treatment-related effects were observed at all MPL treatment levels tested, a no-observed-effect level for MPL was not established in this study.

#### **Study 2 (of 3): Study No. -(b)(4)- 3262.4 - 7-Day Intravenous Dose Range-Finding Toxicity Study in -(b)(4)- Rats with MPL**

**Test Article:** 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL)

**Species/Strain:** -(b)(4)- Rat

**Duration of Dosing:** 7 days

**Initial Age:** 9 weeks

**Duration of Postdose:** N/A

**Date of First Dose:** 23 September 1992

**Method of Administration:** intravenous

**Vehicle/Formulation:** MPL in -----(b)(4)--- in water for injection, USP, and ----(b)(4)----  
-----, USP

**Number of animals:** 24 (3 male and 3 female per group)

**GLP Compliance:** Yes

**Performing Laboratory:** ------(b)(4)-----

**In-life Portion of Study Initiation Date:** September 23, 1992

**Final Report Date:** February 24, 1993

#### **Methods:**

This 7-day intravenous toxicity study consisted of four groups of -(b)(4)- rats with 3 rats/sex/group receiving 0, 40, 200, and 1000 mcg/kg/day in a dosage volume of 4.0 ml/kg.

Animals were administered the test article or control material by intravenous injection, via the lateral tail vein, once daily for seven consecutive days. Doses were administered at a slow, constant rate, approximately 1 ml/60 seconds. Individual doses were calculated based on the most recent body weight data.

#### Clinical Observations

During the treatment period, all animals were observed a minimum of once daily for clinical signs of toxicity, including physical or behavioral abnormalities. Mortality and moribundity checks were performed twice daily, in the morning and afternoon. In addition, during the treatment period, the rats were observed within one hour following dosing for overt signs of toxicity.

#### Body Weights

Individual body weights were measured prior to dose administration on days 1, 2, 4 and 7. Terminal body weights were measured prior to scheduled euthanasia on day 8.

#### Food Consumption

Individual food consumption was measured on study days 1, 2, 4 and 7. Food consumption was calculated and reported as grams/animal/day.

#### Clinical pathology

Blood samples were collected from all animals on the day of scheduled euthanasia (day 8) for evaluation of selected hematology and clinical chemistry parameters. The animals were fasted overnight prior to blood sample collection. Blood samples were obtained via the orbital plexus while the rats were under light isoflurane anesthesia. The following parameters were evaluated:

#### Hematology

- Erythrocyte count (RBC)
- Hematocrit (Hct)
- Hemoglobin concentration (Hgb)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Mean corpuscular volume (MCV)
- Platelet count
- Reticulocyte count
- Total and differential leukocyte counts

#### Clinical Chemistry

- A/G ratio (calculated)
- Alanine aminotransferase (ALT)

- Albumin
- Alkaline phosphatase
- Aspartate aminotransferase (AST)
- Calcium
- Creatinine
- Electrolyte balance (sodium, potassium, chloride)
- Globulin (calculated)
- Glucose (fasting)
- Phosphorus
- Total bilirubin
- Total protein
- Urea nitrogen (BUN)

#### Gross Necropsy

All animals were euthanized on study day 8 by CO<sub>2</sub> inhalation followed by exsanguination. The rats were subjected to a complete gross necropsy examination at the time of death or euthanasia. The gross necropsy included examination of the external surfaces of the body and all internal viscera.

#### Organ weights

Fresh organ weights were obtained from all animals at scheduled euthanasia for the spleen, heart, kidneys, liver and brain. Paired organs were weighed together. Relative organ weights were subsequently calculated.

### **Results:**

#### Survival

All animals survived to scheduled euthanasia on day 8.

#### Clinical Observations

There were no remarkable clinical signs of toxicity in the MPL treated rats. Tail discoloration was noted occasionally at the 40 and 1000 mcg/kg/day levels.

#### Body Weights and Weight Gain

Dose-dependent decreases in mean body weight gain were noted in both males and females of the 40, 200 and 1000 mcg/kg/day groups during days 1-2. Subsequent weight gain in these groups (days 2-4 and 4-7) was comparable to or exceeded control values.

#### Food Consumption

Dose-dependent reductions in mean food consumption (grams/animal/day) were observed in both males and females of the 40, 200 and 1000 mcg/kg/day groups during days 1-2. Additional reductions in food consumption were observed in the 200 mcg/kg/day males and

1000 mcg/kg/day females during days 2-4 and in the 1000 mcg/kg/day males during days 2-4 and 4-7.

