

**Toxicology Review of Respiratory Syncytial Virus Vaccine Recombinant, Adjuvanted  
(Final Report)**

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4.2.3.7 Other toxicity studies

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### Introduction:

RSV, a member of the *Orthopneumovirus* genus, family *Pneumoviridae*, order *Mononegavirales*, is an enveloped virus with a single-stranded negative-sense RNA genome of 15.2kb. It is a major cause of respiratory infection in both infants and older adults. RSV infection follows a seasonal pattern causing illness primarily in the cooler months of the year in temperate regions and during the wet season in tropical countries with seasonal rainfall (1). RSV has 2 subgroups, A and B (either can cause severe disease), which co-circulate.

In infants, less than 1 year of age, respiratory syncytial virus (RSV) is the leading cause of hospitalization. Globally, RSV causes more than 34 million new acute respiratory illness and up to 200,000 deaths each year (2). In immunosuppressed children, immunosuppressed adults, and elderly, RSV causes severe respiratory illness (3-5).

Two indications are proposed to protect against RSV disease:

(b) (4)

2- Prevention of RSV-associated moderate to severe lower respiratory tract disease in adults 60 years of age and older by active immunization.

All ages could be affected by the RSV virus, but young infants below 1 year of age have the highest incidence of severe disease (bronchiolitis, pneumonia), peaking at 1- 3 months of age. Severe disease often leads to hospitalization and may be life threatening. In infants below 6 months of age, the risk for severe RSV-induced lower respiratory tract infections is highest and it is the most common cause of hospitalization in this age group.

Previous maternal vaccines showed that vaccinating mothers with RSV vaccine could reduce RSV associated morbidity and mortality in infants less than 6 months of age.

A Type B Pre-IND meeting was held on May 10, 2018. The minutes of the meeting are located in section 1.6.3. Additionally, a GSK response table is located within section 1.6.3, which includes GSK's responses to each of the comments from the agency on the pre-IND meeting package. The following questions were related to nonclinical toxicology:

#### *Nonclinical: GSK Question No. 6*

Section 15 includes a summary of available results from the ongoing pharmacology studies and the preliminary results of the GLP repeat-dose toxicity study conducted in rabbits with the (b) (4). In addition, a summary of the planned pharmacology and toxicity studies (developmental and reproductive toxicity) are included.

Does CBER agree that the proposed nonclinical pharmacology and nonclinical toxicology packages support the initiation of (b) (4)

#### CBER response

We agree that the nonclinical toxicology package supports the initiation of the proposed clinical study.



*Nonclinical: GSK Question No. 7*

Does CBER agree that the proposed (b) (4) toxicity studies will be sufficient to initiate (b) (4) (see table 35)?

CBER response

The proposed dosing regimen in the (b) (4) toxicity study is acceptable. However, no details of the study design and the end points were reported. Thus, the acceptance of the final study will be pending the study protocol submission. Please submit the study protocol when available.

**Note:** An earlier study was submitted for our review in 2018 using a lower (120µg) dose level with the following study title and number.

Title and study number: Repeat Dose Toxicity Study with RSV (b) (4)  
Given Intramuscularly Alone or with AS01B to the Rabbit Followed by a 4-Week Treatment-Free Period. Study number: 8363131.

**Clinical studies:**

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
<b>RSV OA=ADJ- 006 (212494)</b> – ongoing  <i>DLP for safety analysis: April 30, 2022</i>	<b>Northern hemisphere</b> : US, Canada, Mexico, Belgium, Estonia, Finland, Italy, Germany, Poland, UK, Spain, Russia, South Korea, Japan  <b>Southern hemisphere</b> : Australia, New Zealand, South Africa	<p>Phase 3, randomized, placebo-controlled, observer- blind, multi-center, efficacy study with 4 parallel groups (RSVPreF3 L1/L2/L3<sup>a</sup>: Placebo = 1:1) before Season 1 The RSVPreF3 groups will be re-randomized before Season 2 into 2 subgroups with a 1:1 ratio (RSV_ annual and RSV_1dose).</p> <p>Study duration is planned to be approximately 3 years for participants in the Northern hemisphere and 2.5 to 3 years for participants in the Southern hemisphere.</p> <p>Only objectives relevant to this Application are listed below.</p> <p><u>Primary objective (confirmatory):</u></p> <p>a. VE of a single dose of RSVPreF3 OA in the prevention of RSV-confirmed LRTD during the first season.</p> <p><u>Secondary objectives (descriptive):</u></p> <p>b. VE of a single dose of RSVPreF3 OA in the prevention of RSV-confirmed LRTD for each RSV subtype (A and B) separately, by age category, evolution over time, by baseline comorbidities and by baseline frailty status,</p>	<p>Older Adults <math>\geq 60</math> YOA</p> <p>Single dose of either RSVPreF3 OA or Placebo at Day 1 in all groups and annual revaccination with either RSVPreF3 OA or Placebo depending on the group</p>	<p><u>Season 1</u>  <b>RSVPreF3:</b>  RSVPreF3 OA</p> <p><b>Placebo (Control):</b>  Placebo</p> <p><u>Seasons 2 and 3</u>  <b>RSV annual:</b>  RSVPreF3 OA annual pre-season revaccination doses  <b>RSV_1dose:</b>  Placebo annual pre-season administration  <b>Placebo (Control):</b>  Placebo annual pre-season administration</p>	<p><b>12 467 (12 466)</b></p> <p>12 499 (12 494)</p> <p>Not yet initiated at the time of preparation of this Application</p>	<p><b>850</b></p> <p>852</p> <p>Not yet initiated at the time of preparation of this Application</p>

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
		<p>c. VE of a single dose RSVPreF3 OA in the prevention of hMPV-confirmed LRTD,</p> <p>d. VE of a single dose RSVPreF3 OA in the prevention of severe RSV-confirmed LRTD, RSV-confirmed ARI, any ARI and any LRTD, hospitalization due to RSV-confirmed respiratory diseases or respiratory diseases during the RSV seasons<sup>b</sup>, complications related to RSV-confirmed ARI and any ARI during the RSV seasons,</p> <p>e. Impact of RSVPreF3 OA on lower respiratory tract symptoms, ARI total symptoms, health utility score and physical functioning in participants with RSV- confirmed ARI in the RSVPreF3 groups compared to the Placebo group,</p> <p>f. Description of RSV-confirmed ARI cases and RSV-confirmed LRTD cases in the RSVPreF3 and Placebo groups,</p> <p>g. Humoral immune response to RSVPreF3 OA in terms of RSV-A / -B NAb titers and RSVPreF3- specific IgG Ab concentrations in a subset of participants,</p> <p>h. Reactogenicity in a subset of participants and safety in all participants of RSVPreF3 OA.</p>				

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
<a href="#">RSV OA=ADJ- 004 (212496)</a> – ongoing  <i>DLP for safety analysis: February 11, 2022</i>	US, Finland, German y, Japan, Taiwan	Phase 3; randomized (3:1:1), open-label, multi-center, immunogenicity study with 3 parallel groups. Study duration is planned to be approximately 3 years for participants in all groups. <u>Primary objective (descriptive):</u> <ul style="list-style-type: none"> <li>Humoral immune response following a 1-dose primary schedule of RSVPreF3 OA up to 12 months post-Dose 1 in terms of RSV-A and RSV-B NAb titers in a subset of participants.</li> </ul> <u>Secondary objectives (descriptive):</u> <ul style="list-style-type: none"> <li>Humoral immune response to RSVPreF3 OA up to 12 months post-Dose 1 in terms of RSVPreF3- specific IgG Ab concentrations in a subset of participants,</li> <li>CMI response following 1 dose of RSVPreF3 OA up to study end in terms of RSVPreF3-specific</li> </ul>	Older Adults $\geq 60$ YOA  Single dose of RSVPreF3 OA at Day 1 followed by 3 possible revaccination schedules	<b>RSV_annual:</b> RSVPreF3 OA at Day 1, Month 12, and Month 24  <b>RSV_flexible revaccination:</b> RSVPreF3 OA at Day 1 and a revaccination dose at Month 24  <b>RSV_1dose:</b> RSVPreF3 OA at Day 1	<b>993</b>  <b>329</b>  <b>331</b>	<b>323 / 323</b>  <b>294 / 101</b>  <b>312 / 106</b> Humoral / Cellular Month 6 PPSi in a subset of participants <sup>e</sup>
		polypositive CD4+/CD8+ T cells <sup>e</sup> in a subset of participants, i. Reactogenicity and safety of each vaccination schedule of RSVPreF3 OA in all participants.				

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
RSV OA=ADJ- 007 (214488) – completed	New Zealand, Panama, South Africa	Phase 3; randomized (1:1), controlled, open-label, multi- center, co-administration study with 2 parallel groups. Study duration was 6 to 7 months (i.e., 6 months after last vaccination in all groups). <u>Co-primary objectives (confirmatory):</u> <ul style="list-style-type: none"> <li>Non-inferiority of RSVPreF3 OA when co-administered with FLU-QIV compared to RSVPreF3 OA administered alone in terms of RSV-A NAb titers, 1 month after RSVPreF3 OA.</li> <li>Non-inferiority of FLU-QIV when co-administered with RSVPreF3 OA compared to FLU-QIV administered alone in terms of HI Ab titers for each Flu strain, 1 month after FLU-QIV.</li> </ul> <u>Secondary objectives (descriptive):</u> <ul style="list-style-type: none"> <li>Non-inferiority of FLU-QIV when co-administered with RSVPreF3 OA compared to FLU-QIV administered alone in terms of HI seroconversion status for each Flu strain, 1 month after FLU-QIV.</li> <li>Humoral immune response to RSVPreF3 OA when co-administered with FLU-QIV or administered alone in terms of RSV-A and RSV-B NAb titers, 1 month after the RSVPreF3 OA.</li> </ul>	Older Adults ≥ 60 YOA  Single dose of RSVPreF3 OA either co-administered with or given a month apart from a single dose of FLU-QIV	<b>Co-Ad:</b> FLU-QIV + RSVPreF3 OA at Day 1  <b>Control:</b> FLU-QIV at Day 1 + RSVPreF3 OA at Day 31	<b>442</b>	<b>427</b> (Day 31)
		<ul style="list-style-type: none"> <li>Humoral immune response to FLU-QIV when co-administered with RSVPreF3 OA or administered alone in terms of HI Ab titers expressed as GMT, MGI, seroconversion and seroprotection status for each Flu strain, 1 month after FLU-QIV.</li> </ul>			<b>443</b>	<b>411</b> (Day 31 for FLU-QIV evaluation) 398 (Day 61 for RSV evaluation) <sup>d</sup>

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
		j. Reactogenicity and safety following administration of the RSVPreF3 OA and FLU-QIV, co-administered or administered alone.				
RSV OA=ADJ- 009 (217131) – concluded (last participant last visit performed but end-of- study data not yet reported)  <i>DLP for safety analysis: March 9, 2022</i>	US, Canada , Sweden	Phase 3; randomized (1:1:1), double-blind, multi-center, lot-to-lot consistency study with 3 parallel groups. Study duration was 6 months in all groups. <u>Primary objectives (confirmatory):</u> <ul style="list-style-type: none"> <li>Lot-to-lot consistency of 3 lots of RSVPreF3 OA in terms of RSVPreF3-specific IgG Ab concentrations at Day 31.</li> </ul> <u>Secondary objectives (descriptive):</u> <ul style="list-style-type: none"> <li>Humoral immune response of the 3 lots of RSVPreF3 OA in terms of RSVPreF3-specific IgG Ab concentrations at Day 31.</li> <li>Reactogenicity and safety following administration of RSVPreF3 OA.</li> </ul>	Older Adults ≥ 60 YOA  Single dose of RSVPreF3 OA at Day 1 in all groups	<b>RSVPreF3_Grp1:</b> RSVPreF3 OA Lot 1  <b>RSVPreF3_Grp2:</b> RSVPreF3 OA Lot 2  <b>RSVPreF3_Grp3:</b> RSVPreF3 OA Lot 3	<b>251</b>  <b>253</b>  <b>253</b>	<b>234</b>  <b>237</b>  <b>237</b>

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
RSV OA=ADJ- 002 (208851) – completed	US, Belgium	Phase 1/2, randomized, placebo-controlled, observer-blind, multi-center, dose selection and formulation study, with 4 parallel groups in Part A (1:1:1:1) and 10 parallel groups in Part B (1:1:1:1:1:1:1:1:1:1). Study duration was 3 months for participants in Part A and 14 months for participants in Part B. <u>Primary objectives:</u> <ul style="list-style-type: none"> <li>Reactogenicity and safety of 2 doses of RSVPreF3 OA administered on a 0, 2-month schedule, up to 1 month after the last dose (Day 91).</li> </ul> <u>Secondary objectives (descriptive):</u> <ul style="list-style-type: none"> <li>Humoral immune responses (in terms of RSV-A NAb titers and RSVPreF3-specific IgG Ab concentrations) to the different RSVPreF3 OA formulations up to 1</li> </ul>	<b>Part A:</b> Adults 18-40 YOA  <b>Part B:</b> Older Adults 60-80 YOA  2 doses of RSVPreF3 OA or Placebo at Day 1 and Day 61 depending on the group	<u>Part A:</u> <b>30-Plain_A:</b> 30 µg RSVPreF3	12	11
				<b>60-Plain_A:</b> 60 µg RSVPreF3	12	9
				<b>120-Plain_A:</b> 120 µg RSVPreF3	12	11
				<b>Placebo_A (Control):</b> Placebo	101	12
				<u>Part B:</u> <b>30-Plain_B:</b> 30 µg RSVPreF3	97	88
				<b>60-Plain_B:</b> 60 µg RSVPreF3		85

Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
		<p>month after the last dose (Day 91).</p> <p>k. CMI responses to the different RSVPreF3 OA formulations up to 1 month after the last dose (Day 91) in terms of RSVPreF3-specific CD4+/CD8+ T cells<sup>c</sup>.</p> <p>l. Reactogenicity and safety of 2 doses of RSVPreF3 OA administered up to the end of follow-up (Month 14) in Part B.</p> <p>m. Occurrence of RSV-associated RTI during the RSV season in nasal/throat swab samples collected during the assessment visit for potential RSV-RTI in Part B.</p> <p>Note that RSV-B NAb titers, RSVPreF3 RSB1-specific Ab concentrations and persistence of the humoral and cellular immune response were assessed as tertiary endpoints in this study.</p>		<p><b>120-Plain_B:</b> 120µg RSVPreF3</p> <p><b>30-AS01E_B:</b> 30 µg RSVPreF3 / AS01E</p> <p><b>60-AS01E_B:</b> 60µg RSVPreF3 / AS01E</p> <p><b>120-AS01E_B:</b> 120 µg RSVPreF3 / AS01E</p> <p><b>30-AS01B_B:</b> 30 µg RSVPreF3 / AS01B</p> <p><b>60-AS01B_B:</b> 60 µg RSVPreF3 / AS01B</p> <p><b>120-AS01B_B:</b> 120 µg RSVPreF3 / AS01B</p> <p><b>Placebo_B (Control):</b> Placebo</p>	<p>100</p> <p>101</p> <p>101</p> <p><b>100</b></p> <p>103</p> <p>100</p> <p>101</p> <p>101</p>	<p>87</p> <p>84</p> <p>92</p> <p><b>87</b></p> <p>85</p> <p>93</p> <p>92</p> <p>93</p>



Study ID (number) – status	Study countries	Study design Objectives	Population (age)  Schedule of vaccination	Study groups	Number of participants	
					ES (mES)	PPS for immunogenicity
RSV OA=ADJ- 011 EXT:002 MTH20 (213569) – completed	US, Belgium	Phase 2b, open-label, multi-center extension study with 3 parallel groups from RSV OA=ADJ-002. Study duration was 6 months after Dose 3 in all groups. <u>Primary objectives (descriptive):</u> <ul style="list-style-type: none"> <li>Reactogenicity and safety up to 1 month post-Dose 3 of RSVPreF3 OA for all participants of 3 groups in RSV OA=ADJ-002 (30-AS01E_B, 60-AS01E_B and 120-AS01E_B),</li> <li>Humoral immune response (in terms of RSV-A and RSV-B NAb titers) up to 1-month post-Dose 3 of RSVPreF3 OA for participants vaccinated with 2 doses of RSVPreF3 OA in RSV OA=ADJ-002 (120- AS01E_B)</li> </ul> <u>Secondary objectives (descriptive):</u> <ul style="list-style-type: none"> <li>Humoral (in terms of RSVPreF3-specific IgG Ab concentrations) and cellular (in terms of RSVPreF3- specific polypositive CD4+/CD8+ T cells<sup>a</sup>) immune response up to 1-month post-Dose 3 of RSVPreF3 OA for participants in 120-AS01E_B</li> </ul> Reactogenicity and safety following administration of RSVPreF3 OA up to study end for all participants	Older Adults ≥ 60 YOA  Single dose of RSVPreF3 OA approximately 18 months after Dose 2 in RSV OA=ADJ-002	<b>120_AS01E_B:</b> RSVPreF3 OA <b>30_AS01E_B:</b> RSVPreF3 OA <b>60_AS01E_B:</b> RSVPreF3 OA	<b>40</b>  <b>39</b>  <b>43</b>	<b>34</b> (Immunogenicity assessed for 120_AS01E_B group only)

Data source: M5.3.5.1 and M5.3.5.2, RSV OA=ADJ-006 (212494) Report (13-AUG-2022), RSV OA=ADJ-004 (212496) Report Amendment 1 (02-AUG-2022), RSV OA=ADJ-007 (214488) Report Amendment 1 (02-AUG-2022), RSV OA=ADJ-009 (217131) Report (17-MAY-2022), RSV OA=ADJ-002 (208851) Report (12-MAY-2021), RSV OA=ADJ-011 EXT:002 (213569) Report (31-MAR-2022)

Ab = Antibody; ARI = Acute Respiratory Infection, CMI = Cell-mediated immune response, CSR = Clinical Study Report; DLP = Data Lock Point; ES = Exposed Set; FLU-QIV = Seasonal Quadrivalent Influenza Vaccine, HI = Hemagglutination Inhibition, hMPV = human metapneumovirus; IgG = Immunoglobulin G, LRTD = Lower Respiratory Tract Disease, mES = modified Exposed Set; NAb = Neutralizing antibody, PPS = Per Protocol Set, RSV = Respiratory Syncytial Virus, RSVPreF3 OA = RSV PreFusion protein 3 Older Adult, RTI = Respiratory Tract Infection, UK : United Kingdom; US = United States; VE = Vaccine Efficacy, YOA = Years of Age.

a. 3 separate lots were used for Dose 1 in RSV OA=ADJ-006 but combined data for the 3 lots are shown.

b. The RSV seasons defined for RSV OA=ADJ-006 are from 1 October to 30 April in Northern hemisphere and from 1 March to 30 September in Southern hemisphere.

- c. In RSV OA=ADJ-004, as defined in the protocol HI and CMI was assessed in subsets of participants from each group.
- d. For RSV OA=ADJ-007, numbers shown are for the final analysis PPSi (1-month post-last vaccination) of the co-primary objectives. At the end-of-study analysis, 1 additional participant from the Control group was eliminated compared to the final analysis PPSi.
- e. RSVPreF3-specific polypositive CD4+/CD8+ T cells expressing at least 2 activation markers including at least 1 cytokine among CD40L, IL-2, TNF- $\alpha$ , IFN- $\gamma$  in the Phase ½ studies and also including IL13, IL-17 and 4-1BB in the Phase 3 study.
- f. In RSV OA=ADJ-009, 3 different and randomly selected RSVPreF3 antigen lots were extemporaneously reconstituted with 3 different and randomly selected AS01E adjuvant lots, resulting in 3 unique random combinations.

This table only presents study objectives relevant to the current Application.

Number of participants shown in **bold** received the final RSVPreF3 OA candidate vaccine formulation.

Table 1: Clinical studies; sponsor provided

**Studies reviewed for this BLA:**

***General toxicology studies***

- 1- Repeat Dose Toxicity Study with RSV (b) (4) Given Intramuscularly Alone or with AS01B to the Rabbit Followed by a 4-Week Treatment-Free Period. Study number: 8363131. Original submission.
- 2- A Repeated Dose Toxicity Study with RSVPreF3 Candidate Vaccines (RSVPreF3, RSVPreF3/AS01B or RSVPreF3 Co-administered with Boostrix) Given Intramuscularly to the Rabbit followed by a 4-Week Treatment Free Period. Study number: 8384096. (Submitted in amendment 31).
- 3- Repeated-dose Toxicity Study with Zoster Candidate Vaccine (gE/AS01B) Administered Subcutaneously (Four Times) or Intramuscularly (Four Times) to Male and Female Rabbits Followed by a 4-week Treatment Free Period. Study number: 20094.
- 4- Repeated-dose Toxicity Study with AS01B Administered Intramuscularly (Seven Times) to Male and Female Rats Followed by a 4-week Treatment Free Period. Study number: 20165.
- 5- AS01B Versus AS02V Toxicity Study by Repeated (5 Times) Intramuscular Administration to Rabbits. Study number: (b) (4) 045/022412.
- 6- Repeated Dose Toxicity Study with DQ Administered Intramuscularly to Male and Female rats. Study number: 20154.
- 7- Repeated Dose Toxicity Study with DQ Administered Intramuscularly to Male and Female rabbits. Study number: 20155.
- 8- 7-Day Intravenous, Dose Range-finding Toxicity Study in (b) (4) Rats with (b) (4) Study number: 3262.4.
- 9- 8-Day Intravenous Toxicity Study of (b) (4) in Rats. Study number: 3262.2.
- 10- 14-Day Intravenous Toxicity of (b) (4) in Dogs. Study number: 3262.1
- 11- The Acute Intraperitoneal Toxicity of Monophosphoryl Lipid A (MPLA) in Rats. Study number: (b) (4)
- 12- Repeated Dose Toxicity and Local Tolerance Study with QS-21 and with DQ Administered Intramuscularly to Male and Female Rats-reported in MF (b) (4). Study number: (b) (4).

***Reproductive Studies***

- 1- Zoster Candidate Vaccine (gE/AS01B): Study of Effects on Embryo-fetal, Pre- and Post-natal Development in CD Rats by Intramuscular Administration (Including Pre-mating Immunization Phase). Study number: (b) (4). Serological Report: Zoster Candidate Vaccine (gE/AS01B): Study of Effects on Embryo Fetal, Pre- and Post-natal Development in CD Rats by Intramuscular Administration (Including Pre-mating Immunization Phase); Ref. HLS study number: (b) (4).
- 2- Zoster Candidate Vaccine: Study of Effects on the Fertility of Male CD Rats by Intramuscular Administration (Including Pre-mating Immunization Phase). Study number: (b) (4).
- 3- A Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) of RSVPreF3 or AS01B by Intramuscular Injection in Rabbits. Study number: 20152506.
- 4- A Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) of RSVPreF3 by Intramuscular Injection in Rats. Study number: 20152507.

- 5- MPL: Subcutaneous Study of Embryo-Fetal Development in the Rat. (b) (4) report number 1729/7-D6154.
- 6- MPL: Subcutaneous Study of Embryo-Fetal Development in the Rabbit. (b) (4) report number 1729/8-06154
- 7- MPL: Subcutaneous Study of Pre- and Postnatal Development in the Rat. (b) (4) report number 1729/17-D6154
- 8- DQ Immunostimulant: Study for Effects on Female Fertility, Embryo-fetal and Pre- and Post-natal Development in the CD Rat by Intramuscular Administration (Including Pre-mating Immunization Phase). Study number: (b) (4) .
- 9- DQ – Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) by the Intramuscular Route in the Rabbit. Study number: AB14898.

***Genotoxicology studies: in vivo***

Study # 1: Genetic toxicology (micronucleus test). Report number: (b) (4) 317/032657. (Reviewed by Nabil in IND 13857).

Study # 2: Genetic toxicology (bone marrow and blood cells assessment). Report number: (b) (4) 681/043748


Study # 3: MPL (b) (4) rat micronucleus test (Reviewed by Steve Kunder in 2009)

Study # 4: An assessment of the effects of AS01B on red blood cells in peripheral blood and bone marrow (study number (b) (4) 0026/070209). Genetic toxicology (Reviewed by Nabil).