#### Clinical pathology

##### Hematology

In MPL treated males, a possible, slight decrease in platelets was observed at the 1000 mcg/kg/day level. In addition, in males of the 40 mg/kg/day group, slightly higher total leukocytes, segmented neutrophils and lymphocytes were observed; however, similar changes were not observed in the 200 and 1000 mcg/kg/day males.

In MPL treated females, erythrocytes, hemoglobin and hematocrit appeared to be decreased slightly, but in a dose-dependent manner, at the 200 and 1000 mcg/kg/day levels. Similar trends toward decreased erythrocytes, hemoglobin and hematocrit were noted in the 40 mcg/kg/day females; however, these differences were only marginal and may have been due to biological variation. A slight but dose-related increase in segmented neutrophils was noted in the 40, 200, and 1000 mcg/kg/day females. In the 1000 mcg/kg/day females, possible slight increases in nucleated RBCs and reticulocytes were noted. In addition, in the 200 and 1000 mcg/kg/day females, apparent slight increases in the occurrence of polychromasia (slight to moderate) were observed.

##### Clinical Chemistry

There were no apparent differences in clinical chemistry data among the groups for either sex.

##### Gross Necropsy Observations

Enlarged spleens were observed at necropsy in 2/6 rats at the 40 mcg/kg/day level (2 males); 3/6 rats at the 200 mcg/kg/day level (2 males and 1 female); and 6/6 rats at the 1000 mcg/kg/day level (3 males and 3 females). Other necropsy findings were generally unremarkable.

##### Organ Weights

Dose-dependent increases in absolute and relative spleen weights (relative to final body weights and relative to brain weights) were observed in the MPL treated males and females at the 40, 200, and 1000 mcg/kg/day levels. Mean liver weights of the MPL treated males and females also appeared to be increased as compared to controls; however, these increases did not follow any consistent dose-related pattern.

##### ***FDA Reviewer's comments:***

Complete study reports were reviewed. I concur that the No Observed Adverse Effect Level (NOAEL) was not determined.

**Study 3 (of 3): Study No. -(b)(4)- 3262.1 - 14-Day Intravenous Toxicity Study of MPL in dogs**  
**Test Article: 3-O-desacyl-4'-monophosphoryl lipid A (MPL)**

**Species/Strain:** Dog/-(b)(4)-

**Duration of Dosing:** 14 days

**Initial Age:** approximately 8 months

**Duration of Postdose:** N/A

**Date of First Dose:** 4 November 1991

**Method of Administration:** intravenous

**Vehicle/Formulation:** -----(b)(4)----- in water for injection

**GLP Compliance:** Yes

## Methods:

### Study Design and Treatment

Three animals per sex/group received 0, 6, 120, and 1200 mcg/kg/day in a dosage volume of 1.2 ml/kg. Animals were administered the test or control materials by slow intravenous injection via the cephalic veins once a day for at least 14 days. The first day of administration was designated as study day 1. Intravenous injection of the test article was selected since it was a potential route of administration in humans.

### Clinical Observations

Detailed clinical observations were performed and recorded at least once daily starting on day -7. In addition, the animals were observed twice daily for mortality and moribundity. During the dosing period, animals were observed at least once during the 2 hour post-dose period for overt signs of toxicity.

### Body Weights

Individual body weights were measured on days -7, -1, 1, 2, 8 and 14. A terminal body weight was determined on the day of scheduled sacrifice.

### Food Consumption

Individual food consumption was measured daily beginning on day -7 and continuing to day 14. Each dog was offered 400 g of food for a period of approximately 4 hours per day beginning approximately 2 hours after dosing. Food consumption was calculated in daily intervals as grams/animal/day and grams/kg/day.

### Clinical Pathology

Hematology and biochemistry parameters were evaluated for all animals once prior to study initiation (on day -7), and on days 2, 8 and 15/16. The dogs were fasted overnight prior to each sampling day. An additional blood sample was collected from each dog on day 3 (nonfasted) for evaluation of prothrombin time. All blood samples were taken via the jugular vein. On each day of blood collection for clinical pathology (days 2, 8 and 15/16), an extra serum sample was obtained and sent to the Sponsor.

An 18-hour urine sample was collected from each dog once prior to study initiation (day -6) and on days 2 and 14. During the collection, food was withheld, but water was available. The following parameters were evaluated:



Hematology

- Erythrocyte Count (RBC)
- Hematocrit (Hct)
- Hemoglobin (Hgb)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Mean corpuscular volume (MCV)
- Platelet count
- Reticulocyte count
- Total and differential leukocyte counts

Coagulation

- Activated partial thromboplastin time
- Fibrinogen
- Prothrombin time

Biochemistry

- Albumin/globulin (A/G) ratio
- Alanine aminotransferase (ALT)
- Albumin
- Alkaline phosphatase
- Aspartate aminotransferase (AST)
- Calcium
- Creatinine
- Electrolyte balance (sodium, potassium, chloride)
- Globulin (calculated)
- Glucose (fasting)
- pH
- Phosphorus
- Serum amylase
- Total bilirubin
- Total protein
- Urea nitrogen (BUN)

Urinalysis

- 18-hour volume
- Bilirubin (qualitative)
- Blood (qualitative)
- Glucose (qualitative)
- Gross appearance
- Ketone (qualitative)
- Microscopic examination of sediment
- pH

Protein (qualitative)  
Specific gravity

#### Cardiology

Electrocardiogram (ECG) recordings of lead 2, heart rate, and blood pressure were recorded for each dog once during the pretest period (day -5 or -4) and at approximately 1 hour following dosing on days 1 and 14.

#### Gross Necropsy

All animals were subjected to a complete gross necropsy examination which included examination of the external surfaces of the body and all viscera. Fasted dogs were exsanguinated following an intravenous overdose of sodium pentobarbital. The following organs and tissues were collected from all animals and preserved in 10% neutral buffered formalin:

- Accessory genital organs (epididymides, prostate or uterus and vagina)
- Adrenals
- All gross lesions
- Aorta
- Bone marrow (smear from femur)
- Brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex)
- Cecum
- Colon
- Duodenum
- Ear (for identification only)
- Esophagus
- Eyes (including optic nerve)
- Femur (including articular surface)
- Gall bladder
- Heart
- Ileum
- Jejunum
- Kidneys
- Liver
- Lungs (infused with fixative)
- Mammary gland
- Mesenteric lymph node
- Pancreas
- Peripheral nerve (sciatic)
- Pituitary
- Rectum
- Skeletal muscle (thigh)
- Skin: Site 1 (nonfrictional surface-dorsal thorax)
  - Site 2 (frictional surface - elbow)
  - Site 3 (injection site - including vein)

- Spinal cord (cervical, midthoracic, lumbar)
- Spleen
- Sternum with bone marrow
- Stomach
- Submaxillary salivary gland
- Testes/Ovaries (including oviducts)
- Thymus
- Thyroid/parathyroid
- Trachea
- Urinary bladder

#### Organ Weights

The adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid and parathyroids from all surviving animals were weighed wet; paired organs were weighed together. Organ weights relative to final body weights were subsequently calculated.

#### Histopathology

All tissues collected from each animal on study were processed for histopathological examination. Tissue sections were cut from paraffin blocks, mounted on microscope slides and stained with hematoxylin and eosin. Histology was performed by ----b(4)-----  
----- The tissues were subsequently examined by a board certified veterinary pathologist.

### **Results:**

#### Survival

All animals survived to scheduled sacrifice at study termination.

#### Clinical Observations

No overt clinical signs of toxicity were observed during the study. Signs of gastrointestinal disturbance such as vomitus, mucoid stools and soft stools were observed among the groups. Possible slight increases in the occurrence of these signs were observed at the 120 and 1200 mcg/kg/day levels.

#### Body Weights and Weight Gain

No statistically significant differences in mean body weights were noted for males or females during the study. Mean weight gain was statistically reduced in the 1200 mcg/kg/day females during study days 1-2, however, for both males and females there were no consistent patterns of reduced weight gain that would indicate an effect of MPL treatment.

#### Food Consumption

No statistically significant differences were observed in male food consumption, however, food consumption of the 1200 mcg/kg/day males calculated as grams/animals/day and grams/kg/day was moderately decreased during the first few days of treatment when compared to the control group. In females, occasional statistical reductions in food consumption (grams/animal/day and grams/kg/day) were noted at the 120 and 1200 mcg/kg/day levels. Although statistical significance was not demonstrated at other times, daily food consumption of the 120 and 1200 mcg/kg/day females was slightly, but consistently lower than controls throughout the study. No treatment-related differences in food consumption were observed for the 6 mcg/kg/day females.