Study # 5: Bone marrow micronucleus test with DQ in rats (Study no.: V20204/04). Genetic toxicology (Reviewed by Nabil in IND 13857)

***Genotoxicology studies: in vitro***

(b) (4)



***Local Tolerance:***

Study # 1: (b) (4) Single dose toxicity and local tolerance study with DQ administered intramuscularly to male and female rats

Study # 2: (b) (4) Single dose toxicity and local tolerance study with DQ administered intramuscularly to male and female rabbits

Study # 3: (b) (4) Single dose toxicity and local tolerance study with a VZV candidate vaccine (gE 100 µg / AS01B) administered intramuscularly to male and female rabbits (v 6812/02)

Study # 4: (b) (4) Single dose toxicity and local tolerance study with Zoster candidate vaccine (gE/AS01B) administered subcutaneously to male and female rabbits

**General Toxicology Studies Reviews****Study number 1:**

**Title and study number:** Repeat Dose Toxicity Study with RSV (b) (4) Given Intramuscularly Alone or with AS01B to the Rabbit Followed by a 4-Week Treatment-Free Period. Study number: 8363131.

**Performing laboratory:** (b) (4)

**Study initiation date:** July 18, 2017

**Final report date:** July 05, 2018

**Test article batch/lot:**

Test Article	Storage	Batch No.	Expiration Date	Content <sup>a</sup>
RSV (b) (4) Candidate Vaccine (also known as RSV (b) (4) (b) (4) RSVPreF3)	In a refrigerator, set to maintain 2° to 8°C	TRSV001A	31 March 2018	0.455 mg/mL

<sup>a</sup> via (b) (4)

Vehicle Control Article Components	Storage	Batch No.	Expiration Date
0.9% Sodium Chloride for Injection, USP (sterile saline)	In a refrigerator, set to maintain 2° to 8°C	(b) (4)	14 September 2018
AS01B	In a refrigerator, set to maintain 2° to 8°C		31 August 2018 30 April 2018

Table 2: Test article batch numbers (study # 1); sponsor provided

**Animal species and strain:** (b) (4) rabbits

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

**Number of animal per group and sex:** 10/sex/group

**Age:** 15 weeks old

**Body weight range:** 2109 to 2655 g for males and 1987 to 2592 g for females

**Route and site of administration:** Intramuscular (IM).

**Volume of injection:** 0.5 mL's

**Frequency of administration and study duration:** Animals were dosed on study days 1, 15, and 29

**Dose:** 0.12 mg/dose.

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. For batch number (b) (4), a shelf life of (b) (4) months, with expiration date of 30<sup>th</sup> April, 2018, was reported.

**Means of administration:** Intramuscular (IM)

**Report status:** Final

**Experimental design:**

Animals were randomized and assigned to 3 different groups. Each group consisted of 10 animals/sex. Animals were dosed by intramuscular (IM) route on study days 1, 15, and 29. The details of the study design are listed in the following table:

Group	No. of Animals <sup>b,c</sup>		Vaccine Dose Level (mg/dose)	Injection Volume (mL/injection)
	Male	Female		
1 Control-Saline <sup>a</sup>	10	10	0	0.5
2 RSV (b) (4) Candidate Vaccine + Saline <sup>d</sup>	10	10	0.120	0.5
3 RSV (b) (4) Candidate Vaccine + AS01B <sup>e</sup>	10	10	0.120	0.5

Note: While the last day of dosing was day 29, the dosing phase continued through day 32. The recovery phase began on study day 32.

g. Group 1 received vehicle control article 1 only.

h. Animals were dosed on days 1, 15, and 29 of the dosing phases.

i. Five males and 5 females from each group were euthanized on day 32 (3 days after the last injection - terminal euthanasia). The remaining animals were euthanized on day 26 of the recovery phase (study day 57) - recovery euthanasia.

j. Group 2 was reconstituted in saline

k. Group 3 was reconstituted in AS01B

Table 3: Experimental design (study # 1); sponsor provided

## Methods:

**Randomization procedure:** No.

**Statistical analysis plan:** Yes.

**The following parameters were evaluated:** Cage side observations (twice daily), clinical observations (twice daily), body weights (days 1, 2, 3, 4, 8, 11, 15, 16, 17, 18, 22, 25, 29, 30, 31 and 32 of the dosing phases, and twice weekly after day 32), food consumption (daily), ophthalmology (pre-dose, once during the last week of the dosing phase following the last dose, and once during the last week of the recovery phase), rectal temperature (pre-dose and approximately 6, 24, and 48 hours after each dose), dermal scoring (at approximately 6, 24, and 48 hours post each dose during the dosing phase), clinical chemistry, hematology, and coagulation (pre-dose, days 2, 8, and 30 of the dosing phase, and on days 5 and 26 of the recovery phase), serology (pre-dose and on days 32 and 57). Postmortem evaluations were performed on days 32 and 57.

Parameters	Frequency of Testing
Cageside observation <sup>1</sup>	Twice daily
Clinical observations <sup>2</sup>	Twice daily
Body weight	Days 1, 2, 3, 4, 8, 11, 15, 16, 17, 18, 22, 25, 29, 30, 31 and 32 of the dosing phases, and twice weekly after day 32
Food consumption	Daily
Rectal temperature	Pre-dose and approximately 6, 24, and 48 hours after each dose
Ophthalmologic exam	Pre-dose, once during the last week of the dosing phase following the last dose, and

<sup>1</sup> Cageside observations include mortality, morbidity, general health and signs of toxicity.

<sup>2</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

	once during the last week of the recovery phase
Clinical chemistry*	Pre-dose, days 2, 8, and 30 of the dosing phases, and on days 5 and 26 of the recovery phase
Hematology*	Pre-dose, days 2, 8, and 30 of the dosing phases, and on days 5 and 26 of the recovery phase
Coagulation*	Pre-dose, days 2, 8, and 30 of the dosing phases, and on days 5 and 26 of the recovery phase
Dermal scoring	At approximately 6, 24, and 48 hours post each dose during the dosing phase
Serology*	Pre-dose and on days 32 and 57
Postmortem study evaluations	Days 32 and 57

\* Blood samples were collected from the medial auricular artery.

Table 4: Parameters evaluated (study # 1)

**Postmortem procedures:**

Organ/Tissue		Organ/Tissue	
adrenal (2)	W P,E	mammary gland (females)	P,E
aorta	P,E	muscle, biceps femoris (right) <sup>e</sup>	P,E
bone, femur with bone marrow (articular surface of the distal end)	P,E	optic nerve (2) <sup>a</sup>	P,E
bone, sternum with bone marrow	P,E	ovary (2)	W P,E
brain	W P,E	oviducts (2)	P,E
cecum	P,E	pancreas	P,E
cervix	P,E	parathyroid glands	P,E
colon	P,E	pituitary gland	W P,E
diaphragm	P,E	prostate <sup>b</sup>	W P,E
duodenum	P,E	rectum	P,E
epididymis (2)	W P,E	salivary gland [mandibular, parotid and sublingual (2)]	P,E
esophagus	P,E	sciatic nerve	P,E
eye(2) <sup>a</sup>	P,E	seminal vesicle <sup>b</sup>	W P,E
gall bladder (drained)	P,E	skin/subcutis (hind limb, right)	P,E
gut-associated lymphoid tissue (GALT; Peyer's Patch)	P,E	spinal cord (cervical, thoracic, and lumbar)	P,E
Harderian gland <sup>a</sup>	P,E	spleen	W P,E
heart	W P,E	stomach	P,E
ileum	P,E	testis (2) <sup>a</sup>	W P,E
injection sites including overlying skin and muscle (en bloc, skin/subcutis/muscle) <sup>c</sup>	P,E	thymus	W P,E
jejunum	P,E	thyroid (2 lobes) with parathyroid	W P,E
kidney (2)	W P,E	tongue	P,E
larynx	P,E	trachea	P,E
lesions	P,E	ureter (2)	P,E
liver	W P,E	urinary bladder	P,E
lung with large bronchi	W P,E	uterus (with cervix) <sup>b</sup>	W P,E
lymph node (iliac, inguinal, popliteal, mesenteric and mandibular) <sup>d</sup>	W P,E	vagina	P,E

E = Examined microscopically; P = Processed; W = Weighed.

a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

b Organs weighed together; uterus with cervix and prostate with seminal vesicle.

c All injection sites were collected, but only the last 1 was analyzed

d Lymph nodes from each category were weighed separately

e see [Protocol Deviations](#)

Table 5: Postmortem procedures (study # 1); sponsor provided



**Results:**

No test article-related morbidity and/or mortality were reported.

**Clinical chemistry, hematology, and coagulation:**

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ )	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Aspartate aminotransferase (AST or SGOT) SD8 M $\uparrow$ = 1.6 G3	Alanine aminotransferase (ALT or SGPT)
B) HEPATOBILIARY		Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS	C-reactive protein*	Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Lactate dehydrogenase SD30 M $\downarrow$ = 0.5 G2 SD30 M $\downarrow$ = 0.5 G3 SD35 M $\downarrow$ = 0.6 G3 SD8 F $\uparrow$ = 1.6 G2 SD30 F $\downarrow$ = 0.6 G2  Total Cholesterol SD35 M $\downarrow$ = 0.5 G2 SD56 M $\downarrow$ = 0.6 G2	Albumin (A) Total protein Carbon dioxide Globulin A/G ratio Creatine kinase Fasting triglycerides GGT

\*See section below (page 10)

Table 6: Clinical chemistry results (study # 1)

Clinical chemistry results show an increase in AST levels in group 3 males at study day 8. Lactate dehydrogenase levels were decreased in groups 2 and 3 males at study day 30. Lactate dehydrogenase levels were decreased in group 3 males at study day 35. Lactate dehydrogenase levels were increased in group 2 females at study day 8. Lactate dehydrogenase levels were decreased in group 2 females at study day 30. Cholesterol levels were decreased in group 2 males at study days 35 and 56.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.53, ie, $\geq 1.6$ or $\leq 1.6$	Not of NOTE
Red blood cells		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
White blood cells	Monocyte count: SD2 M $\uparrow$ = 1.9 G3 SD8 F $\uparrow$ = 1.6 G3  Neutrophil count SD2 M $\uparrow$ = 2.3 G3 SD30 M $\uparrow$ = 1.6 G3 SD2 F $\uparrow$ = 2.3 G3 SD30 F $\uparrow$ = 1.8 G3  Eosinophils count Pre-dose M $\downarrow$ = 0.5 G2 SD30 M $\downarrow$ = 0.3 G3 SD35 M $\uparrow$ = 1.7 G3 Pre-dose F $\uparrow$ = 1.7 G3 SD30 F $\downarrow$ = 0.5 G3	Macrophage White Blood Cells (WBC) Lymphocyte count Leukocytes Basophils Large Unstained Cells (LUC)
Clotting potential	Fibrinogen SD30 M $\uparrow$ = 1.8 G3 SD56 F $\downarrow$ = 0.6 G3  Platelet count SD35 M $\uparrow$ = 1.9 G3  Prothrombin time SD35 M $\downarrow$ = 0.6 G3	Activated partial-thromboplastin time clotting time
Others		Bone marrow cytology

Table 7: Hematology results (study # 1)

Hematology results show an increase in monocyte levels in group 3 males and females at study days 2 and 8, respectively. Neutrophil levels were increased in group 3 males and females at study days 2 and 30, respectively.

Eosinophil levels were decreased in groups 2 and 3 males at pre-dose and at study day 30, respectively. Eosinophil levels were increased in group 3 males and females at study day 35 and at pre-dose, respectively. Eosinophil levels were decreased in group 3 females at study day 30.

<sup>3</sup> With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

Fibrinogen levels were increased in group 3 males at study day 30. Fibrinogen levels were decreased in group 3 females at study day 56. Platelet levels were increased in group 3 males at study day 35. Prothrombin time were decreased in group 3 males at study day 35.

#### CRP:

Group/ Sex	Phase Day	CRP mg/dL					
		Predose	Dosing		Recovery		
		8	2	8	30	5	26
1/M	Mean	0.2	0.3	0.2	<0.3	0.3	0.7
	SD	0.17	0.05	0.00	0.18	0.14	0.09
	N	10	10	10	10	5	5
	P(overall)	-	<0.0001	-	-	0.2012	-
2/M	Mean	<0.2	0.2	0.3	<0.3	0.5	0.6
	SD	0.07	0.05	0.07	0.16	0.25	0.04
	N	10	10	10	10	5	5
	P(v1)	-	0.3359	-	-	-	-
	P(v3)	-	<0.0001*	-	-	-	-
3/M	Mean	0.2	1.8	0.3	2.4	0.3	0.7
	SD	0.08	0.56	0.03	0.78	0.13	0.08
	N	10	10	10	10	5	5
	P(v1)	-	<0.0001*	-	-	-	-
	Statistics	X1	APT	X2	X5	AP	X2

\* P<=0.05  
X1 = No analysis required  
AP = ANOVA and protected t-tests  
T = Rank-transformed data  
X2 = Not analyzed (too few distinct values)  
X5 = Not analyzed (values above/below the limit of quantitation)

Table 8: CRP levels in males (study # 1); sponsor provided

Group/ Sex	Phase Day	CRP mg/dL					
		Predose	Dosing		Recovery		
		8	2	8	30	5	26
1/F	Mean	0.2	0.5	0.4	0.4	0.5	0.6
	SD	0.06	0.24	0.12	0.22	0.11	0.04
	N	10	10	10	10	5	5
	P(overall)	-	<0.0001	0.4443	-	0.3403	0.6160
2/F	Mean	0.3	0.7	1.0	<0.4	0.3	0.7
	SD	0.24	0.36	1.60	0.17	0.12	0.18
	N	10	10	10	10	5	5
	P(v1)	-	0.1426	-	-	-	-
	P(v3)	-	<0.0001*	-	-	-	-
3/F	Mean	0.5	3.1	0.6	3.0	0.4	0.7
	SD	0.45	0.91	0.42	0.93	0.23	0.27
	N	10	10	10	10	5	5
	P(v1)	-	<0.0001*	-	-	-	-
	Statistics	X1	APT	AP	X5	AP	AP

\* P<=0.05  
X1 = No analysis required  
AP = ANOVA and protected t-tests  
T = Rank-transformed data  
X5 = Not analyzed (values above/below the limit of quantitation)

Table 9: CRP levels in females (study # 1); sponsor provided

One day after test article administration (i.e., day 2 and/or 30 of the dosing phase), increases in C-reactive protein concentration (up to approximately 8.00x relative to mean control values) were reported in group 3. This effect recovered within 1 week after administration. These findings are indicative of an acute inflammatory response.

#### Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, dermal scores, food consumption, body temperature, ophthalmoscopic parameters, gross pathology were reported.

Transient changes (increases and decreases) in male's mean body weight gain occurred from days 3 to 4, 4 to 8, and days 17 to 18. Mean body weight gain was increased (approximately 15%) for group 2 females over the in-life study period (days 1 to 32 of the dosing phase) relative to the control group.

At the end of the recovery phase, there were no differences in body weight gain for groups 2 and 3 males or group 2 females. Group 3 females gained less body weight (19%) across the recovery phase, relative to control group.

### Organ Weight:

SEX		Males <sup>(b) (4)</sup> (32/57)		
GROUPS		1 (CONTROL)	2	3
NUMBER OF ANIMALS		5/5	5/5	5/5
BODY WEIGHT (terminal)		3030/3384	3024/3435	3074/3449
BRAIN		9.38/10.0	9.47/10.3	9.63/9.84
ADRENALS		0.291/0.324	0.240/0.288	0.320/0.272
EPIDIDYMIDES		1.65/2.13	1.33/2.99	1.48/2.26
HEART		7.46/7.25	7.08/7.43	7.45/7.78
KIDNEYS		18.2/20.3	17.8/19.6	19.6/20.4
LIVER		112.1/114.2	109.7/120.2	110.8/116.2
LUNGS		11.54/10.5	12.84/10.5	11.07/10.5
ILIAIC LYMPH NODES		0.039/0.032	0.039/0.022	0.079/0.044
INGUINAL LYMPH NODES		0.063/0.038	0.053/0.038	0.044/0.046
MANDIBULAR LYMPH NODES	LEFT	0.124/0.072	0.139/0.100	0.128/0.114
MESENTERIC LYMPH NODES		0.539/0.518	0.650/0.696	0.634/0.428
POPLITEAL LYMPH NODES		0.210/0.173	0.222/0.225	0.308/0.197
PROSTATE		1.36/2.59	1.71/2.76	1.91/2.70
SPLEEN		1.33/1.22	1.30/1.44	1.58/1.10
TESTES		4.34/4.57	3.33/5.28	4.68/5.72
PITUITARY		0.023/0.039	0.040/0.040	0.029/0.036
THYROID and PARATHYROID		0.305/0.270	0.266/0.349	0.290/0.295
THYMUS		5.22/4.25	5.96/5.55	5.95/4.61
OVARIES				
UTERUS				

Table 10: Male's organ weights (study # 1): Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight). Male's weight

Iliac lymph node weight was increased 102% in group 3 at study day 32. Iliac lymph node weight was decreased 31% in group 2 at study day 57. Iliac lymph node weight was increased 38% in group 3 at study day 57. Inguinal lymph node weight was decreased 16% in group 2 at

study day 32. Inguinal lymph node weight was decreased 30% in group 2 at study day 57. Inguinal lymph node weight was increased 21% in group 3 at study day 57. Mandibular lymph node weight was increased 39% in group 2 at study day 57. Mandibular lymph node weight was increased 58% in group 3 at study day 57. At study day 32, mesenteric lymph node weight was increased 21% and 18% in groups 2 and 3, respectively. Mesenteric lymph node weight was increased 34% in group 2 at study day 57. Mesenteric lymph node weight was decreased 17% in group 3 at study day 57. Popliteal lymph node weight was increased 47% in group 3 at study day 32. Popliteal lymph node weight was increased 30% in group 2 at study day 57. Popliteal lymph node weight was increased 14% in group 3 at study day 57. At study day 32, prostate weight was increased 26% and 40% in groups 2 and 3, respectively. Spleen weight was increased 19% in group 3 at study day 32. Spleen weight was increased 18% in group 2 at study day 57. Testis's weight was decreased 23% in group 2 at study day 32. At study day 57, testes weight was increased 16% and 25% in groups 2 and 3, respectively. At study day 32, pituitary weight was increased 74% and 26% in groups 2 and 3, respectively. Thyroid weight was increased 29% in group 2 at study day 57. Thymus weight was increased 14% in groups 2 and 3 at study day 32. Thymus weight was increased 31% in group 2 at study day 57.

SEX		Females <sup>(b) (4)</sup> (32/57)		
GROUPS		1 (CONTROL)	2	3
NUMBER OF ANIMALS		5/5	5/5	5/5
BODY WEIGHT (terminal)		2846/3381	2971/3427	2890/3202
BRAIN		9.35/9.47	9.74/9.68	8.95/9.48
ADRENALS		0.234/0.308	0.265/0.341	0.281/0.380
EPIDIDYMIDES				
HEART		6.44/7.10	6.95/7.18	6.41/6.31
KIDNEYS		16.9/17.5	16.1/18.7	16.5/16.1
LIVER		90.0/107.4	86.3/115.5	104.9/88.7
LUNGS		10.0/10.6	9.73/11.0	10.7/9.93
ILIAC LYMPH NODE		0.028/0.039	0.042/0.047	0.063/0.078
INGUINAL LYMPH NODE		0.071/0.055	0.094/0.065	0.060/0.043
MANDIBULAR LYMPH NODE	Left	0.153/0.156	0.181/0.120	0.123/0.126
MESENTERIC LYMPH NODE		0.714/0.574	0.815/0.806	0.636/0.708
POPLITEAL LYMPH NODE		0.220/0.403	0.324/0.309	0.295/0.313
PROSTATE AND SEMINAL VESICLE				
SPLEEN		1.559/1.906	1.439/2.012	1.727/1.348
TESTES				
PITUITARY		0.031/0.032	0.029/0.043	0.033/0.033
THYROID and PARATHYROID		0.209/0.252	0.276/0.258	0.270/0.344
THYMUS		4.196/5.440	4.143/5.318	4.736/4.553
OVARIES		0.276/0.341	0.278/0.288	0.245/0.319

SEX	Females <sup>(b) (4)</sup> (32/57)		
GROUPS	1 (CONTROL)	2	3
NUMBER OF ANIMALS	5/5	5/5	5/5
UTERUS	3.93/6.38	4.78/7.16	4.43/6.89

Table 11: Female's organ weights (study # 1): Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight).

#### Female's weight

At study day 32, adrenal weight was increased 13% and 20% in groups 2 and 3, respectively. At study day 57, adrenal weight was increased 11% and 23% in groups 2 and 3, respectively. Liver weight was increased 17% in group 3 at study day 32. Liver weight was decreased 17% in group 3 at study day 57.

At study day 32, iliac lymph node weight was increased 50% and 125% in groups 2 and 3, respectively. At study day 57, iliac lymph node weight was increased 21% and 100% in groups 2 and 3, respectively. Inguinal lymph node weight was increased 32% in group 2 at study day 32. Inguinal lymph node weight was decreased 15% in group 3 at study day 32. Inguinal lymph node weight was increased 18% in group 2 at study day 57. Inguinal lymph node weight was decreased 22% in group 3 at study day 57. Mandibular lymph node weight was increased 18% in group 2 at study day 32. Mandibular lymph node weight was decreased 20% in group 3 at study day 32. At study day 57, mandibular lymph node weight was decreased 23% and 19% in groups 2 and 3, respectively. At study day 32, mesenteric lymph node weight was increased 14% and decreased 11% in groups 2 and 3, respectively. At study day 57, mesenteric lymph node weight was increased 40% and 23% in groups 2 and 3, respectively. At study day 32, popliteal lymph node weight was increased 47% and 34% in groups 2 and 3, respectively. At study day 57, popliteal lymph node weight was decreased 23% and 22% in groups 2 and 3, respectively.

Spleen weight was decreased 19% in group 3 at study day 57. Pituitary weight was increased 34% in group 2 at study day 57. At study day 32, thyroid weight was increased 32% and 29% in groups 2 and 3, respectively. Thyroid weight was increased 37% in group 3 at study day 57. Thymus weight was increased 13% in group 3 at study day 32. Thymus weight was decreased 16% in group 3 at study day 57. Ovary weight was decreased 16% in group 2 at study day 57. At study day 32, uterus weight was increased 22% and 13% in groups 2 and 3, respectively. Uterus weight was increased 12% in group 2 at study day 57.

Sex		RSV <sup>(b) (4)</sup> Vaccine Treatment Group	
Dose Level (mg/kg/dose)		Males/Females	
		0	0.12 + Saline      0.12 + AS01B
Lymph node, iliac, internal			
Absolute Weight (g)		0.039/0.028	101/151      204/229
Body Weight Ratio (%)		0.0013/0.001	98/141      195/227
Brain Weight Ratio (%)		0.4131/0.293	100/144      203/241*
Lymph node, popliteal			
Absolute Weight (g)		0.21/0.22	106/147      147/134
Body Weight Ratio (%)		0.0069/0.0075	106/144      143/134
Brain Weight Ratio (%)		2.239/2.362	105/141      147/140

\* = Statistically significant difference (absolute or relative) compared with respective control mean value.  
 $P \leq 0.05$ .

Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage of control mean value

Table 12: RSV (b) (4) candidate vaccine-related changes in organ weight. Parameters at terminal euthanasia (study # 1); sponsor provided

Sex	RSV (b) (4) vaccine Treatment Group		
	Males/Females		
Dose Level (mg/kg/dose)	0	0.12 + Saline	0.12 + AS01B
Lymph node, iliac, internal			
Absolute Weight (g)	0.032/.039	69/121	136/200
Body Weight Ratio (%)	0.0009/.0012	68/118	133/214
Brain Weight Ratio (%)	0.3241/0.411	66/117	136/199
Lymph node, popliteal			
Absolute Weight (g)	0.173/0.403	130/76	114/78
Body Weight Ratio (%)	0.0051/0.0119	126/76	113/82
Brain Weight Ratio (%)	1.7335/4.264	123/75	116/78

Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage of control mean value

Table 13: RSV (b) (4) vaccine-related changes in organ weight. Parameters at recovery euthanasia (study # 1); sponsor provided

### Gross pathology:

Macroscopic findings are listed below:

#### Terminal sacrifice// recovery sacrifice (Males)

Groups	Findings
1M	Red discoloration at injection sites A and B (1/5)// no findings
2M	Red discoloration at injection sites A and B (1/5); large thymus (1/5)// no findings
3M	Red discoloration at injection sites A and B (1/5)// no findings

Table 14: Male's gross pathology findings (study # 1).

#### Terminal sacrifice// recovery sacrifice (Females)

Groups	Findings
1F	No findings// no findings
2F	Bilateral large popliteal lymph node (2/5)// no findings
3F	Red discoloration at injection site C (1/5); bilateral large popliteal lymph node (1/5)// no findings

Table 15: Female's gross pathology findings (study # 1).

Large thymus in 1 group 2 males was reported. This was correlated with increased individual thymic weight. In 1 group 3 females, injection site C was discolored (red and tan), which correlated with the microscopic finding of a moderate, mixed cell infiltrate in the subcutis overlying the intramuscular injection site. In 2 group 2 females and 1 group 3 female, the popliteal lymph nodes were large, which correlated with the microscopic finding of minimal increased lymphocytes and with increased lymph node weights.

In 1 group 1, 2, and 3 males, injection sites A and B were described as discolored red at the terminal euthanasia. These findings might be related to the ink used to mark the injection sites at the time of necropsy rather than being a true morphologic change.

**Microscopic findings are listed below:**

Terminal sacrifice (Males)

Groups	Findings
1M	Minimal myofiber degeneration/regeneration in diaphragm (1/5); minimal mixed cell infiltrate in diaphragm (2/5); minimal mineralization in kidney (3/5); minimal tubule cell vacuolation in kidney (1/5); minimal mononuclear cell infiltrate in larynx (1/5); minimal bronchiole-associated lymphoid tissue increases in lungs (1/5); minimal bronchus/bronchiole epithelium increases in lungs (1/5); minimal mixed cell infiltrate in biceps femoris muscle (1/5); minimal (1/5) and slight (1/5) lymphocyte infiltrate in stomach; peripubertal testis (5/5); ectopic parathyroid thymus (1/5); congenital cyst in thyroid (2/5); minimal myofiber degeneration/regeneration in tongue (1/5); minimal mixed cell infiltrate in tongue (2/5); minimal mineralization in urinary bladder (1/5)
2M	Cyst in cortex of adrenal (1/5); minimal choroid plexus mononuclear cell infiltrate in brain (1/5); minimal myofiber degeneration/regeneration in diaphragm (1/5); minimal mixed cell infiltrate in diaphragm (1/5); minimal muscle mononuclear cell infiltrate in esophagus (1/5); minimal pigmented macrophages infiltrate in GALT/Peyer's patch (1/5); minimal atrophy in Harderian glands (1/5); minimal mononuclear cell infiltrate in Harderian glands (1/5); slight myocardium degeneration in heart (1/5); minimal fibroplasia in heart (1/5); minimal valve hemorrhage in heart (1/5); slight mixed cell infiltrate in heart (1/5); minimal mononuclear cell infiltrate in heart (1/5); minimal (1/5) and slight (2/5) myofiber degeneration/necrosis in injection site C; minimal (2/5) and slight (1/5) mixed cell infiltrate in injection site C; minimal basophilic tubule in kidney (1/5); minimal glomerulosclerosis in kidney (1/5); minimal lymphocyte infiltrate in kidney (1/5); minimal mineralization in kidney (2/5); minimal mixed cell infiltrate in larynx (2/5); minimal mononuclear cell infiltrate in larynx (1/5); hepatocytes decreased glycogen in liver (1/5); minimal (1/5) and slight (1/5) alveolus epithelium hyperplasia in lung; minimal alveolar macrophages infiltrate in lungs (2/5); minimal (1/5) and slight (1/5) interstitial mixed cell infiltrate in lungs; minimal osseous metaplasia in lungs (2/5); minimal lymphocytes hyperplasia in iliac lymph node (1/5); minimal increased heterophils infiltrate in mandibular lymph node (1/5); minimal sinus erythrophagocytosis in mandibular lymph node (1/5); minimal sinus erythrophagocytosis in mesenteric lymph node (2/5); minimal mixed cell infiltrate in sciatic nerve (2/5); minimal dermal and/or follicular mixed cell inflammation in rectum (1/5); minimal mixed cell infiltrate in skin/subcutis (1/5); slight degeneration/necrosis in stomach (1/5); immature testis (4/5); minimal multinucleated spermatids in testis (1/5); peripubertal testis (1/5); congenital cyst in thyroid (3/5); minimal myofiber



Groups	Findings
	degeneration/regeneration in tongue (1/5); minimal mixed cell infiltrate in tongue (2/5); minimal lymphoplasmacytic infiltrate in trachea (3/5)
3M	Minimal nodular hyperplasia in cortex adrenal (1/5); minimal myofiber degeneration/regeneration in diaphragm (3/5); minimal (2/5) and slight (1/5) mixed cell infiltrate in diaphragm; slight myofiber degeneration/regeneration in esophagus (1/5); minimal atrophy in Harderian glands (2/5); minimal mononuclear cell infiltrate in Harderian glands (2/5); minimal mononuclear cell infiltrate in heart (1/5); minimal fibroplasia in injection site C (3/5); slight hemorrhage in injection site C (1/5); minimal (1/5) and slight (3/5) mixed cell infiltrate in injection site C; minimal basophilic tubule in kidney (1/5); minimal proteinaceous cast in kidney (1/5); minimal lymphocyte infiltrate in kidney (2/5); minimal mineralization in kidney (3/5); hepatocytes decreased glycogen in liver (1/5); minimal hemorrhage in lung (1/5); minimal alveolar macrophages infiltrate in lungs (2/5); minimal interstitial mixed cell infiltrate in lungs (3/5); minimal sinus erythrocytes in iliac lymph node (2/5); minimal heterophils infiltrate in iliac lymph node (1/5); minimal lymphocytes depletion/necrosis in mesenteric lymph node (1/5); minimal sinus erythrophagocytosis in mesenteric lymph node (1/5); minimal (1/5) and slight (1/5) mixed cell infiltrate in sciatic nerve; minimal mononuclear cell infiltrate in parotid salivary gland (1/5); mineralization in prostate (1/5); minimal mixed cell infiltrate in skin/subcutis (1/5); slight degeneration/necrosis in stomach (1/5); immature testis (2/5); minimal multinucleated spermatids in testis (1/5); peripubertal testis (4/5); congenital cyst in thyroid (3/5); minimal follicle cyst in thyroid (1/5); minimal myofiber degeneration/regeneration in tongue (1/5); minimal mixed cell infiltrate in tongue (2/5); minimal lymphoplasmacytic infiltrate in trachea (2/5); minimal mineralization in urinary bladder (1/5)

Table 16: Male's microscopic findings (study # 1)

## Terminal sacrifice (Females)

Groups	Findings
1F	Minimal myofiber degeneration/regeneration in diaphragm (1/5); minimal mixed cell infiltrate in diaphragm (2/5); minimal myofiber degeneration/regeneration in esophagus (1/5); minimal muscle mononuclear cell infiltrate in esophagus (1/5); minimal choroid mixed cell infiltrate in eyes (2/5); minimal pigmented macrophages infiltrate in GALT/Peyer's patch (1/5); minimal mineralization in GALT/Peyer's patch (1/5); minimal atrophy in Harderian glands (1/5); minimal mononuclear cell infiltrate in Harderian glands (1/5); minimal mononuclear cell infiltrate in heart (1/5); minimal basophilic tubule in kidney (1/5); minimal tubule dilatation in kidney (1/5); minimal lymphocyte infiltrate in kidney (2/5); minimal mineralization in kidney (2/5); hepatocytes decreased glycogen in liver (1/5); minimal bronchiole-associated lymphoid tissue increases in lungs (1/5); minimal sinus erythrocytes in iliac lymph node (2/5); minimal heterophils infiltrate in iliac lymph node (1/5); minimal increased germinal centers in mesenteric lymph node (1/5); minimal mixed cell infiltrate in sciatic nerve (1/5); minimal mineralization in ovary (1/5); cyst in

Groups	Findings
	pituitary (1/5); minimal mixed cell infiltrate in skin/subcutis (1/5); congenital cyst in thyroid (1/5); minimal mineralization in urinary bladder (2/5)
2F	Minimal lymphocyte infiltrate in cortex adrenal (1/5); minimal adipose tissue mononuclear cell infiltrate in cortex adrenal (1/5); minimal myofiber degeneration/regeneration in diaphragm (2/5); minimal mixed cell infiltrate in diaphragm (3/5); minimal myofiber degeneration/regeneration in esophagus (1/5); minimal muscle mononuclear cell infiltrate in esophagus (1/5); minimal pigmented macrophages infiltrate in GALT/Peyer's patch (3/5); minimal mononuclear cell infiltrate in heart (2/5); minimal myofiber degeneration/necrosis in injection site C (1/5); minimal mixed cell infiltrate in injection site C (2/5); minimal basophilic tubule in kidney (3/5); minimal tubule dilatation in kidney (1/5); minimal glomerulosclerosis in kidney (1/5); minimal (3/5) and slight (1/5) mineralization in kidney; minimal mononuclear cell infiltrate in larynx (2/5); hepatocytes decreased glycogen in liver (1/5); slight sinus erythrocytes in iliac lymph node (1/5); minimal (1/5) and moderate (1/5) heterophils infiltrate in iliac lymph node; minimal increased heterophils infiltrate in inguinal lymph node (1/5); minimal sinus erythrophagocytosis in mandibular lymph node (1/5); minimal increased germinal centers in mesenteric lymph node (2/5); minimal (2/5) and slight (1/5) increased lymphocytes in popliteal lymph node; minimal myofiber degeneration/regeneration in biceps femoris muscle (2/5); minimal myofiber degeneration/regeneration in biceps femoris muscle (1/5); minimal lymphocyte/macrophages infiltrate in biceps femoris muscle (2/5); minimal mixed cell infiltrate in sciatic nerve (1/5); corpora lutea in ovary (1/5); congenital cyst in parathyroid (1/5); minimal cortex increased lymphocytes in thymus (1/5); congenital cyst in thyroid (4/5); minimal mononuclear cell infiltrate in thyroid (1/5); minimal myofiber degeneration/regeneration in tongue (1/5); minimal mixed cell infiltrate in tongue (2/5); minimal mixed cell infiltrate in ureter (1/5); minimal mineralization in urinary bladder (1/5); minimal increased subendometrial mixed cell infiltrate in uterus (1/5); minimal intraepithelial-subepithelial mixed cell infiltrate in vagina (2/5); minimal muscle mixed cell infiltrate in vagina (1/5)
3F	Minimal meninges mononuclear cell infiltrate in brain (2/5); minimal myofiber degeneration/regeneration in diaphragm (2/5); minimal mixed cell infiltrate in diaphragm (2/5); minimal myofiber degeneration/regeneration in esophagus (1/5); minimal limbus increased mononuclear cell infiltrate in eyes (1/5); minimal mixed cell infiltrate in heart (1/5); minimal mononuclear cell infiltrate in heart (2/5); moderate mixed cell infiltrate in injection site C (1/5); minimal basophilic tubule in kidney (2/5); minimal lymphocyte infiltrate in kidney (1/5); minimal mineralization in kidney (2/5); minimal tubule cell vacuolation in kidney (2/5); minimal mixed cell infiltrate in larynx (2/5); hepatocytes decreased glycogen in liver (1/5); minimal hepatocytes increased glycogen in liver (3/5); minimal (1/5) and slight (1/5) periportal mononuclear cell infiltrate in liver; minimal alveolar macrophages infiltrate in lungs (1/5); minimal interstitial mixed cell infiltrate in lungs (2/5); minimal (4/5) and slight (1/5)

Groups	Findings
	<p>heterophils infiltrate in iliac lymph node; minimal increased heterophils infiltrate in inguinal lymph node (1/5); minimal sinus erythrophagocytosis in mandibular lymph node (2/5); slight increased heterophils infiltrate in mandibular lymph node (1/5); minimal increased germinal centers in mesenteric lymph node (1/5); minimal (2/5) and slight (1/5) increased lymphocytes in popliteal lymph node; minimal mixed cell infiltrate in mammary gland (1/5); minimal myofiber degeneration/regeneration in biceps femoris muscle (1/5); minimal (1/5) and slight (1/5) mixed cell infiltrate in sciatic nerve; minimal mineralization in ovary (1/5); minimal pigment-laden macrophages in ovary (1/5); slight dermal and/or follicular mixed cell inflammation in rectum (1/5); minimal mixed cell infiltrate in skin/subcutis (1/5); congenital cyst in thyroid (1/5); minimal follicle cyst in thyroid (1/5); minimal lymphoplasmacytic infiltrate in trachea (1/5); minimal mononuclear cell infiltrate in urinary bladder (1/5); minimal mineralization in urinary bladder (2/5)</p>

Table 17: Female's microscopic findings (study # 1)

		Treatment Group	
Sex		Males/Females	
		RSV (b) (4) candidate vaccine +Saline	RSV (b) (4) candidate vaccine +AS01B
Dose Level (0.12 mg/dose)	0		
Intramuscular injection site, C			
Number Examined	5/5	5/5	5/5
Degeneration/necrosis, myofiber			
Minimal	0/0	1/1	0/0
Slight	0/0	2/0	5/0
Fibroplasia			
Minimal	0/0	0/0	3/0
Hemorrhage			
Slight	0/0	0/0	1/0
Infiltrate, mixed cell			
Minimal	0/0	2/2	1/0
Slight	0/0	1/0	3/0
Moderate	0/0	0/0	0/1
Sciatic nerve (subjacent to injection site C)			
Number Examined	5/5	5/5	5/5
Infiltrate, mixed cell			
Minimal	0/1	2/1	1/1
Slight	0/0	0/0	1/1
Lymph node, popliteal			
Number Examined	4/5	5/5	5/5
Lymphocytes, increased			
Minimal	0/0	0/2	0/2
Slight	0/0	0/1	0/1
Lymph node, iliac, internal			
Number Examined	5/5	5/5	5/5
Infiltrate, heterophils			
Minimal	0/1	0/1	1/4
Slight	0/0	0/0	0/1
Moderate	0/0	0/1	0/0

Table 18: Incidence and severity of RSV (b) (4) candidate vaccine ± AS01B-related microscopic findings at terminal euthanasia (study # 1); sponsor provided

Table of incidence and severity of RSV (b) (4) candidate vaccine ± AS01B-related microscopic findings at recovery euthanasia

	Sex		Treatment Group	
			Males/Females	
			RSV (b) (4) candidate vaccine +Saline	RSV (b) (4) candidate vaccine +AS01B
Dose Level (0.12 mg/dose)		0		
Intramuscular injection site, C				
Number Examined		5/5	5/5	5/5
Degeneration/necrosis, myofiber				
Minimal		0/0	1/0	1/0
Hemorrhage				
Minimal		0/0	1/0	0/0
Infiltrate, mixed cell				
Minimal		0/0	1/0	1/1

Table 19: Incidence and severity of RSV (b) (4) candidate vaccine ± AS01B-related microscopic findings at recovery euthanasia (study # 1); sponsor provided

An extensive number of tissues were examined for histology. Except for injection sites and lymph node (popliteal and iliac) findings, no increased incidences of histological findings indicative of potential adverse events were reported in the treated groups relative to the controls.

#### Body temperature:

No test article-related effect on body temperature was reported.

#### Serology:

To determine anti-(b) (4) IgG titers, (b) (4) assay was used. Anti-(b) (4) antibody titers were below the limits of detection (BLOD) in control group (group1) at all time points and in all groups before immunization (at day -10).

In group 2 males, anti-(b) (4) antibody titers levels were 2617 and 3815 EU/mL at days 32 and 57, respectively. In group 3 males, anti-(b) (4) antibody titers levels were 125120 and 195120 EU/mL at days 32 and 57, respectively. In group 2 females, anti-(b) (4) antibody titers levels were 10553 and 21537 EU/mL at days 32 and 57, respectively. In group 3 females, anti-(b) (4) antibody titers levels were 136181 and 188749 EU/mL at days 32 and 57, respectively.

In group 2 female animals, 8 of 10 (80%) animals at day 32 and 4 of 5 animals (80%) at day 57 had seroconverted (anti-(b) (4) antibody titers >LOD). In this group, 2 females at day 32 and 1 female at day 57 had anti-(b) (4) IgG antibody titers below the LOD (42 EU/ml) at all time points tested. In group 3 females, 9 of 10 (90%) females at day 32 and 4 of 5 females (80%) at day 57 had anti-(b) (4) IgG antibody titers >42 EU/ml (LOD) and had seroconverted.

The adjuvanted vaccine (group 3) developed a stronger immune response than in the non-adjuvanted vaccine (group 2). Males' and females' immune responses were comparable in group 3 (adjuvanted vaccine). However, females' immune responses were higher than Males' in group 2 (non-adjuvanted vaccine).

Group	Date of sampling		
	-10	32	57 (Recovery)
	(b) (4)	titer (GMT, EU/mL)	
1	BLOD	BLOD	BLOD
2	BLOD	2617	3815
3	BLOD	125120	195120

BLOD = Below limits of detection.

Table 20: Anti-(b) (4) IgG titers in males (study # 1).

Group	Date of sampling		
	-10	32	57 (Recovery)
	(b) (4)	titer (GMT, EU/mL)	
1	BLOD	BLOD	BLOD
2	BLOD	10553	21537
3	BLOD	136181	188749

BLOD = Below limits of detection.

Table 21: Anti-(b) (4) IgG titers in females (study # 1).

**Test article related effects are listed in the table below:**

Test article related effects	Effects considered incidental
↓ LDH ↑ Neutrophils ↑ Eosinophils ↑ CRP ↑ Iliac lymph node weight ↑ Mesenteric lymph node weight ↑ Popliteal lymph node weight ↑ Thymus weight Microscopic findings at injection site C, iliac, and popliteal lymph nodes Immune responses	↑ Prostate weight ↑ Pituitary weight

Table 22: Test article related effects (study # 1).

**Assessment:**

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, dermal scores, food consumption, body temperature, ophthalmoscopic parameters, and gross pathology were reported.

The inter-conversion of [pyruvate](#) and [lactate](#) with concomitant inter-conversion of NADH and [NAD<sup>+</sup>](#) is catalyzed by lactate dehydrogenase (LDH). Pyruvate is the final product of [glycolysis](#) and it is converted to lactate, when oxygen is absent or in short supply, by LDH. LDH performs the reverse reaction during the [Cori cycle](#) in the [liver](#). LDH may be used as a [tumor marker](#) because many cancers can raise LDH levels. Also, measuring LDH levels can be helpful in monitoring treatment for cancer. LDH levels could be raised by heart failure, hypothyroidism, anemia, and lung or liver disease (6). Because [tissue breakdown](#) releases LDH, LDH can be measured as a surrogate for tissue breakdown, e.g. [hemolysis](#). Elevated LDH could be an

indication of other disorders like meningitis, encephalitis, acute pancreatitis, and HIV. It can also be used as a marker of [myocardial infarction](#). Levels of LDH peak at 3–4 days and remain elevated for up to 10 days following a myocardial infarction.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood.

CRP is protein synthesized by the liver, found in the blood, and is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections. Also, a positive CRP test that indicates an inflammation in the body may be used to detect a variety of different conditions, including cancer, connective tissue disease, heart attack, infection, inflammatory bowel disease (IBD), lupus, pneumococcal pneumonia, rheumatoid arthritis, rheumatic fever, or tuberculosis.

The increases in the weights of iliac lymph nodes, mesenteric lymph nodes, and popliteal lymph nodes might be related to the immune response due to test article treatment. The microscopic findings in the iliac (heterophils infiltrate) and popliteal (increased lymphocytes) lymph nodes might also be related to the immune responses.

The thymus is a specialized primary [lymphoid](#) organ of the [immune system](#). Within the thymus, [T cells](#) or T [lymphocytes](#) mature. T cells are critical to the [adaptive immune system](#), where the body adapts specifically to foreign invaders. The thymus is composed of two identical [lobes](#) and is located anatomically in the anterior superior [mediastinum](#), in front of the [heart](#) and behind the [sternum](#). As the thymus is the organ of [T-cell](#) development, any [congenital](#) defect in thymic genesis or a defect in [thymocyte](#) development can lead to a profound [T cell](#) deficiency in primary [immunodeficiency](#) disease. Defects that affect both the [T cell](#) and [B cell lymphocyte](#) lineages result in [severe combined immunodeficiency](#) syndrome (SCIDs). Acquired [T cell](#) deficiencies can also affect [thymocyte](#) development in the thymus.<sup>4</sup>

Microscopic findings at injection site C were reported. This might be related to inflammation and is a relatively common occurrence as part of the acute phase response following administration of some vaccines. Immune responses due to test article treatment were reported.

Adverse gross or microscopic alteration that could be indicative of systemic or local toxicity was not reported.

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<sup>4</sup> <https://en.wikipedia.org/wiki/Thymus>.

Changes in prostate and pituitary weights were not associated with any macroscopic and/or microscopic findings. Thus, it was considered incidental.

Based on the overall findings in this study, it can be concluded that in (b) (4) rabbit's administration of RSV (b) (4) vaccine on study days 1, 15, and 29 had no adverse effects in terms of systemic toxicity at the dose level of 0.12 mg.

**GLP study deviations or amendments:** No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

**Investigators Brochure:** Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes (X) or no ().

**Conclusions:**

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect.

**Internal communication:**

Due to the high levels of CRP reported in this study, there will be a need for increased monitoring of subjects, small cohorts, and conservative stopping rules in any clinical trial. In addition, the sponsor should add a statement in the IB to clarify that the elevated levels of CRP reported in the test article-treated animals suggests an increased likelihood of significant adverse events such as malaise, fatigue, and nausea.

**Study number 2:**

**Title and study number:** A Repeated Dose Toxicity Study with RSVPreF3 Candidate Vaccines (RSVPreF3, RSVPreF3/AS01<sub>B</sub> or RSVPreF3 Co-administered with Boostrix) Given Intramuscularly to the Rabbit Followed by a 4-Week Treatment Free Period. Study number: 8384096.

**Performing laboratory:** (b) (4)

**Study initiation date:** June 18, 2018

**Final report date:** March 28, 2019

**Test article batch/lot:**

Test Article <sup>a</sup>	Storage	Batch Number	Expiration Date	Protein Content
RSVPreF3 vaccine	In a refrigerator, set to maintain 2°C to 8°C	TRSVA003A	31 January 2019	942 µg/mL

<sup>a</sup> Also known as (b) (4).

**Adjuvant**

Adjuvant	Storage	Batch Number	Expiration Date
AS01 <sub>B</sub>	In a refrigerator, set to maintain 2°C to 8°C	AA1BA009A	31 December 2019

**Boostrix Vaccine**

Vaccine	Storage	Batch Number	Expiration Date
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Boostrix <sup>a</sup>	In a refrigerator, set to maintain 2°C to 8°C	AC37B241A	January 2019
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a Tetanus, Diphtheria, and Acellular Pertussis (Tdap) - EU version.

### Saline Control Article

Saline Control Article	Manufacturer	Storage	Batch Number	Expiration Date
0.9% Sodium Chloride for Injection (sterile saline)	GSK HBRIX	Refrigerated	5DT75	06 December 2018

Table 23: Test article's storage condition, batch number, expiration date, and protein content (study # 2); sponsor provided

**Animal species and strain:** (b) (4) rabbits

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

**Number of animal per group and sex:** 10/sex/group

**Age:** 14-15 weeks old

**Body weight range:** 1832 to 2459 g for males and 1968 to 2478 g for females

**Route and site of administration:** Intramuscular (IM).

**Volume of injection:** 0.5 mL's

**Frequency of administration and study duration:** Animals were dosed on study days 1, 15, and 29. All animals were administered 2 injections, which were right/left adjacent in the same leg on each dose day. On each dose day, group 1 was administered 2 injections of saline, group 2 was administered 1 injection of RSVPreF3 and 1 injection of saline, group 3 was administered 1 injection of RSVPreF3/AS01B and 1 injection of saline, and group 4 was administered 1 injection of RSVPreF3 and 1 injection of Boostrix.