## Clinical Pathology Examinations

### Hematology

Very slight to moderate reductions in platelets were observed in the 120 and 1200 mcg/kg/day males and females on days 2, 8 and 15/16, however, only the day 6 platelet level of the 1200 mcg/kg/day females was statistically different from the control group. The lowest platelet levels were observed in the 1200 mcg/kg/day males on day 8 ( $116.7 \times 10^3/\text{cmm}$ ) and day 15 ( $133.3 \times 10^3/\text{cmm}$ ). All other measured platelet levels were  $\geq 170 \times 10^3/\text{cmm}$ . Marked increases in serum fibrinogen levels were noted in the 1200 mcg/kg/day males (day 2) and females (days 2 and 8). These increases were statistically significant in the female, but not the male dogs. A possible trend toward increased fibrinogen levels was also observed in the 120 mcg/kg/day males and females on day 2. The biological significance of these changes was not determined.

Marked increases in leukocytes were noted in the 1200 mcg/kg/day males and females on day 2. For each sex, the leukocytosis was attributed to an increase in segmented neutrophils. According to the Sponsor, these changes are expected pharmacological effects of MPL. On days 8 and 15/16, leukocyte and segmented neutrophil counts remained slightly higher than controls, but were not statistically different.

Other statistical differences in hematology data included decreased prothrombin time in the 1200 mcg/kg/day males on day 3 and decreased segmented neutrophils in the 6 mcg/kg/day females on day 2. These differences appeared to be incidental and unrelated to treatment with MPL.

### Clinical Chemistry

Statistical differences in clinical chemistry data included decreased phosphorus in the 1200 mcg/kg/day males on day 2, decreased AST in the 1200 mcg/kg/day females on day 8, decreased total bilirubin in the 1200 mcg/kg/day females on day 16, decreased pH in the 120 mcg/kg/day females on day 2, increased calcium in the 1200 mcg/kg/day females on day 2, increased sodium in the 1200 mcg/kg/day females on day 16, increased pH level in the 1200 mcg/kg/day females on day 8, increased albumin in the 1200 mcg/kg/day females on day -7, and increased calcium in the 6 mcg/kg/day females on day 2. These differences were not considered to be biologically meaningful since they were relatively minor, did not occur in any dose-related pattern, and they did not correlate with abnormal histopathology.

#### Urinalysis

Urine specific gravity was statistically decreased in the 120 and 1200 mcg/kg/day females on day 2. This change, coupled with increased urine volume in the 120 and 1200 mcg/kg/day females, was indicative of slight treatment-related hypoosmolar diuresis. No other apparent differences in urinalysis data were noted among the groups.

#### Cardiology

There were no indications of test article-related cardiovascular changes in the MPL-treated dogs.

#### Gross Necropsy Observations

Gross necropsy examinations did not reveal any specific changes attributable to MPL treatment.

#### Organ Weights

No statistically significant differences were noted in absolute or relative organ weight data, however, both absolute and relative spleen weight of the 1200 mcg/kg/day males and females were increased when compared to the control group.

#### Histopathology Observations

No test article-related lesions were observed microscopically in the MPL-treated dogs.

#### ***FDA reviewer's comment:***

Complete study reports were reviewed. I concur that the No Observed Adverse Effect Level (NOAEL) of 6 mcg/kg/day was determined in this study in dogs.

#### ***Overall conclusions from toxicology studies:***

The results from the toxicology studies conducted with MPL alone confirm that MPL is a detoxified form of bacterial LPS. Significant difference in the results from the tox studies conducted with MPL alone (included in the BLA) as compared to the results reported for LPS by Ribí et al, 1986 were noted. Repeat-dose toxicity studies were performed in which MPL was given once daily for up to 8 days in rats and 14 days in -(b)(4)- dogs, by the intravenous route to maximize the exposure. These studies showed a no-observed effect level at 6 mcg/kg/day in -(b)(4)- dogs and that the 40 mcg/kg/day dose was well-tolerated in rats. Effects seen were generally dose-related. The very few effects seen at the lowest dose of 40 mcg/kg/day in rat were minor and consistent with an immunostimulatory action of MPL, such as increased WBC count and spleen weight. These results may be contrasted with those reported for LPS by Ribí et al, 1986 where doses as low as 1 mcg/kg body weight of LPS induce death in rabbits.

MPL caused death when administered intravenously to rats at very high doses (5 mg/kg/day), which was attributed to endotoxic shock because of the findings of edema and hemorrhage in the brains of the dead animals. In this case, the MPL was administered intravenously and it was thought to be in a -----(b)(4)----- form as it was dissolved in -----(b)(4)----- . This is in

contrast to the MPL in Cervarix, which rather than being administered intravenously and in a soluble form, is being administered intramuscularly and in a non-soluble, particulate form, i.e., adsorbed onto aluminum hydroxide. In addition, in Cervarix, MPL is to be administered at a much lower dose (i.e., 50 mcg per dose, which equates to 1.7 to 0.7 mcg/kg dose for 30-70 kg-weighting individuals). In addition, the peak systemic exposure to MPL after a human intramuscular vaccination with an AS04-containing vaccine, administered according to a 0, 1, 6 month immunization schedule, for example, is expected to be significantly reduced compared to daily intravenous MPL injections used in the repeated-dose toxicity studies.