**Dose:** 240 µg RSVPreF3/dose.

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. For batch number AA1BA007A, a shelf life of (b) (4) months was reported. No information for the stability of batch number TRSVA003A was provided.

**Means of administration:** Intramuscular (IM)

**Report status:** Final

**Experimental design:**

Animals were randomized and assigned to 4 different groups. Each group consisted of 10 animals/sex. Animals were dosed by intramuscular (IM) route on study days 1, 15, and 29. The details of the study design are listed in the following table:

Group <sup>a</sup>	No. of Animals <sup>b</sup>		Dose Level (µg RSVPreF3/dose)	Injection Volume (mL/injection)
	Male	Female		
1 Vehicle Control (Saline) <sup>c</sup>	10	10	0	0.5
2 RSVPreF3 <sup>d</sup>	10	10	240	0.5
3 RSVPreF3/AS01B <sup>e</sup>	10	10	240	0.5
4 RSVPreF3 + Boostrix <sup>f</sup>	10	10	240	0.5

a. Animals were dosed on days 1, 15, and 29.

b. Five males and 5 females from each group were euthanized on day 32 (3 days after the last injection - terminal euthanasia). The remaining animals were euthanized on day 26 of the treatment-free period - recovery euthanasia (study day 57).

c. Group 1 was administered saline only (2 injections in the same leg on each dose day).

- d. The RSVPreF3 for group 2 was reconstituted in saline. Animals were administered 1 injection of RSVPreF3 and 1 injection of saline in the same leg on each dose day.
- e. The RSVPreF3 for group 3 was reconstituted in AS01<sub>B</sub>. Animals were administered 1 injection of RSVPreF3/AS01<sub>B</sub> and 1 injection of saline in the same leg on each dose day.
- f. The RSVPreF3 for group 4 was reconstituted in saline. The Boostrix vaccine for group 4 was used as supplied. Animals were administered 1 injection of RSVPreF3 and 1 injection of Boostrix in the same leg on each dose day.

Note: While the last day of dosing was day 29, the dosing phase continued through day 32. The recovery phase began on study day 32.

Table 24: Experimental design (study # 2).

## Methods:

**Randomization procedure:** Yes

**Statistical analysis plan:** Yes.

**The following parameters were evaluated:** Cage side observations (twice daily), clinical observations (twice daily), detailed observations (pre-dose and on days 1, 8, 15, 22, 29 31, 32, 39, 46, 53, and 56), body weights (days 1, 2, 3, 4, 8, 11, 15, 16, 17, 18, 22, 25, 29, 30, 31, 32, 35, 39, 42, 46, 53, and 56), food consumption (daily), ophthalmology (pre-dose phase, once within 3 days after the last dose, and once within 7 days of the end of the treatment free period), rectal temperature (pre-dose and approximately 6, 24, and 48 hours after each dose), dermal scoring (at approximately 6, 24, and 48 hours post each dose during the dosing phase), clinical chemistry, hematology, and coagulation (pre-dose and on days 2, 8, 30, 36, and 57), serology (pre-dose and on days 32 and 57). Postmortem evaluations were performed on days 32 and 57.

Parameters	Frequency of Testing
Cageside observation <sup>5</sup>	Twice daily
Clinical observations <sup>6</sup>	Twice daily
Detailed observations	Pre-dose and on days 1, 8, 15, 22, 29 31, 32, 39, 46, 53, and 56
Body weight	Days 1, 2, 3, 4, 8, 11, 15, 16, 17, 18, 22, 25, 29, 30, 31, 32, 35, 39, 42, 46, 53, and 56
Food consumption	Daily
Rectal temperature	Pre-dose and approximately 6, 24, and 48 hours after each dose
Ophthalmologic exam	Pre-dose phase, once within 3 days after the last dose, and once within 7 days of the end of the treatment free period
Clinical chemistry*	Pre-dose and on days 2, 8, 30, 36, and 57
Hematology*	Pre-dose and on days 2, 8, 30, 36, and 57
Coagulation*	Pre-dose and on days 2, 8, 30, 36, and 57
Dermal scoring	At approximately 6, 24, and 48 hours post each dose during the dosing phase
Serology*	Pre-dose and on days 32 and 57
Postmortem study evaluations	Days 32 and 57

<sup>5</sup> Cageside observations include mortality, morbidity, general health and signs of toxicity.

<sup>6</sup> Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

\* Blood samples were collected from the medial auricular artery.

Table 25: Parameters evaluated (study # 2).

**Postmortem procedures:**

Organ/Tissue		Organ/Tissue	
adrenal (2)	P,E	lymph node, and mandibular	P,E
aorta	P,E	mammary gland (females)	P,E
bone, femur with bone marrow (articular surface of the distal end)	P,E	muscle, biceps femoris (right thigh)	P,E
bone, sternum with bone marrow	P,E	optic nerve (2) <sup>a</sup>	P,E
brain	P,E	ovary (2)	P,E
cecum	P,E	oviducts (2)	P,E
cervix	P,E	pancreas	P,E
colon	P,E	pituitary gland	P,E
diaphragm	P,E	prostate	P,E
duodenum	P,E	rectum	P,E
epididymis (2)	P,E	salivary gland, mandibular (2)	P,E
esophagus	P,E	salivary gland, parotid (2)	P,E
eye(2) <sup>a</sup>	P,E	salivary gland, sublingual (2)]	P,E
gall bladder (drained)	P,E	sciatic nerve	P,E
gut-associated lymphoid tissue (GALT /Peyer's Patch)	P,E	seminal vesicle	P,E
Harderian gland <sup>a</sup>	P,E	skin/subcutis (hind limb, right)	P,E
heart	P,E	spinal cord (cervical, thoracic, and lumbar)	P,E
ileum	P,E	spleen	P,E
dose sites <sup>b</sup> including overlying skin and muscle ( <i>en bloc</i> , skin/subcutis/muscle)	P,E	stomach	P,E
jejunum	P,E	testis (2) <sup>a</sup>	P,E
kidney (2)	P,E	thymus	P,E
larynx	P,E	thyroid (2 lobes) with parathyroid	P,E
lesions	P,E	tongue	P,E
liver	P,E	trachea	P,E
lung with large bronchi	P,E	ureter (2)	P,E
lymph node, iliac (internal)	P,E	urinary bladder (see <a href="#">Protocol Deviations</a> )	P,E
lymph node inguinal	P,E	uterus	P,E
lymph node, popliteal	P,E	vagina	P,E
lymph node, mesenteric	P,E		

E = Examined microscopically; P = Processed.

a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

b All dose sites were collected but only the last dose site (i.e. left thigh) was analyzed. The biceps femoris from the right thigh was analyzed as part of the skeletal muscle examination .

Table 26: Postmortem procedures (study # 2); sponsor provided

**Results:**

No test article-related morbidity and/or mortality were reported.

**Clinical chemistry, hematology, and coagulation:**

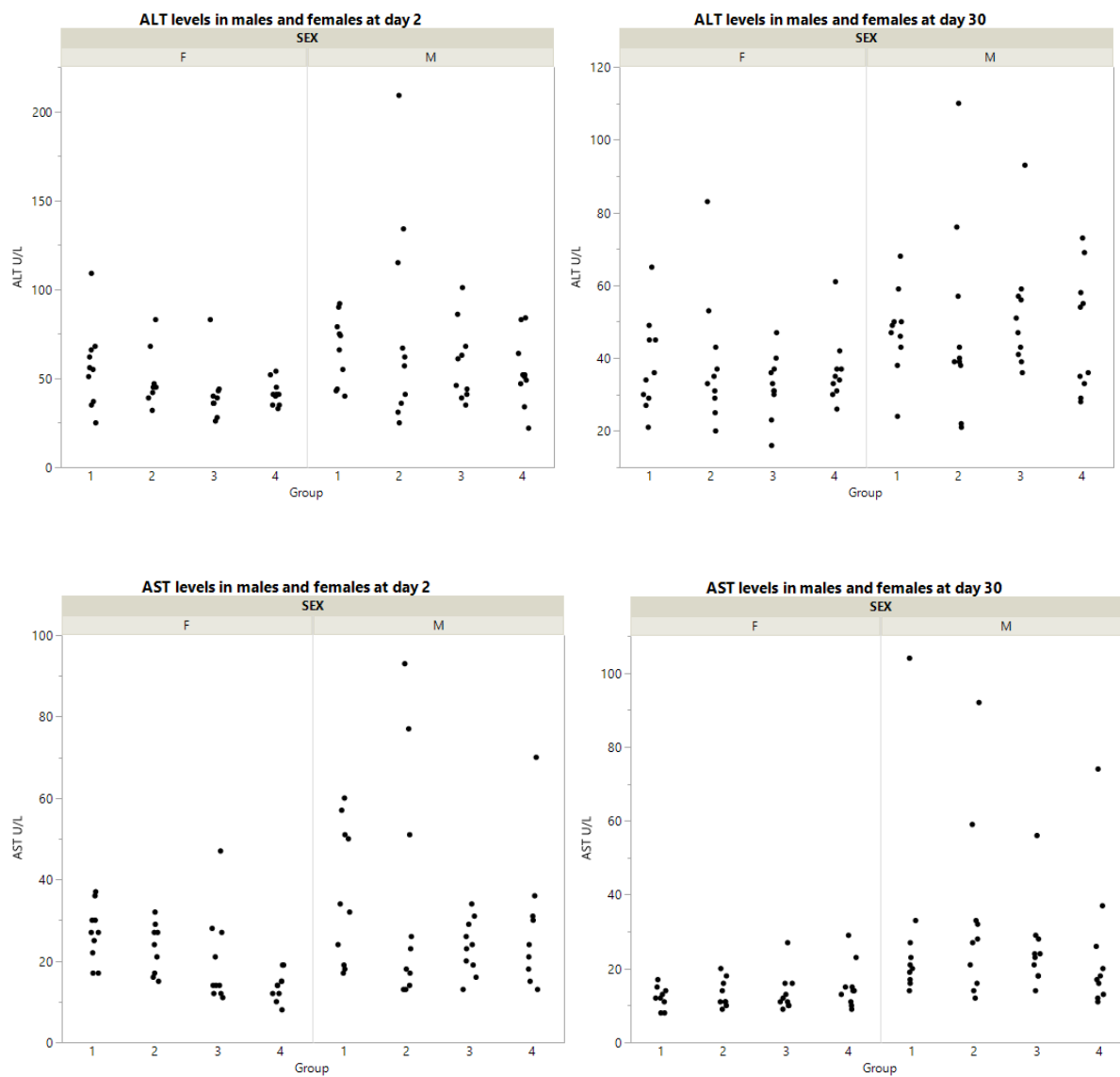
CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP ( <b>G</b> ), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ )	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR  B) HEPATOBILIARY	Aspartate aminotransferase (AST or SGOT) SD36 M $\uparrow$ = 2.2 G2 SD2 M $\downarrow$ = 0.5 G4  Alanine aminotransferase (ALT or SGPT) SD36 M $\uparrow$ = 1.6 G2	
		Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS	C-reactive protein*	Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Globulin SD57 M $\downarrow$ = 0.6 G3  A/G ratio SD57 M $\uparrow$ = 1.9 G3 SD57 M $\uparrow$ = 1.6 G4  Lactate dehydrogenase (LDH) SD57 M $\uparrow$ = 1.9 G3 SD57 M $\uparrow$ = 1.9 G4 SD57 F $\downarrow$ = 0.6 G2 SD57 F $\downarrow$ = 0.5 G3 SD57 F $\downarrow$ = 0.4 G4  Total Cholesterol	Albumin (A) Total protein Carbon dioxide

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise $\leq 1.5$ ))	NOT OF NOTE
	<p>SD2 M <math>\downarrow \leq 0.6</math> G3</p> <p>Fasting triglycerides  SD2 M <math>\downarrow = 0.6</math> G3  SD2 M <math>\downarrow = 0.6</math> G4  SD57 M <math>\downarrow = 0.5</math> G3  SD57 M <math>\downarrow = 0.5</math> G4  SD36 F <math>\uparrow = 1.6</math> G3  SD57 F <math>\downarrow = 0.6</math> G4</p> <p>PLIP  SD2 M <math>\downarrow = 0.6</math> G3  SD2 M <math>\downarrow = 0.6</math> G4</p> <p>GGT  SD57 M <math>\downarrow = 0.6</math> G4</p> <p>Creatine kinase (CK)  SD36 M <math>\downarrow = 0.6</math> G2  SD36 M <math>\downarrow = 0.5</math> G3  SD36 M <math>\downarrow = 0.6</math> G4  SD57 M <math>\downarrow = 0.6</math> G2  SD57 M <math>\downarrow = 0.6</math> G3  SD57 M <math>\downarrow = 0.6</math> G4  SD2 F <math>\uparrow = 1.6</math> G4  SD36 F <math>\uparrow = 2.6</math> G4  SD57 F <math>\downarrow = 0.6</math> G2  SD57 F <math>\downarrow = 0.6</math> G3  SD57 F <math>\downarrow = 0.5</math> G4</p>	

\*See section below (page 12)

Table 27: Clinical chemistry results (study # 2).

Graphs for ALT, AST, and CK levels in males (M) and females (F) at study days 2 and 30:



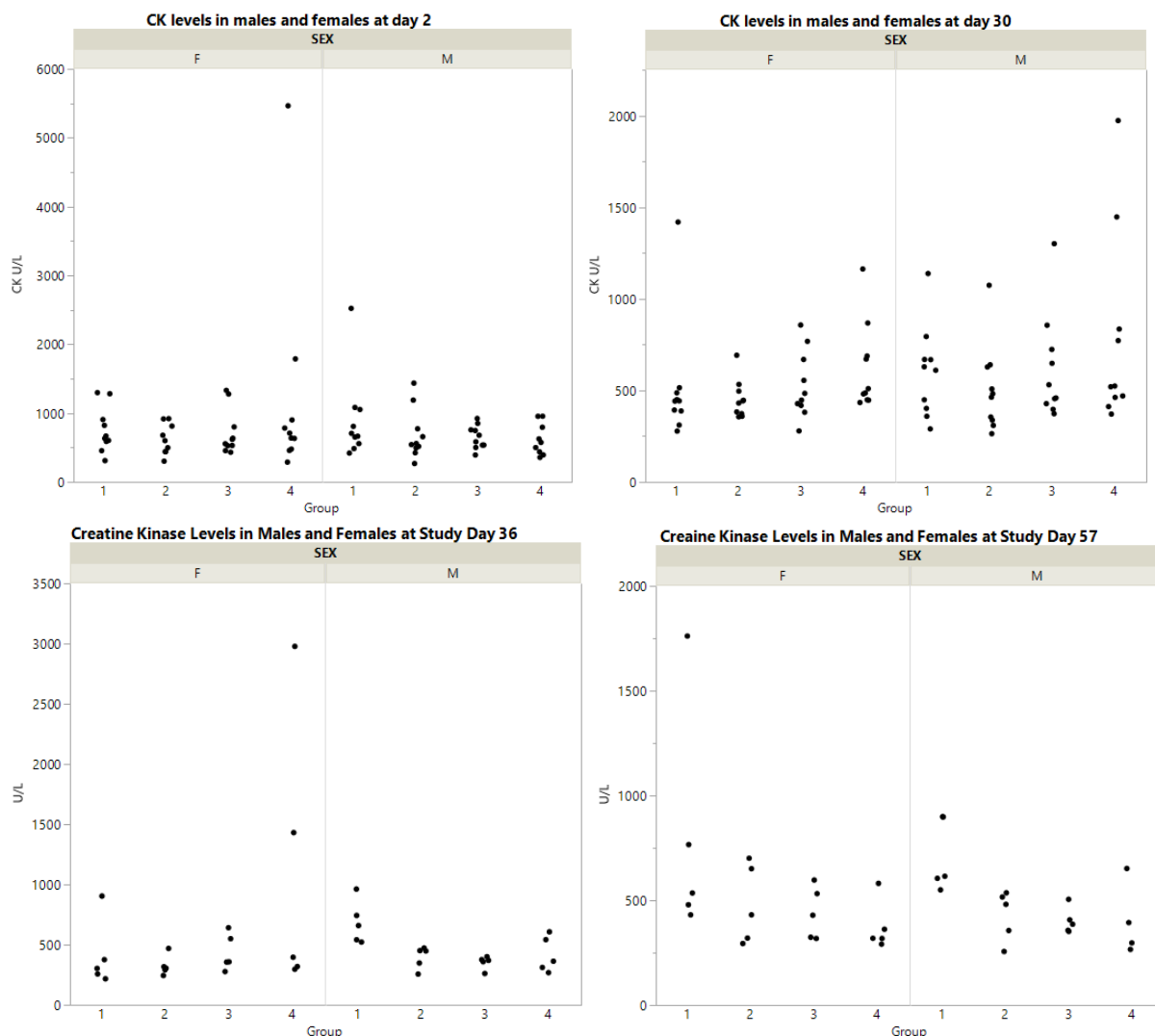


Figure 1: Graphs for ALT, AST, and CK levels in males (M) and females (F) at study days 2 and 30 (study # 2).

Clinical chemistry results show an increase in AST levels in group 2 males at study day 36. AST levels were decreased in group 4 males at study day 2. ALT levels were increased in group 2 males at study day 36. Globulin levels were decreased in group 3 males at study day 57. A/G ratio were increased in groups 3 and 4 males at study day 57. Lactate dehydrogenase levels were increased in groups 3 and 4 males at study day 57. Lactate dehydrogenase levels were decreased in groups 2, 3, and 4 females at study day 57. Triglyceride levels were decreased in groups 3 and 4 males at study day 2. Triglyceride levels were decreased in groups 3 and 4 males at study day 57. In females, triglyceride levels were increased in group 3 and decreased in group 4 at study days 36 and 57, respectively. Phospholipid (PLIP) levels were decreased in groups 3 and 4 males at study day 2. Gamma glutamyl transferase (GGT) levels were decreased in group 4 males at study day 57. Creatine kinase (CK) levels were decreased in groups 2, 3, and 4 males at study days 36 and 57. Creatine kinase levels were increased in group 4 females at study days 2 and 36. Creatine kinase levels were decreased in groups 2, 3, and 4 females at study day 57.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.57, ie, $\geq 1.6$ or $\leq 1.6$	Not of NOTE
Red blood cells	Reticulocytes SD57 M $\downarrow = 0.5$ G3 SD57 M $\downarrow = 0.5$ G4 SD30 F $\uparrow = 1.8$ G3 SD30 F $\uparrow = 1.6$ G4	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
White blood cells	<p>Monocyte count: SD2 M <math>\uparrow = 1.6</math> G4 SD8 M <math>\uparrow = 1.8</math> G3 SD8 M <math>\uparrow = 2.2</math> G4 SD36 M <math>\uparrow = 1.7</math> G2 SD36 M <math>\uparrow = 2.0</math> G3 SD2 F <math>\uparrow = 2.3</math> G3 SD2 F <math>\uparrow = 2.1</math> G4 SD8 F <math>\uparrow = 2.3</math> G3 SD30 F <math>\downarrow = 0.4</math> G3 SD30 F <math>\downarrow = 0.3</math> G4 SD36 F <math>\downarrow = 0.5</math> G3 SD36 F <math>\downarrow = 0.6</math> G4 SD57 F <math>\uparrow = 1.6</math> G2</p> <p>Neutrophil count SD30 M <math>\uparrow = 2.0</math> G3 SD57 M <math>\downarrow = 0.6</math> G3 SD57 M <math>\downarrow = 0.4</math> G4 SD2 F <math>\uparrow = 1.6</math> G3 SD30 F <math>\uparrow = 2.2</math> G3 SD30 F <math>\uparrow = 1.7</math> G4</p> <p>Eosinophils count SD30 M <math>\downarrow = 0.6</math> G4 SD30 F <math>\downarrow = 0.4</math> G3 SD30 F <math>\downarrow = 0.4</math> G4 SD36 F <math>\uparrow = 2.4</math> G3 SD36 F <math>\uparrow = 2.2</math> G4</p> <p>Basophils SD30 F <math>\downarrow = 0.6</math> G4 SD57 F <math>\downarrow = 0.6</math> G4</p> <p>Large Unstained Cells (LUC) SD30 M <math>\uparrow = 2.5</math> G3 SD-9 F <math>\downarrow = 0.5</math> G4 SD8 F <math>\uparrow = 1.7</math> G3 SD8 F <math>\uparrow = 1.7</math> G4 SD30 F <math>\downarrow = 0.3</math> G3</p>	<p>Macrophage White Blood Cells (WBC) Lymphocyte count Leukocytes Large Unstained Cells (LUC)</p>

<sup>7</sup> With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.



HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.57, ie, $\geq 1.6$ or $\leq 1.6$	Not of NOTE
	SD30 F ↓ = 0.3 G4 SD36 F ↓ = 0.5 G3 SD36 F ↓ = 0.5 G4	
Clotting potential	Fibrinogen SD30 M ↑ = 1.6 G3	Activated partial-thromboplastin time clotting time Platelet count Prothrombin time
Others		Bone marrow cytology

Table 28: Hematology results (study # 2).

Hematology results show a decrease in reticulocyte levels in groups 3 and 4 males at study day 57. Reticulocyte levels were increased in groups 3 and 4 females at study day 30. Monocyte levels were increased in group 4 males at study days 2 and 8. Monocyte levels were increased in group 3 males at study day 8. Monocyte levels were increased in groups 2 and 3 males at study day 36. Monocyte levels were increased in groups 3 and 4 females at study day 2. Monocyte levels were increased in group 3 females at study day 8. Monocyte levels were decreased in groups 3 and 4 females at study days 30 and 36. Monocyte levels were increased in group 2 females at study day 57. Neutrophil levels were increased in group 3 males and females at study days 30 and 2, respectively. Neutrophil levels were decreased in groups 3 and 4 males at study day 57. Neutrophil levels were increased in groups 3 and 4 females at study day 30.

Eosinophil levels were decreased in group 4 males at study day 30. Eosinophil levels were decreased in groups 3 and 4 females at study day 30. Eosinophil levels were increased in groups 3 and 4 females at study day 36. Basophil levels were decreased in group 4 females at study days 30 and 57.

Absolute large unstained cells (LUC) levels were increased in group 3 males at study day 30. LUC levels were decreased in group 4 females at study day -9. LUC levels were increased in groups 3 and 4 females at study day 8. LUC levels were decreased in groups 3 and 4 females at study days 30 and 36. Fibrinogen levels were increased in group 3 males at study day 30.

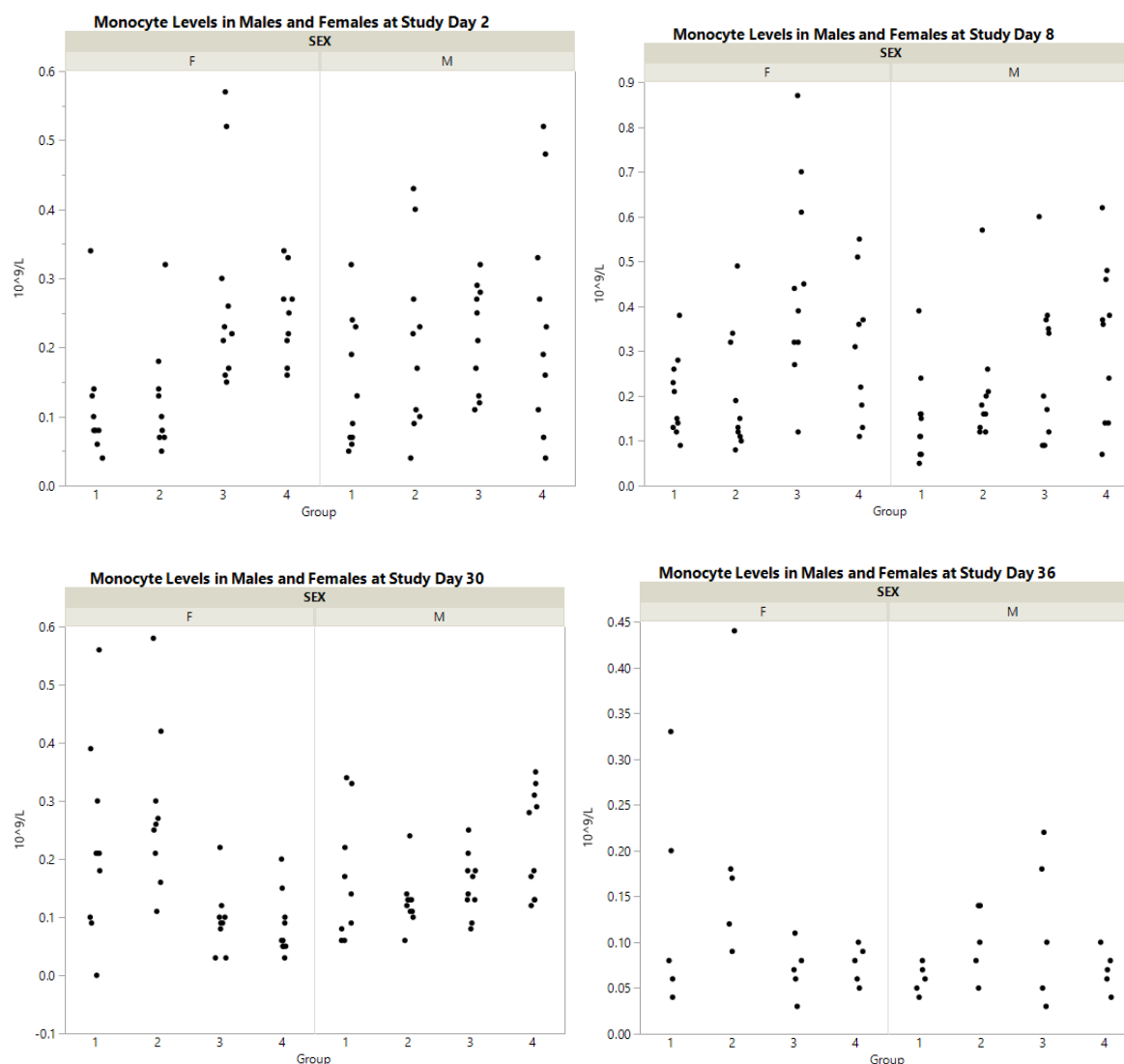


Figure 2: Graphs for monocyte levels in males (M) and females (F) at study days 2, 8, 30, and 36 (study # 2).

#### CRP:

C reactive protein (CRP) levels were increased significantly in group 3 males and females at study days 2 and 30. However, this increase was higher in females (3.5 and 3.6 mg/dL at study days 2 and 30, respectively) than males (1.7 and 2.7 mg/dL at study days 2 and 30, respectively) in this group. In group 4, the increase in CRP levels were lower than group 3. These results show the difference in the effect of AS01B (group 3) and Boostrix (group 4) on CRP levels.

Group/ Sex	Phase Day	CRP mg/dL					
		Predose	Dosing				Recovery
		9	2	8	30	5	26
1/M	Mean	<0.3	<0.3	<0.1	<0.3	0.2	0.2
	SD	0.22	0.30	0.09	0.16	0.00	0.04
	N	10	10	10	10	5	5
	P(overall)	-	-	-	-	-	<0.0001
2/M	Mean	<0.2	<0.2	<0.1	<0.2	0.2	0.4
	SD	0.13	0.41	0.00	0.08	0.04	0.08
	N	10	10	10	10	5	5
	P(v1)	-	-	-	-	-	0.0001*
3/M	Mean	<0.2	1.7	<0.2	2.7	0.2	0.3
	SD	0.28	0.66	0.16	0.67	0.04	0.05
	N	10	10	10	10	5	5
	P(v1)	-	-	-	-	-	0.0551
	P(v2)	-	-	-	-	-	0.0070*
4/M	Mean	<0.2	<0.3	<0.1	0.8	0.2	0.4
	SD	0.23	0.44	0.07	0.71	0.04	0.05
	N	10	10	10	10	5	4
	P(v1)	-	-	-	-	-	<0.0001*
	P(v2)	-	-	-	-	-	0.2875
	Statistics	X1	X5	X5	X5	X2	AP

\* P<=0.05  
X1 = No analysis required  
X5 = Not analyzed (values above/below the limit of quantitation)  
X2 = Not analyzed (too few distinct values)  
AP = ANOVA and protected t-tests

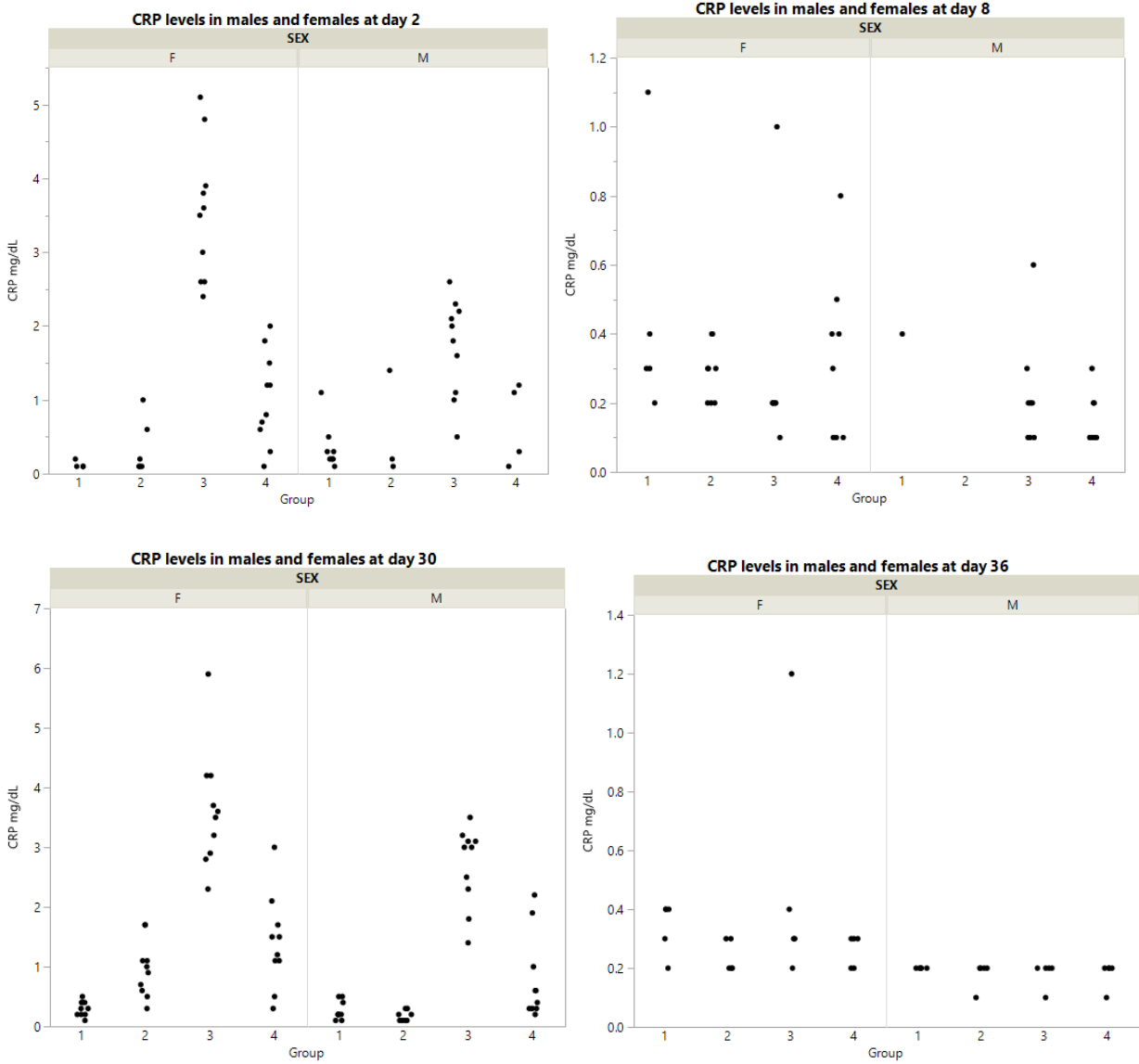
Table 29: CRP levels in males (study # 2); sponsor provided

Group/ Sex	Phase Day	CRP mg/dL					
		Predose	Dosing				Recovery
		9	2	8	30	5	26
1/F	Mean	<0.3	<0.1	<0.3	0.3	0.3	0.4
	SD	0.14	0.03	0.32	0.12	0.09	0.04
	N	10	10	9	10	5	5
	P(overall)	-	-	-	<0.0001	-	0.2170
2/F	Mean	<0.3	<0.3	<0.3	1.0	0.2	0.4
	SD	0.44	0.32	0.11	0.47	0.05	0.11
	N	10	9	10	10	5	5
	P(v1)	-	-	-	<0.0001*	-	-
3/F	Mean	<0.4	3.5	<0.2	3.6	0.5	0.3
	SD	0.20	0.92	0.27	1.00	0.41	0.23
	N	10	10	10	10	5	5
	P(v1)	-	-	-	<0.0001*	-	-
	P(v2)	-	-	-	<0.0001*	-	-
4/F	Mean	<0.4	1.0	<0.3	1.4	0.3	0.2
	SD	0.41	0.63	0.24	0.77	0.05	0.04
	N	10	10	10	10	5	5
	P(v1)	-	-	-	<0.0001*	-	-
	P(v2)	-	-	-	0.1106	-	-
	Statistics	X1	X5	X5	APT	X2	AP

\* P<=0.05  
X1 = No analysis required  
X5 = Not analyzed (values above/below the limit of quantitation)  
AP = ANOVA and protected t-tests  
T = Rank-transformed data  
X2 = Not analyzed (too few distinct values)

Table 30: CRP levels in females (study # 2); sponsor provided

Graphs for CRP levels in males (M) and females (F) at study days 2, 8, 30, 36, and 57:



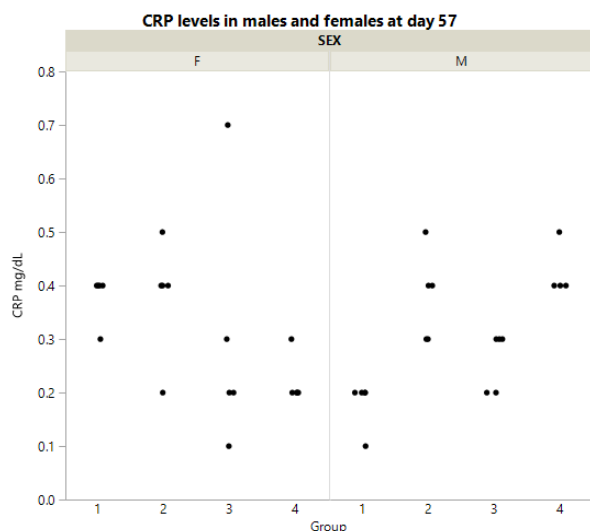


Figure 3: Graphs for CRP levels in males (M) and females (F) at study days 2, 8, 30, 36, and 57 (study # 2).

#### Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight, dermal scores, food consumption, body temperature, ophthalmoscopic parameters, gross pathology were reported.

Statistically significant increases or decreases in body weight and body weight gain at certain intervals were reported in groups 3 and 4 when compared with group 1. However, no group differences were present over the 32-day dosing phase or during the recovery period.

#### Organ Weight:

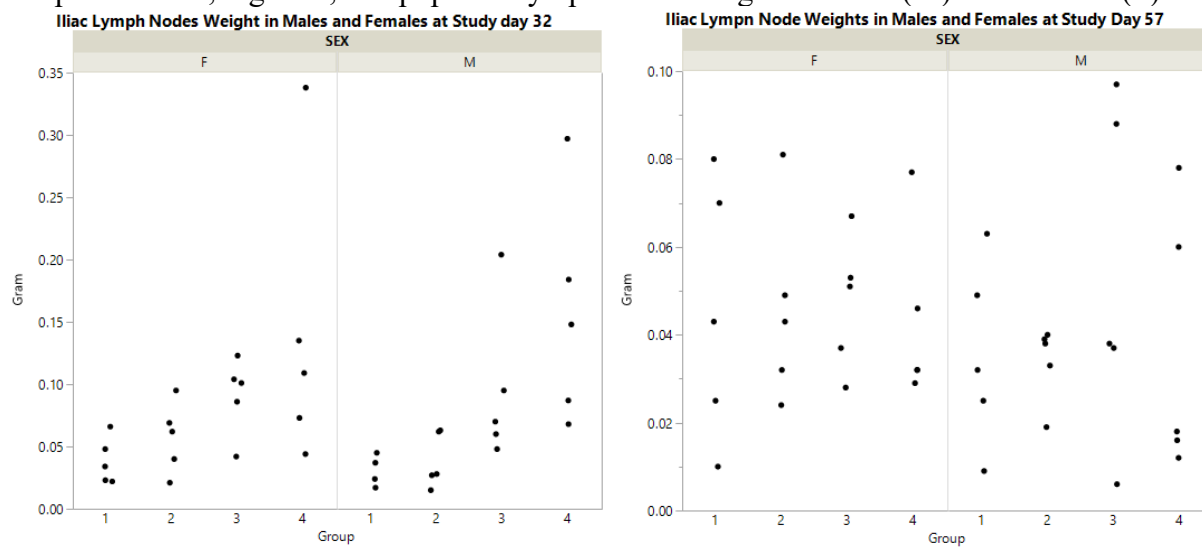
SEX	Males <sup>(b) (4)</sup> (32/57)			
GROUPS	1 (CONTROL)	2	3	4
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	2800/3071	2773/3105	2800/3034	2768/3144
BRAIN	9.92/9.25	9.32/9.79	9.92/9.59	9.79/9.97
ADRENALS	0.238/0.279	0.242/0.279	0.249/0.269	0.264/0.352
EPIDIDYMIDES	1.33/1.89	1.52/1.90	1.39/1.91	1.35/1.90
HEART	6.76/7.06	6.08/7.15	6.34/6.55	6.20/7.05
KIDNEYS	16.98/17.33	17.02/18.02	17.86/15.90	17.24/17.66
LIVER	88.26/99.89	92.66/102.5	90.14/78.75	94.40/87.12
LUNGS	10.59/11.27	10.22/12.26	11.77/10.22	11.74/10.56
ILIAC LYMPH NODES	0.031/0.036	0.039/0.034	0.095/0.053	0.157/0.037
INGUINAL LYMPH NODES	0.021/0.032	0.024/0.038	0.032/0.043	0.045/0.026
MANDIBULAR LYMPH NODES	0.101/0.150	0.085/0.115	0.090/0.140	0.107/0.097
MESENTERIC LYMPH NODES	0.617/0.769	0.693/0.853	0.898/0.776	0.606/0.829

SEX	Males <sup>(b) (4)</sup> (32/57)			
GROUPS	1 (CONTROL)	2	3	4
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5
POPLITEAL LYMPH NODES	0.134/0.139	0.185/0.188	0.241/0.203	0.220/0.190
PROSTATE	1.53/2.40	1.68/2.76	2.01/3.17	2.12/2.77
SPLEEN	1.18/1.11	1.40/1.20	1.28/1.23	1.35/1.04
TESTES	3.21/5.78	3.20/5.04	3.91/4.58	4.37/5.01
PITUITARY	0.027/0.025	0.019/0.023	0.023/0.027	0.025/0.021
THYROID and PARATHYROID	0.285/0.353	0.226/0.256	0.240/0.280	0.249/0.265
THYMUS	4.91/5.47	6.11/5.34	4.14/4.01	5.69/5.11
OVARIES				
UTERUS				

Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight).

Table 31: Male's organ weights (study # 2)

Graphs for iliac, inguinal, and popliteal lymph nodes weight in males (M) and females (F):



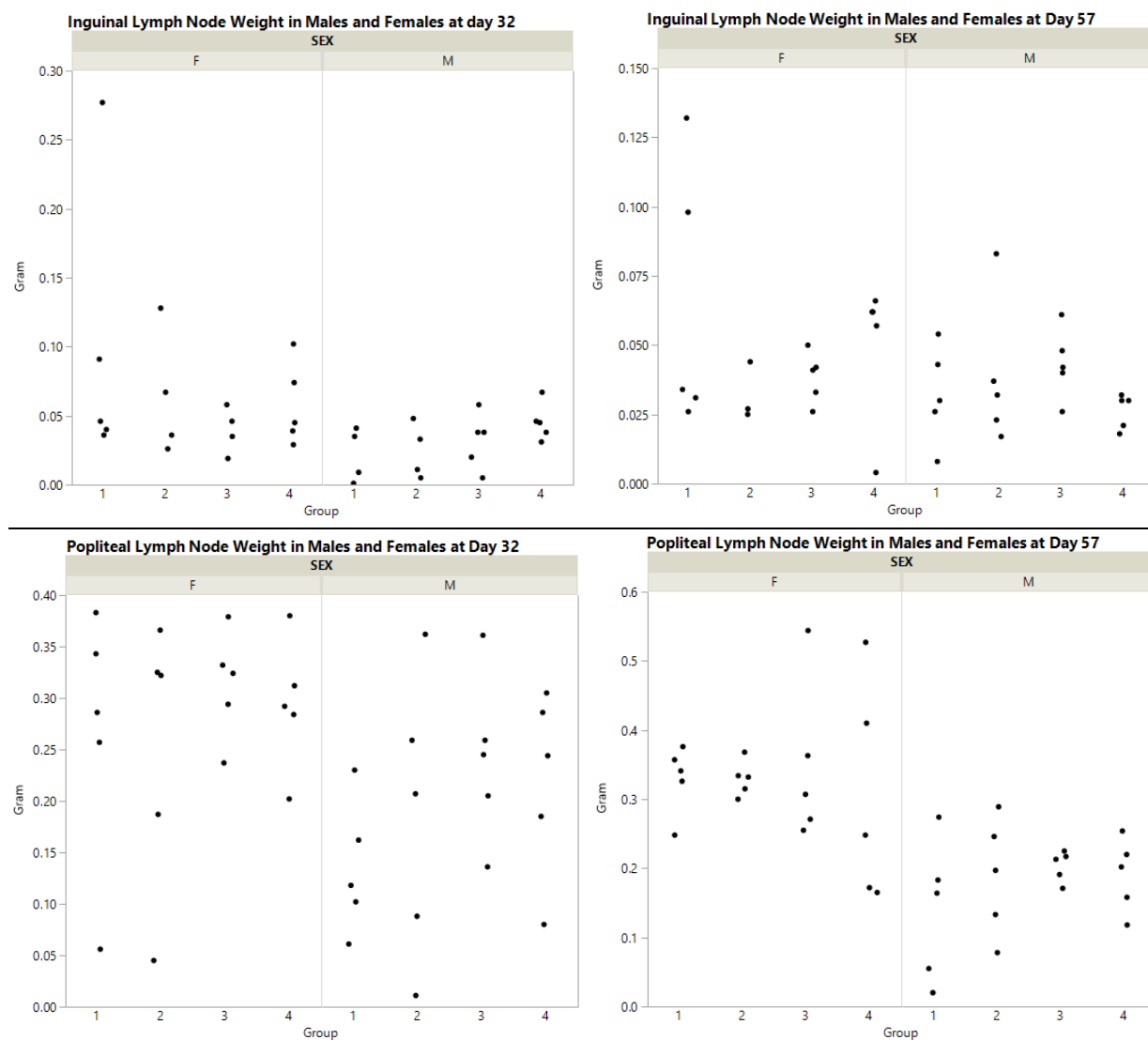


Figure 4: Graphs for iliac, inguinal, and popliteal lymph nodes weight in males (M) and females (F) (study # 2).

#### Males weight

Adrenal weight was increased 11% and 26% in group 4 males at study days 32 and 57, respectively. Epididymides lymph node weight was increased 14% in group 2 at study day 32. At study day 57, liver weight was decreased 21% and 13% in groups 3 and 4, respectively. At study day 32, iliac lymph node weight was increased 26%, 206%, and 406% in groups 2, 3, and 4, respectively. Iliac lymph node weight was increased 47% in group 3 at study day 57. At study day 32, inguinal lymph node weight was increased 52% and 114% in groups 3 and 4, respectively. At study day 57, inguinal lymph node weight was increased 19% and 34% in groups 2 and 3, respectively. Inguinal lymph node weight was decreased 19% in group 4 at study day 57. At study day 32, mandibular lymph node weight was decreased 16% and 11% in groups 2 and 3, respectively. At study day 57, mandibular lymph node weight was decreased 23% and 35% in groups 2 and 4, respectively. At study day 32, mesenteric lymph node weight was increased 12% and 46% in groups 2 and 3, respectively. Mesenteric lymph node weight was

increased 11% in group 2 at study day 57. At study day 32, popliteal lymph node weight was increased 38%, 80%, and 64% in groups 2, 3, and 4, respectively. At study day 57, popliteal lymph node weight was increased 35%, 46%, and 37% in groups 2, 3, and 4, respectively. At study day 32, prostate weight was increased 31% and 39% in groups 3 and 4, respectively. At study day 57, prostate weight was increased 15%, 32%, and 15% in groups 2, 3, and 4, respectively. At study day 32, spleen weight was increased 19% and 14% in groups 2 and 4, respectively. Spleen weight was increased 11% in group 3 at study day 57. At study day 32, testes weight was increased 22% and 36% in groups 3 and 4, respectively. At study day 57, testes weight was decreased 13%, 21%, and 13% in groups 2, 3, and 4, respectively. At study day 32, pituitary weight was decreased 30% and 15% in groups 2 and 3, respectively. Pituitary weight was decreased 16% in group 4 at study day 57. At study day 32, thyroid weight was decreased 21%, 16%, and 13% in groups 2, 3, and 4, respectively. At study day 57, thyroid weight was decreased 27%, 21%, and 25% in groups 2, 3, and 4, respectively. At study day 32, thymus weight was increased 24% and 16% in groups 2 and 4, respectively. Thymus weight was decreased 16% in group 3 at study day 32. Thymus weight was decreased 27% in group 3 at study day 57.

SEX	Females <sup>(b) (4)</sup> (32/57)			
GROUPS	1 (CONTROL)	2	3	4
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5
BODY WEIGHT (terminal)	2812/3184	2776/3229	2809/3208	2895/3230
BRAIN	9.86/9.71	9.40/9.64	9.86/9.96	9.51/10.0
ADRENALS	0.324/0.286	0.290/0.308	0.284/0.320	0.269/0.299
EPIDIDYMIDES				
HEART	5.98/7.08	5.87/5.98	6.05/9.81	6.07/6.64
KIDNEYS	14.98/17.3	16.43/16.5	16.78/17.0	15.55/17.2
LIVER	81.45/86.93	87.05/99.41	78.29/94.77	90.53/88.16
LUNGS	10.8/10.3	10.3/11.1	10.5/11.2	11.8/10.7
ILIAC LYMPH NODE	0.038/0.046	0.057/0.046	0.091/0.047	0.140/0.043
INGUINAL LYMPH NODE	0.098/0.064	0.064/0.032	0.040/0.038	0.058/0.050
MANDIBULAR LYMPH NODE	0.113/0.159	0.116/0.109	0.125/0.105	0.106/0.134
MESENTERIC LYMPH NODE	1.645/1.091	0.923/0.929	0.977/1.323	1.195/0.973
POPLITEAL LYMPH NODE	0.265/0.330	0.249/0.330	0.313/0.348	0.294/0.304
PROSTATE AND SEMINAL VESICLE				
SPLEEN	1.412/1.442	1.424/1.677	1.589/1.448	1.559/1.658
TESTES				
PITUITARY	0.031/0.030	0.028/0.028	0.038/0.029	0.028/0.029
THYROID and PARATHYROID	0.242/0.290	0.248/0.270	0.238/0.252	0.244/0.244
THYMUS	5.182/4.46	4.771/4.24	5.302/5.24	4.750/4.72
OVARIES	0.253/0.272	0.238/0.314	0.271/0.468	0.263/0.335



SEX	Females <sup>(b) (4)</sup> (32/57)			
GROUPS	1 (CONTROL)	2	3	4
NUMBER OF ANIMALS	5/5	5/5	5/5	5/5
UTERUS	3.50/5.93	3.61/5.08	5.23/7.48	4.90/6.84

Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight).