In conclusion, the data reported here showed expected findings associated with a strong immunostimulant (increased spleen weight and white blood cell value), and for these changes, there is a high safety margin for the proposed use of MPL. These data therefore support the use of MPL as a vaccine adjuvant in Human Papillomavirus Vaccine, AS04 Adjuvant-Adsorbed (Cervarix).for human vaccination against HPV infections and related clinical outcomes.

**Summary of GSK’s Response to Item 3 of FDA’s letter dated August 3, 2007, regarding MPL and MPL-containing products**

**This response is located in Module 2.6.6 (Toxicology Written Summary Section) in Amendment 12 to BLA 125259, received 27-AUG-2007**

1. Introduction

FDA’s letter of August 3, 2007, regarding MPL and MPL-containing products, which referenced BB-INDs -----(b)(4)-----, requested the following of GSK:

“In addition, please provide a summary of all animal data available to you that may reflect neurological and autoimmune-based adverse reactions to these investigational products.”

GSK chose to address this letter by submitting responses to each of the INDs and to the BLA for Cervarix (STN 125259). As it relates to the toxicological information in this review, a summary of the response regarding item 3 of FDA’s letter dated August 3, 2007 follows.

GSK examined the preclinical data for all their MPL-containing vaccine formulations (adjuvant plus antigen) as well as preclinical studies conducted with various formulations of the ---(b)(4)--- ----- adjuvant systems (both of which contain MPL) as well as the MPL component alone. A comprehensive listing of these studies is included in this submission in the tables provided.

These tables indicate the product/formulation/substance that was studied, the laboratory in which each test was performed (for MPL-containing vaccines), the type of animal study performed, species and strain and route of administration. The tables also provide GSK’s observations, where applicable regarding individual studies (row just below study description) relevant to FDA’s request.

2. Background Information

GSK’s adjuvant systems are unique combinations of different components, one of which is MPL. The firm states that the appropriate adjuvant system is chosen according to several criteria which include the following: the disease to be prevented, the target population, the antigen, the route of administration, the type of desired immune response and the desired duration of immunity.

GSK’s Tables 1 and 2 in this submission provide an overview of all adjuvant systems developed by GSK Biologicals or Corixa that contain MPL. GSK states that the most commonly used adjuvant system in GSK Biologicals’ clinical programs to date (based on the number of subjects receiving at least one dose of adjuvanted vaccine) is the AS04 adjuvant system. The second most commonly used adjuvant system is -(b)(4)-, a combination of MPL, -----(b)(4)-----  
-----.

### 3. Summary of Preclinical Studies for MPL-Containing Vaccine Formulations, Formulations of -----(b)(4)----- and MPL Alone

#### 3.1. General Principles of Toxicology Studies Conducted by GSK Biologicals

This section summarized GSK's routine approach to repeated dose studies, local tolerance studies, reproductive toxicity studies, and safety pharmacology studies.

#### 3.2. Summary of Preclinical Studies with Vaccine formulations Containing MPL

GSK's 3<sup>rd</sup> table in their response lists the preclinical studies conducted with vaccine formulations containing MPL. GSK concluded that none of these studies show evidence of adverse neurological, immunological or autoimmune-based (AID) reactions.

#### 3.3. Summary of Preclinical Studies with MPL-containing Adjuvants and MPL Alone

GSK's Table 4 in this submission lists the preclinical studies conducted with MPL-containing adjuvants (such as -----(b)(4)-----) and MPL alone (all of which have been submitted to the various Master Files for the respective adjuvants). GSK concluded that none of these studies show evidence of adverse neurological, immunological or autoimmune-based (AID) reactions.

#### 4. Overall Conclusion of GSK's Response

GSK concludes that none of the preclinical studies with vaccine formulations containing MPL, various -----(b)(4)----- formulations or MPL itself show evidence of adverse neurological, immunological or autoimmune-based (AID) reactions. There were no findings in the preclinical studies, for any vaccine formulation or for ----(b)(4)---- or MPL studied alone, that specifically directed any monitoring for diseases of potential neuroinflammatory or AID etiology in ensuing clinical protocols.

#### ***FDA reviewer's comment:***

I found GSK's response to item 3 of our August 3, 2007 letter to be complete and adequate and I concur in general with their overall conclusion.



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