Table 32: Female's organ weight (study # 2)

#### Female's weight

At study day 32, adrenal weight was decreased 12% and 17% in groups 3 and 4, respectively. At study day 57, adrenal weight was increased 12% in group 3. Heart weight was decreased 16% in group 2 and increased 39% in group 3 at study day 57. Kidney weight was increased 12% in group 3 at study day 32. Liver weight was increased 11% in group 4 at study day 32. Liver weight was increased 14% in group 2 at study day 57.

At study day 32, iliac lymph node weight was increased 50%, 139%, and 268% in groups 2, 3, and 4, respectively. At study day 32, inguinal lymph node weight was decreased 35%, 59%, and 41% in groups 2, 3, and 4, respectively. At study day 57, inguinal lymph node weight was decreased 50%, 41%, and 22% in groups 2, 3, and 4, respectively. Mandibular lymph node weight was increased 11% in group 3 at study day 32. At study day 57, mandibular lymph node weight was decreased 31%, 34%, and 16% in groups 2, 3, and 4, respectively. At study day 32, mesenteric lymph node weight was decreased 44%, 41%, and 27% in groups 2, 3, and 4, respectively. At study day 57, mesenteric lymph node weight was decreased 15% and 11% in groups 2 and 4, respectively. Mesenteric lymph node weight was increased 21% in group 3 at study day 57. At study day 32, popliteal lymph node weight was increased 18% and 11% in groups 3 and 4, respectively.

Spleen weight was increased 13% in group 3 at study day 32. At study day 57, spleen weight was increased 16% and 15% in groups 2 and 4, respectively. Pituitary weight was increased 23% in group 3 at study day 32. At study day 57, pituitary weight was decreased 13% and 16% in groups 3 and 4, respectively. Thymus weight was increased 17% in group 3 at study day 57. At study day 57, ovary weight was increased 15%, 72%, and 23% in groups 2, 3, and 4, respectively. At study day 32, uterus weight was increased 49% and 40% in groups 3 and 4, respectively. Uterus weight was decreased 14% in group 2 at study day 57. At study day 57, uterus weight was increased 26% and 15% in groups 3 and 4, respectively.

#### **Gross pathology:**

Macroscopic findings are listed below:

Terminal sacrifice// recovery sacrifice (Males)

Groups	Findings
1M	Discolored lung (1/5)// no findings
2M	Thickened wall in cecum (1/5)// no findings

Groups	Findings
3M	Small epididymis (1/5); discolored iliac lymph node (1/5); small testis (2/5)// abnormal contents in gall bladder (1/5); discolored kidney (1/5); large urethra (1/5)
4M	Discolored iliac lymph node (1/5); large iliac lymph node (1/5)// discolored heart (1/5); thickened mammary gland (1/5); large seminal vesicle (1/5); large urethra (2/5)

Table 33: Male's macroscopic findings (study # 2).

## Terminal sacrifice// recovery sacrifice (Females)

Groups	Findings
1F	Discolored kidney (1/5); discolored iliac lymph node (1/5); cyst in ovary (1/5)// small gall bladder (1/5)
2F	Discolored kidney (1/5); large iliac lymph node (1/5)// no findings
3F	Discolored injection site A (1/5); large iliac lymph node (1/5); cyst in ovary (1/5)// no findings
4F	Cyst in ovary (1/5)// no findings

Table 34: Female's macroscopic findings (study # 2).

In one group 4 males and one group 3 females, a large internal iliac lymph node was reported at day 32 euthanasia. This was correlated with the increased lymph node weights and/or the microscopic finding of increased lymphocytes. At recovery (day 57) euthanasia, internal iliac lymph nodes were macroscopically normal in all groups (consistent with full reversibility).

Because of the low incidence, were randomly distributed across groups (including concurrent controls), and/or were as expected for (b) (4) rabbits, all other macroscopic findings were considered spontaneous and/or incidental.

Microscopic findings are listed below:

## Terminal sacrifice (Males)

Groups	Findings
1M	Slight lumen cellular debris in epididymis (1/5); adrenal ectopic tissue in epididymis (1/5); minimal mixed cell infiltrate in esophagus (2/5); minimal limbus mixed cell infiltrate in eye (1/5); moderate hemorrhage in injection site A (1/5); slight basophilic tubule in kidney (1/5); minimal mineralization in kidney (3/5); minimal sinus erythrocytes in iliac lymph node (2/5); minimal increased heterophils infiltrate in popliteal lymph node (2/5); ectopic spleen in pancreas (1/5); slight acanthosis/hyperkeratosis in skin/subcutis (1/5); slight mixed cell inflammation in skin/subcutis (2/5); minimal mixed cell infiltrate in stomach (1/5); ectopic thymus (1/5); minimal (1/5) and slight (1/5) mineralization in urinary bladder
2M	Adrenal ectopic tissue in epididymis (1/5); minimal mixed cell infiltrate in esophagus (4/5); minimal (1/5) and slight (1/5) mixed cell infiltrate in injection site A; minimal mixed cell infiltrate in injection site B (1/5); slight basophilic tubule in kidney (1/5); minimal mineralization in kidney (4/5); minimal

Groups	Findings
	hemorrhage in lung (1/5); slight perivascular mixed cell infiltrate in lungs (1/5); minimal (2/5), slight (1/5), and moderate (1/5) heterophils infiltrate in iliac lymph node; minimal increased lymphocyte in inguinal lymph node (1/5); minimal increased heterophils infiltrate in popliteal lymph node (2/5); minimal mixed cell infiltrate in sciatic nerve (1/5); slight mixed cell inflammation in skin/subcutis (1/5); slight seminiferous tubule degeneration in testis (1/5); minimal tubular dilatation in testis (1/5); minimal mineralization in urinary bladder (1/5)
3M	Minimal mixed cell infiltrate in aorta (1/5); minimal hypoplasia in epididymis (1/5); minimal mixed cell infiltrate in esophagus (3/5); minimal limbus mixed cell infiltrate in eye (1/5); minimal atrophy in Harderian gland (1/5); minimal hemorrhage in injection site A (1/5); minimal (3/5) and moderate (1/5) mixed cell infiltrate in injection site A; slight mixed cell infiltrate in kidney (1/5); minimal mineralization in kidney (4/5); minimal mixed cell infiltrate in larynx (1/5); minimal sinus erythrocytes in iliac lymph node (3/5); minimal (3/5) and slight (1/5) heterophils infiltrate in iliac lymph node; slight increased lymphocyte in iliac lymph node (4/5); minimal increased heterophils infiltrate in popliteal lymph node (2/5); minimal (2/5) and slight (1/5) mixed cell infiltrate in right femoris biceps muscle; minimal (1/5), slight (1/5), and moderate (2/5) mixed cell infiltrate in sciatic nerve; minimal seminiferous tubule degeneration in testis (1/5); minimal tubular dilatation in testis (2/5); hypoplasia in testis (2/5); minimal mononuclear cell infiltrate in thyroid (1/5); slight myofiber degeneration in rectum (1/5)
4M	Slight lumen cellular debris in epididymis (1/5); adrenal ectopic tissue in epididymis (1/5); minimal erosion in esophagus (1/5); minimal mixed cell infiltrate in esophagus (1/5); minimal myofiber degeneration/necrosis in injection site A (1/5); slight (1/5) and moderate (1/5) mixed cell infiltrate in injection site A; slight basophilic tubule in kidney (1/5); minimal mineralization in kidney (4/5); minimal sinus erythrocytes in iliac lymph node (2/5); minimal (1/5) and slight (3/5) heterophils infiltrate in iliac lymph node; slight increased lymphocyte in iliac lymph node (5/5); minimal increased heterophils infiltrate in popliteal lymph node (2/5); minimal (1/5) and slight (1/5) degeneration/necrosis in right femoris biceps muscle; moderate mixed cell infiltrate in right femoris biceps muscle (2/5); minimal mononuclear cell infiltrate in right femoris biceps muscle (1/5); minimal (3/5) and slight (1/5) mixed cell infiltrate in sciatic nerve; minimal acanthosis/hyperkeratosis in skin/subcutis (1/5); slight mixed cell inflammation in skin/subcutis (1/5); minimal (1/5) and slight (1/5) tubular dilatation in testis; ectopic thymus (1/5); minimal (1/5) and slight (1/5) mineralization in urinary bladder

Table 35: Male's microscopic findings (study # 2).

## Terminal sacrifice (Females)

Groups	Findings
1F	Minimal mixed cell infiltrate in esophagus (2/5); minimal atrophy in Harderian glands (1/5); minimal mixed cell infiltrate in injection site A (1/5); minimal

Groups	Findings
	mineralization in kidney (3/5); minimal peri-portal mixed cell infiltrate in liver (1/5); slight sinus erythrocytes in iliac lymph node (1/5); minimal increased heterophils infiltrate in popliteal lymph node (2/5); minimal mixed cell infiltrate in sciatic nerve (1/5); slight mixed cell inflammation in skin/subcutis (1/5); minimal mineralization in urinary bladder (3/5)
2F	Minimal mixed cell infiltrate in esophagus (1/5); minimal myofiber degeneration/necrosis in injection site A (1/5); minimal (1/5) and slight (1/5) mixed cell infiltrate in injection site A; minimal mineralization in kidney (4/5); minimal chronic inflammation in lungs (1/5); minimal mineralization in lungs (2/5); minimal sinus erythrocytes in iliac lymph node (1/5); minimal (2/5) and slight (1/5) heterophils infiltrate in iliac lymph node; minimal mixed cell infiltrate in sciatic nerve (1/5); ectopic spleen in pancreas (1/5); minimal mononuclear cell infiltrate in thyroid (1/5); moderate chronic inflammation in tongue (1/5)
3F	Slight mixed cell infiltrate in esophagus (1/5); minimal myofiber degeneration/necrosis in injection site A (1/5); minimal and slight hemorrhage in injection site A (1/5); minimal (3/5) and moderate (1/5) mixed cell infiltrate in injection site A; minimal mixed cell infiltrate in injection site B (1/5); slight basophilic tubule in kidney (1/5); minimal (4/5) and slight (1/5) mineralization in kidney; minimal (3/5) and slight (2/5) heterophils infiltrate in iliac lymph node; minimal increased heterophils infiltrate in popliteal lymph node (1/5); minimal mixed cell infiltrate in sciatic nerve (3/5); slight mixed cell inflammation in rectum (1/5); minimal mixed cell inflammation in skin/subcutis (1/5)
4F	Minimal mixed cell infiltrate in esophagus (2/5); minimal (1/5) and slight (1/5) myofiber degeneration/necrosis in injection site A; minimal (1/5), slight (2/5), and moderate (1/5) mixed cell infiltrate in injection site A; minimal mineralization in kidney (3/5); minimal hemorrhage in lung (1/5); minimal (2/5) and slight (3/5) heterophils infiltrate in iliac lymph node; slight increased lymphocyte in iliac lymph node (3/5); minimal increased heterophils infiltrate in popliteal lymph node (3/5); slight degeneration/necrosis in right femoris biceps muscle (1/5); minimal (2/5), slight (1/5), and moderate (1/5) mixed cell infiltrate in right femoris biceps muscle; minimal mixed cell infiltrate in sciatic nerve (1/5); minimal acanthosis/hyperkeratosis in skin/subcutis (1/5); minimal mixed cell inflammation in skin/subcutis (1/5)

Table 36: Female's microscopic findings (study # 2).

An extensive number of tissues were examined for histology. Except for injection sites and lymph node (iliac and popliteal) findings, no increased incidences of histological findings indicative of potential adverse events were reported in the treated groups relative to the controls.

#### **Body temperature:**

No test article-related effect on body temperature was reported. In groups 3 and 4, body temperature fluctuations were reported transiently. However, these fluctuations lacked any consistent trend. Thus, these changes were considered incidental and within the normal variation

of data. Temperature spikes above 40°C occurred in individual animals during the dosing phase (prior to, and subsequent to, dosing on dosing days) were reported in test article-treated animals. However, these increases were transient and were also reported in control group and, thus, considered incidental. Body temperature results are shown in the following graphs:

Graphs for body temperature levels in males (M) and females (F) at study days 1, 15, and 29:

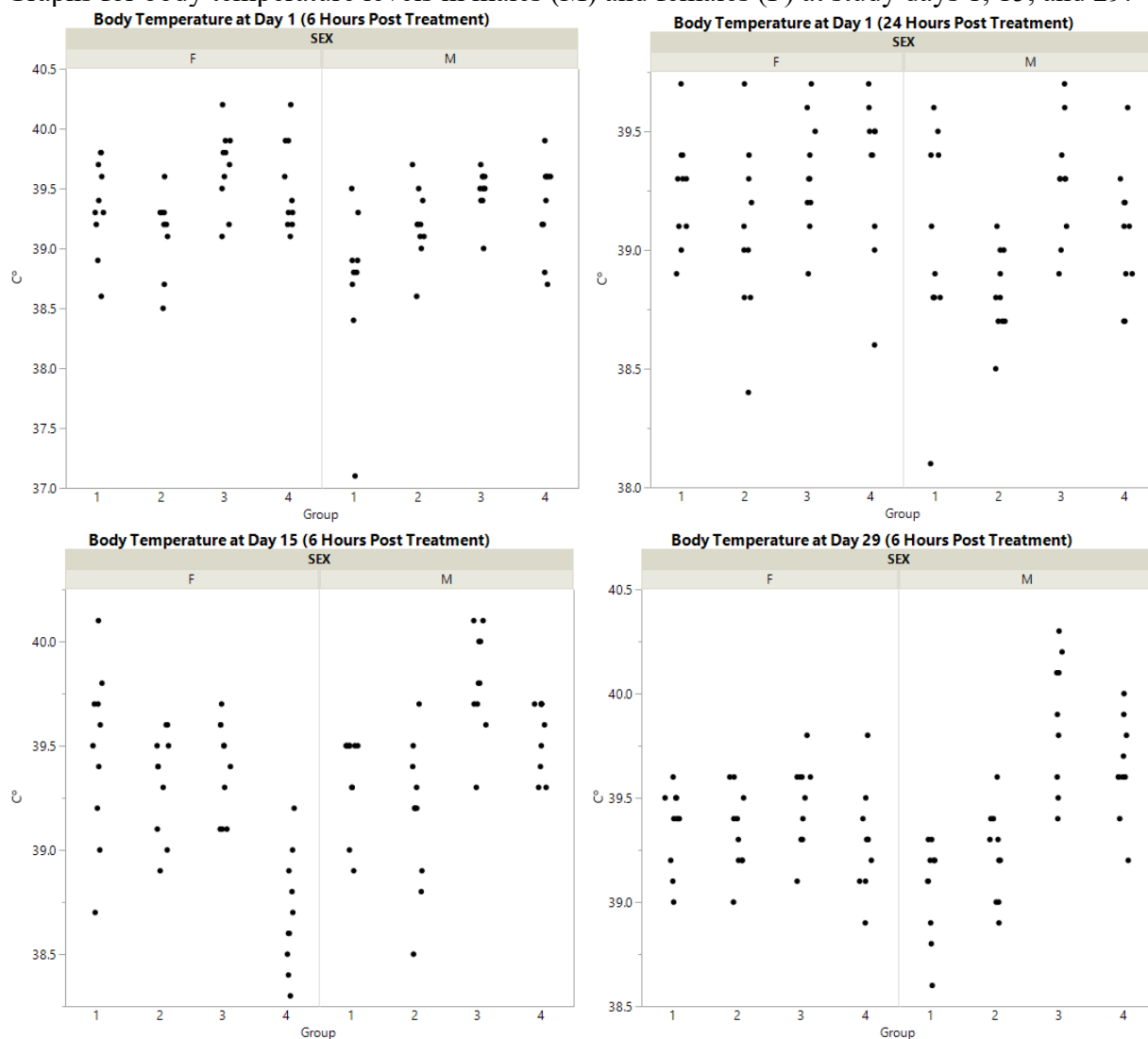


Figure 5: Graphs for body temperature levels in males (M) and females (F) at study days 1, 15, and 29 (study # 2).

**Serology:***RSVPreF3 Immunogenicity Analysis*

To determine anti- RSVPreF3 IgG titers, (b) (4) assay was used. Anti- RSVPreF3 antibody titers were below the limits of detection (BLOD) in control group (group1) at all time points and in all groups before immunization.

In the pre-dose phase (study day -10) or control samples during the dosing phase, no anti-RSVPreF3 IgG antibody titers were reported.

In groups 2 and 3 males and females, there was a consistent anti-RSVPreF3 IgG antibody titer response on days 32 and 57. This increase ranged from 6205 to 995405 EU/mL. In group 4 males (on days 32 and 57), detectable anti-RSVPreF3 IgG antibody titers were reported in all animals. This indicated 100% seroconversion. At days 32 and 57, all females had detectable anti-RSVPreF3 antibody titers and had seroconverted. Therefore, exposure to the vaccine was successfully demonstrated, as measured by an immune response that differed from control or pre-dose phase samples.

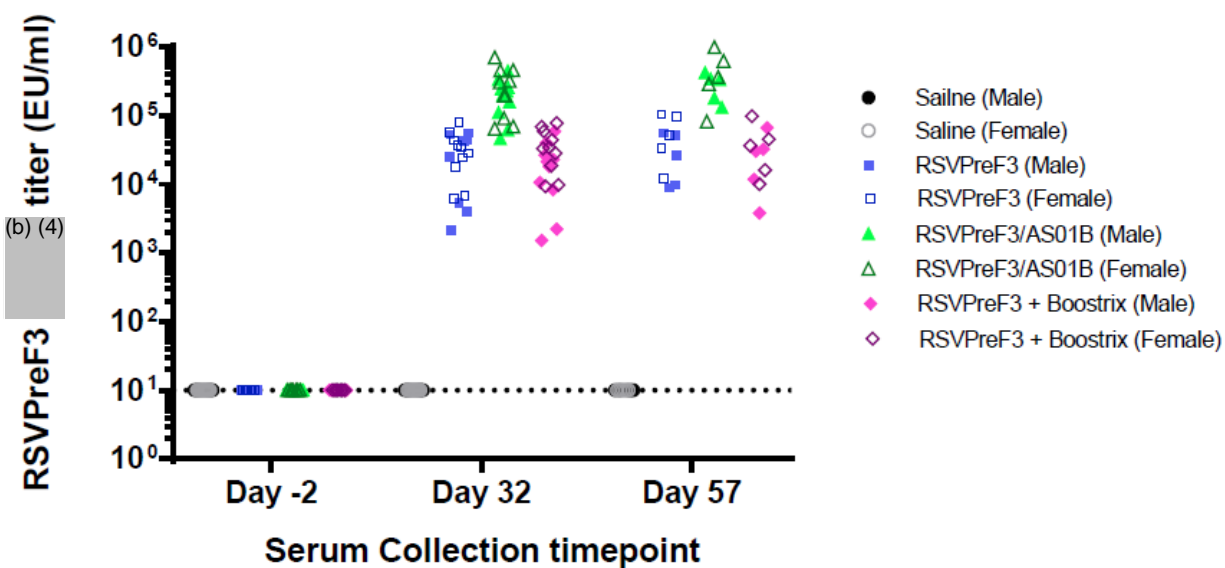


Figure 6: Distribution of RSVPreF3 IgG antibody titers (study # 2); sponsor provided

*Diphtheria Antigen Immunogenicity Analysis*

Anti-DT IgG binding antibody tiers were measured by (b) (4) (EU/mL) at days -2, 32, and 26 of the recovery phases.

Before immunization, no anti-diphtheria specific antibody response was reported in any group. In group 1, no antibody titer against diphtheria was reported. In group 4, an anti-diphtheria specific antibody response was reported. This indicated effective vaccine exposure of all RSVPreF3 + Boostrix-treated animals. A graphical representation of the individual anti-DT IgG titers (EU/mL) is below:

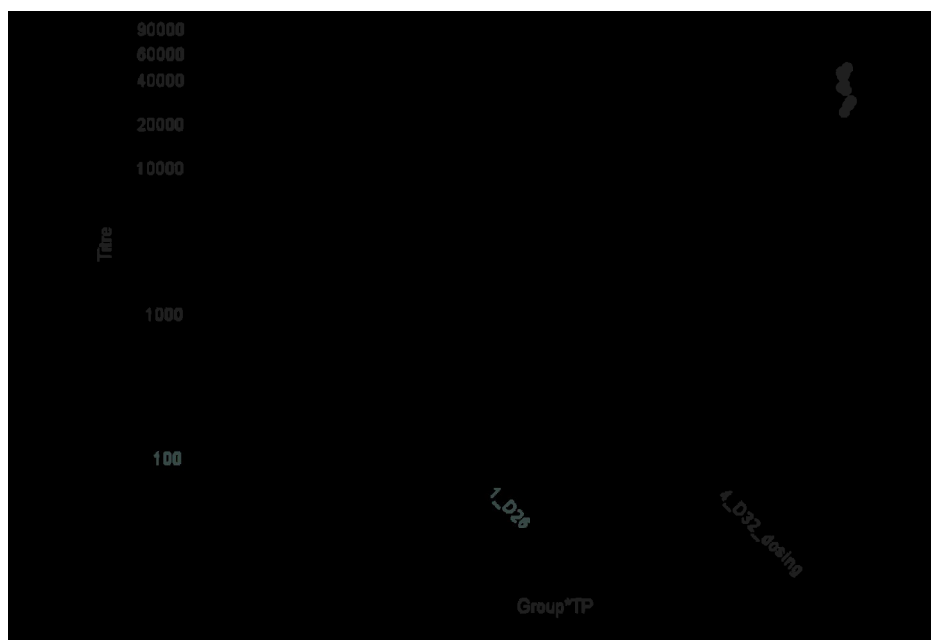


Figure 7: Anti-DT IgG titers per group, per time point (study # 2); sponsor provided

**Test article related effects are listed in the table below:**

Test article related effects	Effects considered incidental
↑ Monocytes ↑ Neutrophils ↓ LUC ↑ CRP (G3 higher than G4) ↑ Iliac lymph node weight ↑ Inguinal lymph node weight ↑ Popliteal lymph node weight ↑ Thymus weight Microscopic findings at injection sites, iliac lymph nodes, and popliteal lymph nodes Immune responses	↑ Prostate weight ↓ Thyroid weight ↓ Uterus weight

Table 37: Test article related effects (study # 2).

**Assessment:**

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight, dermal scores, food consumption, body temperature, ophthalmoscopic parameters, gross pathology were reported.

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increases in the monocyte count might be related to test article treatment.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection.

LUC is a measurement of the large, peroxidase-negative cells which cannot be further characterized (i.e. as large lymphocytes, virocytes, or stem cells) present in a biological specimen. In LUC are found large lymphoid cells, more immature lymphocytes and other cells. If the value is higher than normal, blood counts should be checked under a microscope slide.

CRP is protein synthesized by the liver, found in the blood, and is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections. Also, a positive CRP test that indicates an inflammation in the body may be used to detect a variety of different conditions, including cancer, connective tissue disease, heart attack, infection, inflammatory bowel disease (IBD), lupus, pneumococcal pneumonia, rheumatoid arthritis, rheumatic fever, or tuberculosis. The significant increase in CRP levels in group 4 were lower than group 3. These results show the difference in the effect of AS01<sub>B</sub> (group 3) and Boostrix (group 4) on CRP levels.

The increases in the weights of iliac lymph nodes, inguinal lymph nodes, and popliteal lymph nodes might be related to the immune response due to test article treatment. The microscopic findings in the iliac and popliteal lymph (heterophils infiltrate and/or increased lymphocyte) nodes might also be related to the immune responses.

The thymus is a specialized primary [lymphoid](#) organ of the [immune system](#). Within the thymus, [T cells](#) or T [lymphocytes](#) mature. T cells are critical to the [adaptive immune system](#), where the body adapts specifically to foreign invaders. The thymus is composed of two identical [lobes](#) and is located anatomically in the anterior superior [mediastinum](#), in front of the [heart](#) and behind the [sternum](#). As the thymus is the organ of [T-cell](#) development, any [congenital](#) defect in thymic genesis or a defect in [thymocyte](#) development can lead to a profound [T cell](#) deficiency in primary [immunodeficiency](#) disease. Defects that affect both the [T cell](#) and [B cell lymphocyte](#) lineages result in [severe combined immunodeficiency](#) syndrome (SCIDs). Acquired [T cell](#) deficiencies can also affect [thymocyte](#) development in the thymus.<sup>8</sup>

Microscopic findings at injection sites were reported. This might be related to inflammation and is a relatively common occurrence as part of the acute phase response following administration of some vaccines. Immune responses due to test article treatment were reported.

Adverse gross or microscopic alteration that could be indicative of systemic or local toxicity was not reported.

Changes in prostate, thyroid, and uterus weights were not associated with any macroscopic and/or microscopic findings. Thus, it was considered incidental.

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<sup>8</sup> <https://en.wikipedia.org/wiki/Thymus>.



Based on the overall findings in this study, it can be concluded that in (b) (4) rabbit's administration of RSV PreF3 vaccine on study days 1, 15, and 29 had no adverse effects in terms of systemic toxicity at the dose level of 240 µg.

**GLP study deviations or amendments:** No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

**Investigators Brochure:** Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes ( ) or no (X).

**Conclusions:**

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect.

**Study number 3:**

**Title and study number:** Repeated-dose Toxicity Study with Zoster Candidate Vaccine (gE/AS01B) Administered Subcutaneously (Four times) or Intramuscularly (Four times) to Male and Female Rabbits Followed by a 4-Week Treatment Free Period. Study number: 20094.

**Performing laboratory:** (b) (4)

**Study initiation date:** 12/12/2011

**Final report date:** 06/13/2012

**Experimental design:**

Males:

Group	Color code	Treatment	Animal numbers of males (even nos.)	
			S1 (n=5)	S2 (n=5)
20094/1	White	Saline 0.5 mL	2-10	12-20
20094/2	Blue	gE/AS01B (SC) 0.5 mL	22-30	32-40
20094/3	Green	AS01B (SC) 0.5 mL	42-50	52-60
20094/4	Red	gE/AS01B (IM) 0.5 mL	62-70	72-80
20094/5	Yellow	AS01B (IM) 0.5 mL	82-90	92-100

Females:

Group	Color code	Treatment	Animal numbers of females (odd nos.)	
			S1 (n=5)	S2 (n=5)
20094/1	White	Saline 0.5 mL	1-9	11-19
20094/2	Blue	gE/AS01B (SC) 0.5 mL	21-29	31-39
20094/3	Green	AS01B (SC) 0.5 mL	41-49	51-59
20094/4	Red	gE/AS01B (IM) 0.5 mL	61-69	71-79
20094/5	Yellow	AS01B (IM) 0.5 mL	81-89	91-99

S1 = Subgroup 1 were sacrificed on day 45 (i.e. 3 days post 4<sup>th</sup> injection)

S2 = Subgroup 2 were sacrificed on day 70/71 (i.e. 28/29 days post 4<sup>th</sup> injection)

Route of administration: Subcutaneous (SC) or intramuscular (IM)

Dosing-volume: Approximately 0.5 mL per animal per injection, equivalent to an intended human dose.

Injection: Bolus, new sterile disposable syringe and needle per animal

Table 38: Experimental design (study # 3); sponsor provided

**Results:**

No test article-related effects on clinical signs, body temperature, body weight, food consumption, ophthalmology, and organ weights were reported.

Hematology results showed a transient increase in fibrinogen in all four male and female test groups one day after the first and after the fourth injection. An increase in neutrophils was reported one day after the fourth injection in the AS01B (SC) males and AS01B (IM) males and females. These changes were considered to be part of the immune response to vaccination.

Clinical chemistry results showed a transient increase of CRP concentration in all four male and female test groups one day after the first and after the fourth injection. The increase was more pronounced after the first injection and was the highest in the AS01B (IM) group. The increase in CRP was part of the inflammatory process following vaccination.

In one AS01B (SC) female, the SC injection site showed superficial hemorrhages at necropsy at 3 days post fourth injection. The IM injection site showed superficial petechiae in one AS01B (IM) female, a red area in one AS01B (IM) female and male and a superficial red area in one saline control female.

Slight diffuse mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes) in one gE/AS01B (SC) male and one gE/AS01B female were reported three days after the fourth injection. Diffused mononuclear cell infiltrate was reported in one AS01B (SC) female. These changes were reported in the subcutis/deeper layers of the skin.

In all ten gE/AS01B (IM) males and females, the four consecutive IM injections resulted in slight widespread mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes). In animals that received AS01B (IM) alone, mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes) was reported at various incidence and distribution, being slight to moderate and widespread in a single male and 2/5 females, slight and multifocal in a single male and very slight to slight and localized in 3/5 males and 2/5 females.

The injection sites (either IM or SC) did not show vaccine or adjuvant treatment related histopathological changes at twenty-eight- or twenty-nine-days post fourth injection.

In all gE/AS01B (SC and IM) males, the draining popliteal and inguinal lymph nodes showed an activated appearance. This finding was considered a physiological response against the injection with the candidate vaccine.

No adverse vaccine or adjuvant related histopathological changes were reported in other organs.

**Study number 4:**

**Title and study number:** Repeated-dose Toxicity Study with AS01B Administered Intramuscularly (Seven times) to Male and Female Rats Followed by a 4-Week Treatment Free Period. Study number: 20165.

**Performing laboratory:** (b) (4)

**Study initiation date:** 04/24/2012

**Final report date:** 03/20/2013

**Experimental design:**

**Males:**

Group	Color code	Treatment	Volume	Animal numbers of males (even nos.)	
				S1 (n=10)	S2 (n=5)
20165/1	White	Saline	0.2 mL	2-20	22-30
20165/2	Blue	AS01B	0.2 mL	32-50	52-60

**Females:**

Group	Color code	Treatment	Volume	Animal numbers of females (odd nos.)	
				S1 (n=10)	S2 (n=5)
20165/1	White	Saline	0.2 mL	1-19	21-29
20165/2	Blue	AS01B	0.2 mL	31-49 <sup>1</sup>	51-59

<sup>1</sup> Animal no. 49 did not survive blood collection on day 85 of the study; S1 = Subgroup 1 sacrificed on day 87 (i.e. 3 days post 7<sup>th</sup> injection); S2 = Subgroup 2 sacrificed on day 113 (i.e. 29 days post 7<sup>th</sup> injection).

Table 39: Experimental design (study # 4).

**Route of administration:** Intramuscular

**Dosing-volume:** Approximately 0.1 mL per injection site (2 injections/animal/occasion)

**Injection:** Bolus, new sterile disposable syringe and needle per animal

**Schedule/injection site:**

- day 0, left and right posterior thigh muscles.
- day 14, left and right calf muscles.
- day 28, left and right posterior thigh muscles.
- day 42, left and right calf muscles.
- day 29, left and right posterior thigh muscles.
- day 70, left and right calf muscles.
- day 84, left and right anterior thigh muscles.

**Results:**

No test article-related effects on clinical signs, body temperature, body weight, food consumption, ophthalmology, and organ weights were reported.

Test article-related swollen calf muscle in all males and females after the first injection, in all males after the second injection and in 5/15 males after the third injection were reported.

A higher mean body temperature was reported in group 2 males 4 hours after the first and after the seventh injection and in group 2 females 4 hours after the first injection.

In group 2 males and females, hematology results revealed, in general (i.e. either statistical significant or as a trend), a WBC response, consisting of an increase in neutrophils, a decrease in lymphocytes and an increase in eosinophils (females only) on day 1 after the first and after the seventh injection. In addition, a transient increase in fibrinogen was reported in group 2 males and females on days 1 and 3 after the first and after the seventh injection.

Lower A/G ratios in group 2 males and females on days 1 and 3 after the first and after the seventh injection were reported.

In groups 1 and 2 animals, macroscopic examination at study days 3 or 29 days after the seventh injection revealed sporadic injection related changes (discoloration).

Localized or widespread mononuclear inflammatory cell responses were reported three days post seventh injection at the right and the left anterior thigh muscle injection site. The minimal to mild localized response in group 2 animals did in fact not exceed the minimal to mild localized response in group 1 animals. This might be a reaction to needle injection. The response was considered to be associated with AS01B treatment in the animals showing a minimal to marked widespread response. This was the case in 8/10 AS01B males and 9/10 AS01B females. Local edema or fibrin accumulation was reported in a single animal. Minimal muscle fiber degeneration was reported as part of the local response in few group 2 animals but also in a few group 1 animals. Therefore, this was considered as specific response not related to the test article.

At 29 days post seventh injection, the principal microscopic findings at the right and left anterior thigh muscle injection site was a minimal localized mononuclear inflammatory response. This response in group 2 animals did not exceed the minimal localized response in group 1 animals. Minimal muscle fiber degeneration was reported as part of the local response in few group 2 animals but also, in a few group 1 animals.

No adverse test article-related histopathological changes were reported in other organs.

In conclusion, repeated intramuscular administration (seven injections in duplicate) with AS01B to rats, induced local effects (swollen muscle) reported only in the calf muscles of the animals. Systemically, increased body temperature, few hematology parameters (increased fibrinogen and neutrophils, decreased lymphocytes and increased eosinophils) and one clinical chemistry parameter (decreased A/G ratio) were reported. All these changes were considered to be related to the local inflammation or the immune stimulation following AS01B injection. Microscopically, AS01B caused a widespread mononuclear cell inflammatory response at the injection sites, graded as mild to moderate (marked in one animal). All these changes were not reported in recovery groups.

4 Pages have been determined to be not releasable: (b)(4)

**Study number 6:**

**Title and study number:** Repeated Dose Toxicity Study with DQ Administered Intramuscularly to Male and Female Rats. Study number: 20154.

**Performing laboratory:** (b) (4)

**Study initiation date:** 08/23/2012

**Final report date:** 03/04/2013

**Experimental design:**

Groups	Number of animals		Animal nos.		Treatment (intramuscular; 0.1 mL per site)		Dose <sup>1</sup> of QS21 [µg/Kg/bw]	
	Subgroup 1	Subgroup 2	Males Even nos.	Females Odd nos.	Left leg	Right leg	Males	Females
1	10 males + 10 females	5 males + 5 females	2-20 (S1) 22-30 (S2)	1-19 (S1) 21-29 (S2)	Saline	Saline	-	-
2	10 males + 10 females		32-50 (S1)	31-49 (S1)	DQ; 20 µg/mL	DQ; 20 µg/mL	10	16
3	10 males + 10 females		52-70 (S1)	51-69 (S1)	DQ; 100 µg/mL	DQ; 100 µg/mL	50	80
4	10 males + 10 females	5 males + 5 females	72-90 (S1) 92-100 (S2)	71-89 (S1) 91-99 (S2)	DQ; 200 µg/mL	DQ; 200 µg/mL	100	160

<sup>1</sup> Dose level of QS21 per occasion (two injections)

Table 43: Experimental design (study # 6); sponsor provided

Route of administration: Intramuscular

Dosing-volume: Approximately 0.1 mL per injection (2 injections/occasion)

Injection: Bolus, new sterile disposable syringe and needle per animal.

Injection sites: Day 0, posterior thigh muscle; day 4, calf muscle; day 7, posterior thigh muscle; day 11, calf muscle; day 14, posterior thigh muscle; day 18, anterior thigh muscle.

Species: (b) (4)

Supplier: (b) (4)

Sex and age: 53 males and 53 females, young adult (ca 12 weeks upon arrival)

Body weight range ordered: ca 375-400 g for males and ca 225-250 g for females

Acclimatization period: 12/13 days (males/females)

Caging: Maximum of 5 animals/sex per macrolon cage with wood shavings (Lignocel) as bedding material and environmental enrichment (Enviro-dri and woodblock)

**Results:**

No test article-related effects on clinical signs, body temperature, body weight, food consumption, ophthalmology, and organ weights were reported.

Hematology*Red blood cell (RBC) variables in males*

Fibrinogen: In groups 3 and 4 on day 1 after the sixth injection, and in group 4 on day 3 after the sixth injection higher fibrinogen levels were reported. The fibrinogen concentration tended to be

higher in group 2 on day 1 after the sixth injection and in groups 2 and 3 on day 3 after the sixth injection. The increases in fibrinogen were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

*RBC variables in females:*

Red Blood Cells (RBC): In groups 3 and 4, lower RBC were reported on day 1 after the sixth injection. The RBC tended to be lower in group 2 on day 1 after the sixth injection.

Hemoglobin (Hb): In groups 2, 3, and 4, lower Hb were reported on day 1 after the sixth injection. The toxicological significance of this incidental and transient finding was considered negligible.

Packed Cell Volume (PCV): In group 3, lower PCV was reported on day 1 after the sixth injection. No dose-response effect relationship was reported; thus, the toxicological significance of this incidental and transient finding was considered negligible.

Mean Corpuscular Haemoglobin Concentration (MCHC): In group 4, lower MCHC was reported on day 28 after the sixth injection. The toxicological significance of this incidental finding was considered negligible.

Thrombocytes: In groups 3 and 4, higher number of thrombocytes was reported on day 3 after the sixth injection. The number of thrombocytes tended to be higher in group 2 on day 3 after the sixth injection. Thrombocytosis is often associated with inflammation and, therefore, considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Prothrombin Time (PT): In group 4, A shorter PT was reported on day 1 after the sixth injection. The decreases in PT might be related to the observed increases in fibrinogen. The individual values were within the control range. Therefore, the toxicological significance of this incidental and transient finding was considered negligible. In group 3, a shorter PT was reported on day 3 after the sixth injection. Without a dose-response effect relationship, the toxicological significance of this incidental and transient finding was considered negligible.

Activated Partial Thromboplastin Time (APTT): In group 3, a shorter APTT was reported on day 3 after the sixth injection. Without a dose-response effect relationship, the toxicological significance of this transient finding was considered negligible.

Fibrinogen: In groups 2, 3, and 4, a higher fibrinogen concentration was reported on day 1 after the sixth injection, and in group 4 on day 3 after the sixth injection. The fibrinogen concentration tended to be higher in groups 2 and 3 on day 3 after the sixth injection. The increases in fibrinogen levels were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

*White blood cell (WBC) variables of the males:*

Total White Blood Cell count (WBC): In group 4, a higher WBC was reported on day 1 after the sixth injection. The WBC tended to be higher in groups 2 and 3 on day 1 after the sixth injection.

The increase in WBC was considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute eosinophil count: In groups 3 and 4, a higher absolute eosinophil count was reported on day 1 after the sixth injection. The increase in eosinophils was considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute neutrophil count: In groups 2 and 4, a higher absolute neutrophil count was reported on day 1 after the sixth injection. The absolute neutrophil count tended to be higher in group 3 on day 1 after the sixth injection. In group 4, a lower absolute neutrophil count was reported on day 28 after the sixth injection. The increases in neutrophils were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute monocyte count: In group 4, a higher absolute monocyte count was reported on day 1 after the sixth injection. The monocyte count tended to be higher in groups 2 and 3 on day 1 after the sixth injection. The increases in monocytes were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

*WBC variables of the females:*

Absolute neutrophil count: In groups 3 and 4, a higher absolute neutrophil count was reported on day 1 after the sixth injection. The absolute neutrophil count tended to be higher in the DQ 20 µg/mL group on day 1 after the sixth injection. The increases in neutrophils were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

The RBC (fibrinogen and thrombocytes) and WBC (total WBC, neutrophils, eosinophils, monocytes) responses were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Blood chemistry

Compared to the saline control group, the following statistically significant changes were reported in the males of the DQ test groups:

Aspartate aminotransferase (ASAT): In group 4, a lower ASAT activity was reported on day 28 after the sixth injection.

Lactate dehydrogenase (LDH): In groups 2 and 3, a lower LDH activity was reported on day 1 after the sixth injection.

Total protein (TP): In group 2, a lower TP concentration was reported on day 1 after the sixth injection.

Albumin/Globulin ratio (A/G ratio): In group 4, a lower A/G ratio was reported on day 3 after the sixth injection. This transient decrease in A/G ratio was considered to be part of the inflammatory process upon injection of an immunostimulant.



Bilirubin: In groups 3 and 4, a higher bilirubin concentration was reported on day 1 after the sixth injection.

Creatinine: In groups 2, 3, and 4, a higher creatinine concentration was reported on day 3 after the sixth injection.

Phospholipids: In group 3, a lower phospholipids concentration was reported on day 1 after the sixth injection, and in groups 3 and 4 on day 3 after the sixth injection.

Chloride (Cl): In groups 2, 3, and 4, a higher Cl concentration was reported on day 1 after the sixth injection.

Phosphate (PO<sub>4</sub>): In group 4, a higher PO<sub>4</sub> concentration was reported on day 1 after the sixth injection.

Compared to the saline control group, the following statistically significant changes were reported in the females of the DQ test groups:

Aspartate aminotransferase (ASAT): In group 4, a lower ASAT activity was reported on day 28 after the sixth injection.

Gamma-glutamyl transferase (GGT): In groups 3 and 4, a higher GGT activity was reported on day 1 after the sixth injection.

Lactate dehydrogenase (LDH): In group 2, a lower LDH activity was reported on day 1 after the sixth injection.

Total protein (TP): In groups 3 and 4, a lower TP concentration was reported on day 1 after the sixth injection and in group 4 on day 3 after the sixth injection.

Albumin: In groups 3 and 4, a lower albumin concentration was reported on days 1 and 3 after the sixth injection.

Albumin/Globulin ratio (A/G ratio): In groups 3 and 4, a lower A/G ratio was reported on days 1 and 3 after the sixth injection.

Bilirubin: In groups 2, 3, and 4, a higher bilirubin concentration was reported on day 1 after the sixth injection and a lower bilirubin concentration in group 4 on day 3 after the sixth injection.

Potassium (K): In group 3, a higher K concentration was reported on day 1 after the sixth injection.

Phosphate (PO<sub>4</sub>): In group 4, a higher PO<sub>4</sub> concentration was reported on day 1 after the sixth injection.

## **Organ weights**

### *Subgroup 1 (sacrificed 3 days after the sixth injection)*

A lower relative mean kidneys weight of group 3 males and a higher relative mean left popliteal lymph node weight of group 3 females were reported. Without a dose-response effect relationship and because histopathology of the kidneys and the popliteal lymph nodes did not reveal any treatment-related abnormalities, no toxicological significance was attached to these changed organ weights.

### *Subgroup 2 (sacrificed 28 days after the sixth injection)*

No test article-related effects on organ weights were reported.

## **Macroscopy**

### Subgroup 1, sacrificed day 21, 3 days post sixth injection

No test article-related effects on macroscopic examination were reported.

### Subgroup 2, sacrificed day 45, 28 days post sixth injection

No test article-related effects on macroscopic examination were reported.

## **Microscopy**

### Subgroup 1, sacrificed day 21, 3 days post sixth injection

Right treated anterior thigh muscle: At the injection site, a minimal to mild localized mononuclear cell inflammatory response was reported in 7/10 saline control males, 8/10 DQ 20 µg/mL males, 1/10 DQ 100 µg/mL males, 8/10 saline control females and 10/10 DQ 20 µg/mL females. A mild to moderate widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) mononuclear cell inflammatory reaction was reported in 9/10 DQ 100 µg/mL males, 10/10 DQ 200 µg/mL males, 10/10 DQ 100 µg/mL females and 10/10 DQ 200 µg/mL females. A minimal to mild multifocal mononuclear cell inflammatory response was reported in 3/10 saline control males and in 1/10 saline control females. Mononuclear cell inflammatory reaction consisted of lymphocytes and small macrophages. Minimal hemorrhage or focal muscle fiber degeneration were reported as part of the local response in 1/20 saline control animals, 3/20 DQ 20 µg/mL animals and 3/20 DQ 100 µg/mL animals. These findings were not considered related to the DQ treatment because these reactions were also reported in saline control group.

Left treated anterior thigh muscle: Minimal to mild localized mononuclear cell inflammatory response in 7/10 group 1 males and females, 5/10 group 2 males, and 10/10 group 2 females were reported. A mild to marked widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) mononuclear cell inflammatory reaction was reported in 10/10 group 3 males and females and 10/10 group 4 males and females. A minimal multifocal mononuclear cell inflammatory response was reported in 2/10 group 2 males and in 1/10 group 1 females. Mononuclear cell inflammatory reaction consisted of lymphocytes and small macrophages. The response was considered to be associated with DQ treatment in the animals showing a wider distribution of the mononuclear cell inflammation (reported in all but one groups 3 and 4 animals). The severity of the local response in these animals was slightly higher than that in the groups 1 and 2 animals.

Sciatic nerve: A minimal to moderate inflammatory reaction in the interstitial tissue surrounding the sciatic nerve was reported in 2/20 group 1 animals, 5/20 group 2 animals, 16/20 group 3 animals and 15/20 group 4 animals. The inflammatory reactions in the interstitial tissue surrounding the sciatic nerve in several animals were associated with the local reaction in the nearby muscle tissue.

Mesenteric artery: A minimal to mild inflammatory reaction in the interstitial tissue surrounding the mesenteric artery (periarteritis) was reported in 2/20 group 1 animals, 9/20 group 2 animals, 5/20 group 3 animals and 7/20 group 4 animals. Since there was no apparent dose effect relationship and the lesion also occurred in 2/20 group 1 animals, the periarteritis was not ascribed to the DQ treatment. Hemorrhages, degeneration and/or inflammation in the optic nerve were reported in a few animals of all groups. These findings were ascribed to the orbital puncture 3 days prior to necropsy.

#### Subgroup 2, sacrificed day 45, 28 days post sixth injection

Right treated anterior thigh muscle: Minimal localized or multifocal mononuclear cell inflammatory response in 5/5 group 1 males, 3/5 group 4 males, 5/5 group 1 females and 3/5 group 4 females were reported. Minimal focal muscle fiber degeneration was reported as part of the local response in 4/10 group 1 animals.

Left treated anterior thigh muscle: Minimal localized mononuclear cell inflammatory response in 5/5 group 1 males, 5/5 group 4 males, 4/5 group 1 females and 3/5 group 4 females were reported. Minimal focal muscle fiber degeneration was reported as part of the local response in 4/10 group 1 animals and in 3/10 group 4 animals.

The histopathological changes reported in the other organs and tissues are part of background pathology. They were about equally distributed amongst the groups, or they occurred in one or a few animals only.

#### **Discussion and conclusion**

Treatment of the rats with the three test concentrations of DQ (up to 160 µg of QS21/rat) did not reveal any test substance-related changes in local clinical signs, body temperature, body weight, food intake and organ weights.

In groups 2, 3, and 4 males and females, hematology findings showed (either statistically significant or as a trend) an increase in the number of thrombocytes in the DQ females on day 3 after the sixth injection, a dose-dependent increase in fibrinogen in the DQ males and females on days 1 and 3 after the sixth injection, and a WBC response, consisting of a dose-dependent increase in neutrophils in the DQ males and females (accompanied by an increase in eosinophils and in monocytes in males) on day 1 after the six injection.

The WBC response, increased fibrinogen levels, and the increased number of thrombocytes were considered to be part of the inflammatory process following injection of DQ.

In groups 2, 3, and 4 males and females, clinical chemistry revealed a dose-dependent decrease in A/G ratio in females on day 1 and in both males and females on day 3 after the sixth injection.

The decreased A/G ratios were considered to be part of the non-specific immune response following injection of DQ and were related to increasing globulin levels since the albumin levels were in general not affected in males and decreased in females (also showing a decrease in TP concentration).

No test article-related macroscopic changes at the injection sites and other organs at necropsy, 3 or 28 days, after the sixth injection were reported.

An exacerbation of the inflammatory response at the right and left anterior thigh muscle were reported in groups 2, 3, and 4 as compared to group 1. This was evidenced by a wider distribution of the mononuclear cell inflammation (reported in all but one group 3 and group 4 animals) reported three days post sixth injection.

The severity of the local responses in groups 3 and 4 animals was higher than that in groups 1 and 2 animals. The inflammatory responses reported in group 2 animals were comparable to the responses reported in group 1 animals. Findings of this nature are usually reported in controls as a reaction to insertion of an injection needle.

In the interstitial tissue surrounding the sciatic nerve in several animals, the inflammatory reactions were associated with the local reaction in the nearby muscle tissue. Twenty-eight days post sixth injection, the injection sites at the right and left anterior thigh muscle of groups 2, 3, and 4 animals did not show histopathological changes exceeding those reported in group 1 animals.

#### **Study number 7:**

**Title and study number:** Repeated Dose Toxicity Study with DQ Administered Intramuscularly to Male and Female Rabbits. Study number: 20155.

**Performing laboratory:** (b) (4)

**Study initiation date:** 08/18/2012

**Final report date:** 03/04/2013

#### **Experimental design:**

Groups	Number of animals		Animal nos.		Treatment (intramuscular; 0.5 mL per site)		Dose <sup>1</sup> of QS21 [µg/Kg bw]
	Subgroup 1	Subgroup 2	Males Even nos.	Females Odd nos.	Left leg	Right leg	
1	5 males + 5 females	5 males + 5 females	2-10 (S1) 12-20 (S2)	1-9 (S1) 11-19 (S2)	Saline	Saline	-
2	5 males + 5 females		22-30 (S1)	21-29 (S1)	DQ; 20 µg/mL	DQ; 20 µg/mL	7
3	5 males + 5 females		32-40 (S1)	31-39 (S1)	DQ; 100 µg/mL	DQ; 100 µg/mL	33
4	5 males + 5 females	5 males + 5 females	42-50 (S1) 52-60 (S2)	41-49 (S1) 51-59 (S2)	DQ; 200 µg/mL	DQ; 200 µg/mL	67

<sup>1</sup> Dose level of QS21 per occasion (two injections)

Table 44: Experimental design (study # 7); sponsor provided

**Test article:**

Name: **DQ** (QS21 = 10 µg/dose)

Supplier: GlaxoSmithKline Vaccines

Batch no.: EA10A003A

Labeling: DQ (QS21 = 10 µg/dose)

Amount: 120 syringes of which 65 syringes were reserved for this study

Composition: 20 µg/mL QS21, 400 µg/mL DOPC and 100 µg/mL cholesterol (theoretical concentrations)

Appearance: White turbid liquid

Expiry date: 31.05.2013

Storage conditions: 2-8 °C in the dark

(b) (4)

Name: **DQ** (QS21 = 50 µg/dose)

Supplier: GlaxoSmithKline Vaccines

Batch no.: EA10A002A

Labeling: DQ (QS21 = 50 µg/dose)

Amount: 120 syringes of which 65 syringes were reserved for this study

Composition: 100 µg/mL QS21, 2000 µg/mL DOPC and 500 µg/mL cholesterol

Appearance: White turbid liquid

Expiry date: 31.05.2013

Storage conditions: 2-8 °C in the dark

(b) (4)

Name: **DQ** (QS21 = 100 µg/dose)

Supplier: GlaxoSmithKline Vaccines

Batch no.: EA10A001A

Labeling: DQ (QS21 = 100 µg/dose)

Amount: 200 syringes of which 65 syringes were reserved for this study

Composition: 200 µg/mL QS21, 4000 µg/mL DOPC and 1000 µg/mL cholesterol

Appearance: White turbid liquid

Expiry date: 31.05.2013

Storage conditions: 2-8 °C in the dark

(b) (4)

Route of administration: Intramuscular

Dosing-volume: Approximately 1 mL per injection, given as two injections of 0.5 mL per occasion

Injection: Bolus, new sterile disposable syringe and needle per animal.

Injection sites: Day 0, posterior thigh muscle; day 4, calf muscle; day 7, posterior thigh muscle; day 11, calf muscle; day 14, posterior thigh muscle; day 18, anterior thigh muscle.

Species: (b) (4) rabbits

Supplier: (b) (4)

Sex and age: 33 males and 33 females, young adult (ca 12 weeks at the start of the study).

Body weight range ordered: ca 2000-2300 g

## Results:

No test article-related effects on clinical signs, body temperature, food consumption, ophthalmology, and organ weights were reported.

In group 4 males, a higher mean body weight was reported on day 0 (prior to injection). Post-dose, a higher mean body weight was reported in group 4 males on days 7 and 45 of the study, and in group 4 females on days 3 and 7 of the study. Compared to the respective pre-dose (day 0) body weights, the day 7 mean body weight of group 4 males had slightly less increased compared to group 1. No toxicological significance was attached to these minor changes in body weight.

## Hematology

Red blood cell variables

*Compared to the saline control group, the following statistically significant changes were reported in the RBC variables of the males of the DQ test groups:*

Hemoglobin (Hb): In group 4, lower Hb levels were reported on day 3 after the first injection.

Packed Cell Volume (PCV): In group 4, lower PCV were reported on day 3 after the first injection.

Mean Corpuscular Haemoglobin Concentration (MCHC): In groups 3 and 4, higher MCHC were reported on day 1 after the first injection.

Reticulocytes: In group 4, lower percentage of reticulocytes were reported on day 3 after the first injection and a higher percentage of reticulocytes on day 1 after the sixth injection.

Thrombocytes: In group 4, a higher number of thrombocytes were reported at pre-dose. A lower number of thrombocytes in groups 3 and 4 were reported on day 1 after the first injection.

Prothrombin Time (PT): A shorter PT was reported in all three DQ dose groups on day 1 after the first injection, in group 4 on day 3 after the first injection, and in groups 3 and 4 on day 1 prior to and on day 3 after the sixth injection. In general, decreases in PT are considered to be related to the observed increases in fibrinogen.

Fibrinogen: A higher fibrinogen concentration were reported in group 3 on day 1 after the first injection, and in group 4 on days 1 and 3 after the first injection and on day 1 after the sixth injection. The fibrinogen concentration tended to be higher in group 4 on day 3 after the sixth injection and in group 2 on day 1 after the first injection and in group 3 on day 3 after the first injection and on day 1 after the sixth injection. The increases in fibrinogen were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

*Compared to the saline control group, the following statistically significant changes were reported in the RBC variables of the females of the DQ test groups:*

Mean Corpuscular Volume (MCV): In group 4, lower MCV were reported on day 1 after the first injection.

Mean Corpuscular Hemoglobin (MCH): In group 4, lower MCH were reported on day 1 after the sixth injection.

Reticulocytes: In group 4, lower percentage of reticulocytes were reported on day 3 after the first injection.

Thrombocytes: In group 4, higher number of thrombocytes were reported on day 1 prior to the sixth injection.

Prothrombin Time (PT): In group 4, shorter PT were reported on day 1 after the first injection.

Fibrinogen: In group 4, higher fibrinogen concentration was reported on days 1 and 3 after the first and the sixth injection and on day 1 prior to the sixth injection. The increases in fibrinogen were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

#### White blood cell variables

*Compared to the saline control group, the following statistically significant changes were reported in the WBC variables of the males of the DQ test groups:*

Total White Blood Cell count (WBC): In group 4, higher WBC were reported on day 1 after the first injection. The increase in WBC was considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute neutrophil count: In group 4, higher absolute neutrophil count was reported on day 1 after the first injection. The absolute neutrophil count tended to be higher in group 3 on day 1 after the first injection. On day 1 after the sixth injection, a dose-dependent increase in neutrophils was reported in groups 2, 3, and 4, when compared to their respective values of day 1 prior to the sixth injection. The increases in neutrophils were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute lymphocyte count: In group 4, higher absolute lymphocyte count was reported on day 1 after the first injection.

Absolute monocyte count: In group 4, higher absolute monocyte count was reported on day 3 after the sixth injection.

Absolute basophil count: In group 4, higher absolute basophil counts were reported on day 3 after the first injection and lower absolute basophil counts were reported on day 27 after the sixth injection.

*Compared to the saline control group, the following statistically significant changes were reported in the WBC variables of the females of the DQ test groups:*

Total White Blood Cell count (WBC): In group 4, higher WBC were reported on day 1 after the first injection. The increase in WBC was considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute eosinophil count: In group 4, higher absolute eosinophil count was reported on day 27 after the sixth injection.

Absolute neutrophil count: In group 4, higher absolute neutrophil count was reported on day 1 after the first injection. On day 3 after the first injection, a decrease in the absolute neutrophil count was reported in group 4. On day 1 after the sixth injection, a dose-dependent increase in neutrophils was reported in groups 3 and 4, when compared to their respective values of day 1 prior to the sixth injection. The increases in neutrophils were considered to be part of the local reaction (inflammatory process) upon intramuscular injection of an immunostimulant.

Absolute monocyte count: In group 4, higher absolute monocyte count was reported on day 27 after the sixth injection.

### Blood chemistry

*Compared to the saline control group, the following statistically significant changes were observed in the males of the DQ test groups:*

Alkaline Phosphatase (ALP): In group 4, lower ALP activity was reported on day 3 after the first injection.

Gamma Glutamyl transferase (GGT): At pre-dose, a lower GGT activity in group 2 was reported.

Lactate dehydrogenase (LDH): In group 2, higher LDH activity was reported on day 1 prior to the sixth injection.

Total Protein (TP): In groups 3 and 4, higher TP concentration were reported on day 1 after the first injection.

Albumin/Globulin ratio (A/G ratio): In group 4, lower A/G ratio were reported on days 1 and 3 after the first and after the sixth injection. Compared to the pre-dose values of the group 4, the A/G ratio of day 1 after the first injection had hardly changed. The lower A/G ratios were related to increasing globulin levels since the albumin concentrations were, in general, not affected and TP concentrations had increased or remained unchanged. The transient increases in TP were considered to be part of the inflammatory process upon injection of an immunostimulant.

Glucose: In group 4, higher glucose concentration was reported on day 3 after the first injection.

C-reactive protein (CRP): In groups 2, 3, and 4, a dose-dependent increase in CRP concentration was reported on days 1 and 3 after the first injection (except for group 3 on day 3), and in groups 3 and 4 on day 1 after the sixth injection. The CRP levels tended to be increased in group 4 on day 3 after the sixth injection. The increases in CRP were considered to be part of the inflammatory process upon injection of an immunostimulant.

Calcium (Ca): In groups 2 and 3, higher Ca concentration were reported on day 3 after the first injection.

Potassium (K): In group 4, higher K concentration was reported on day 3 after the first injection.

Sodium (Na): At pre-dose, a lower Na concentration in group 3 was reported.

A lower Na concentration in group 4 on days 1 and 3 after the first injection and in group 3 on day 3 after the first injection were reported. Compared to group 1 and the respective pre-dose values, the Na concentration had increased more in group 3.

Chloride (Cl): In groups 2 and 4, lower Cl concentration were reported on day 3 after the first injection.

*Compared to the saline control group, the following statistically significant changes were observed in the females of the DQ test groups:*

Aspartate alanine transferase (ALAT): At pre-dose, a lower ALAT activity in group 4 was reported.

Gamma Glutamyl transferase (GGT): A lower GGT activity in group 4 was reported on day 27 after the sixth injection.

Lactate dehydrogenase (LDH): In group 3, lower LDH activity was reported on day 1 after the injection.

Total Protein (TP): In group 4, higher TP concentration was reported on days 1 and 3 after the first injection. The transient increases in TP were considered to be part of the inflammatory process upon injection of an immunostimulant.

Albumin: A higher albumin concentration in group 4 was reported on day 1 after the first injection.



Albumin/Globulin ratio (A/G ratio): In group 4, lower A/G ratio was reported on day 3 after the first injection, day 1 prior to and day 1 after the sixth injection. In general, a dose-dependent decrease of the A/G ratio was reported in groups 2, 3, and 4 after the first and sixth injection. The individual values were within or very close to the pre-dose and/or control range or within recent historical control ranges of other studies with rabbits of this strain and age performed at (b) (4)

Therefore, the toxicological significance of these transient findings was considered negligible. The transient decreases in A/G ratio were considered to be part of the inflammatory process upon injection of an immunostimulant.

C-reactive protein (CRP): A dose-dependent increase in CRP concentration was reported in groups 2, 3, and 4 on days 1 and 3 after the first injection and on day 1 after the sixth injection in groups 3 and 4. This increase was not statistically significant in group 2, and not statistically significant in group 3 on day 3 after the first injection. On day 1 prior to the sixth injection, the CRP concentration was still increased in group 4. On day 3 after the sixth injection, the CRP levels in groups 2, 3, and 4 had returned to group 1 level. The increases in CRP were considered to be part of the inflammatory process upon injection.

Bilirubin: In groups 2 and 3, higher bilirubin concentration was reported on day 3 after the sixth injection, and in group 4 on day 27 after the sixth injection.

Calcium (Ca): In group 2, higher Ca concentration was reported on day 3 after the sixth injection.

Potassium (K): In group 4, lower K concentration was reported on day 27 after the sixth injection.

Most of the hematology and clinical chemistry findings above, were within the control range. Therefore, the toxicological significance of these incidental and transient finding was considered negligible.

### Organ weights

#### *Subgroup 1 (sacrificed 3 days after the sixth injection)*

Compared to group 1, no statistically significant changes were reported in groups 2, 3, and 4 males and females.

#### *Subgroup 2 (sacrificed 27 days after the sixth injection)*

Apart from a higher absolute and relative mean prostate weight of group 4 males and a lower relative mean brain weight of group 4 males, no other statistically significant changes were reported in groups 2, 3, and 4 males and females. Histopathology of the prostate and the brain did not reveal any treatment-related abnormalities. Therefore, no toxicological significance was attached to these changed organ weights.

### Pathology

#### Macroscopy

#### *Subgroup 1, sacrificed day 21, 3 days post sixth injection*

Macroscopic examination of the injection site at the right anterior thigh muscle at necropsy 3 days after the sixth injection revealed a hemorrhage in 3/10 group 1 animals, 2/10 group 3 animals and 4/10 group 4 animals. At the injection site of the left anterior thigh muscle a hemorrhage was reported in 3/10 group 1 animals, 1/10 group 2 animals, 1/10 group 3 animals and 2/10 group 4 animals. A hemorrhage was also reported at the injection site at the left

posterior thigh muscle (2/10 group 1 animals) and the left calf muscle (1/10 group 1 animals). The hemorrhages reported in both saline controls and DQ treated animals were considered a reaction to insertion of the injection needle.

*Subgroup 2, sacrificed day 45, 27 days post sixth injection*

At the injection sites, macroscopic examination at necropsy 27 days after the sixth injection did not reveal any gross changes. The gross changes reported in the other organs were unremarkable.

Microscopy

*Subgroup 1, sacrificed day 21, 3 days post sixth injection*

Right treated anterior thigh muscle (3 days post sixth injection):

Minimal to mild localized mononuclear inflammatory response in 1/5 group 1 males, 3/5 group 2 males, 4/5 group 3 males, 4/5 group 1 females, 4/5 group 2 females and 1/5 group 3 females were reported. The mononuclear inflammatory reaction was widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) in 1/5 group 3 males, 5/5 group 4 males, 2/5 group 3 females and 3/5 group 4 females. Mononuclear inflammatory reaction consisted of lymphocytes and small macrophages.

In 2/5 group 3 females and in 2/5 group 4 females, a widespread mixed inflammatory response was reported (besides lymphocytes and macrophages, polymorphonuclear inflammatory cells were also present). Minimal to mild hemorrhage(s) were reported at the injection site of 1/5 group 3 males, 1/5 group 4 males, 2/5 group 1 females and 2/5 group 4 females. Minimal mineralization was reported as part of the local response in 2/10 group 4 animals but also in 1/10 group 1 animals.

Left treated anterior thigh muscle (3 days post sixth injection):

Minimal to mild localized mononuclear inflammatory response in 2/5 group 1 males, 2/5 group 2 males, 1/5 group 3 males, 3/5 group 1 females, 4/5 group 2 females and 1/5 group 4 females were reported. The mononuclear inflammatory reaction was widespread (i.e. extended along the epimysium and diffusely between the muscle fibers) in 4/5 group 3 males, 5/5 group 4 males and 2/5 group 4 females. Mononuclear inflammatory reaction consisted of lymphocytes and small macrophages.

In 5/5 group 3 females and in 2/5 group 4 females a widespread mixed inflammatory response was reported (besides lymphocytes and macrophages, polymorphonuclear inflammatory cells were also present). Minimal to mild hemorrhage(s) were reported at the injection site of 1/5 group 3 males, 2/5 group 4 males, 1/5 group 1 females, 1/5 group 2 females and 1/5 group 4 females. Minimal mineralization was reported as part of the local response in 1/5 group 3 males and 1/5 group 2 females.

The histopathological changes reported in the other organs and tissues are part of background pathology. They were about equally distributed amongst the groups, or they occurred in one or a few animals only.

*Subgroup 2, sacrificed day 45, 27 days post sixth injection*

No test article-related effects were reported in subgroup 2.

**Discussion and conclusion**

No test article-related effects on clinical signs, body temperature, food consumption, ophthalmology, and organ weights were reported.

A higher mean body temperature was reported in group 4 males and females 24 hours after the first injection. In groups 2, 3, and 4 males and females, hematology revealed (either statistically significant or as a trend) a dose-dependent increase in fibrinogen after the first and after the sixth injection. Also, WBC response, consisting of a dose-dependent increase in neutrophils, predominantly on day 1 after the first injection was reported. The WBC response was considered to be part of the inflammatory process following injection of an immunostimulant. The increased fibrinogen was also considered related to the inflammatory process since it is a marker for the acute phase of tissue damage and inflammation linked to the local reaction.

In groups 2, 3, and 4 males and females, clinical chemistry revealed (either statistically significant or as a trend) a dose-dependent decrease in A/G ratio (predominantly on day 3 after the first injection) and an increase in CRP on days 1 and 3 after the first and the sixth injection. Increases in CRP are considered to be part of the inflammatory process following injection of an immunostimulant. The decreased A/G ratios were related to increasing globulin levels since the albumin levels were in general not affected and the TP concentrations were increased, predominantly in the groups 3 and 4 males and group 4 females after the first injection.

No test article-related gross changes were reported at the left and right anterior thigh muscles (injection sites) at necropsy 3 days or 27 days after the sixth injection. Three days post sixth injection, microscopic findings at the right and left anterior thigh muscle injection site were either localized or widespread mononuclear inflammatory responses. The minimal to mild localized response in groups 2, 3, and 4 animals did not exceed the minimal to mild localized response in group 1. These findings might be related to insertion of an injection needle. The response was considered to be associated with DQ treatment in the animals showing a widespread inflammatory response, either mononuclear or mixed. This was the case in 4/5 group 3 males, 5/5 group 4 males, 5/5 group 3 females and 5/5 group 4 females.

In recovery groups (twenty-seven days post sixth injection), the injection sites at the right and left anterior thigh muscle of group 4 animals did not show any test article-related histopathological changes.

In general, it can be concluded that intramuscular treatment with DQ caused mainly dose-dependent, transient effects on general clinical condition (body temperature), hematology (fibrinogen and neutrophils) and clinical chemistry (CRP and A/G ratio). These findings were considered to be related to the local inflammation or the non-specific immune stimulation following DQ injection. Microscopically, widespread inflammation was reported at the injection sites of groups 3 and 4 with complete recovery after 27 days.

**Study number 8:**

**Title and study number:** 7-Day Intravenous, Dose Range-finding Toxicity Study in (b) (4) Rats with (b) (4). Study number: 3262.4.

**Performing laboratory:** (b) (4)

**Study initiation date:** 09/29/1992

**Final report date:** 02/24/1993

**Experimental design:**

The experimental design and dosage levels tested were as follows:

Group	No of animals		Dosage Material	Dosage Level (mg/kg/day)	Final MPL® Conc. (mg/ml)	Dosage Volume (mL/kg)
	Male	Female				
1	3	3	Control <sup>a</sup>	0	0 <sup>a</sup>	4.00
2	3	3	MPL® <sup>b</sup>	0.04	0.01 <sup>b</sup>	4.00
3	3	3	MPL® <sup>c</sup>	0.20	0.05 <sup>c</sup>	4.00
4	3	3	MPL® <sup>d</sup>	1.00	0.25 <sup>d</sup>	4.00

<sup>a</sup> Prepared by diluting the control vehicle with 5% dextrose injection, USP (1:1 v/v).

<sup>b</sup> Prepared by diluting MPL® (0.5 mg/ml) with control vehicle to a concentration of 0.02 mg/ml, then further diluting with 5% dextrose injection, USP (1:1 v/v) to the final concentration of 0.01 mg/ml.

<sup>c</sup> Prepared by diluting MPL® (0.5 mg/ml) with control vehicle to a concentration of 0.10 mg/ml, then further diluting with 5% dextrose injection, USP (1:1 v/v) to a final concentration of 0.05 mg/ml.

<sup>d</sup> Prepared by diluting MPL® (0.5 mg/ml) with 5% dextrose injection, USP (1:1 v/v) to a final concentration of 0.25 mg/ml.

Table 45: Experimental design (study # 8).

(b) (4)

(b) (4)

Dosing concentration and volume: The test article was administered at dosage levels of 0.04, 0.20 and 1.00 mg/kg/day and a dose volume of 4 ml/kg

Injection sites: Lateral tail vein, once daily for seven consecutive days

Twenty-one male and twenty-one female (b) (4) rats were received at (b) (4) on September 10, 1992, from (b) (4).

Body weight range ordered: Male body weights ranged from 271-305 g and female body weights ranged from 190-212 g.

### **Results:**

No test article-related effects on clinical signs were reported.

In groups 2, 3, and 4 males and females, dose-dependent decreases/losses in mean body weight gain were reported during days 1-2. Subsequent weight gain in these groups (days 2-4 and 4-7) was comparable to or exceeded group 1 values.

In groups 2, 3, and 4 males and females, dose-dependent reductions in mean food consumption (grams/animal/day) were reported during days 1-2. Additional reductions in food consumption were reported in group 3 males and group 4 females during days 2-4 and in group 4 males during days 2-4 and 4-7.

### Hematology

In MPL<sup>®</sup> treated males, slight decreases in platelets were reported in group 4. In addition, in males of group 2, slightly higher total leukocytes, segmented neutrophils and lymphocytes were reported. However, similar changes were not reported in groups 3 and 4 males. In groups 3 and 4 females, erythrocytes, hemoglobin, and hematocrit levels decreased slightly, but in a dose-dependent manner. Similar trends toward decreased erythrocytes, hemoglobin and hematocrit were reported in group 2 females, however, these differences were only marginal and may be due to biological variation. A slight but dose related increase in segmented neutrophils was reported in groups 2, 3, and 4 females. In group 4 females, slight increases in nucleated RBCs and reticulocytes were reported. In conclusion, in groups 3 and 4 females, slight increases in the occurrence of polychromasia (slight to moderate) were reported.

### Clinical chemistry

No test article-related effects on clinical chemistry parameters were reported.

### Gross necropsy observations

Enlarged spleens were reported at necropsy in 2/6 of group 2 (2 males); 3/6 of group 3 (2 males and 1 female); and 6/6 of group 4 (3 males and 3 females) rats. 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 reported in groups 2, 3, and 4 males and females. Mean liver weights of groups 2, 3, and 4 males and females also increased as compared to controls, however, these increases did not follow any consistent dose-related pattern.

**Conclusion:**

No mortality was reported during the study. Dose dependent reductions in body weight gain and food consumption were reported in groups 2, 3, and 4 males and females during study days 1-2. Subsequent weight gain in these groups was comparable to or exceeded control values. However, additional reductions in food consumption were reported in group 3 males and in groups 3 and 4 males and females. Decreases in platelets levels was reported in group 4 males. In groups 3 and 4 females, erythrocytes, hemoglobin and hematocrit levels decreased slightly, but in a dose-dependent manner. A slight but dose-related increase in segmented neutrophils was also reported in groups 2, 3, and 4 females. No test article-related effects on clinical chemistry parameters were reported. Dose-dependent increases in absolute and relative spleen weights were reported in groups 2, 3, and 4 males and females. Mean liver weights also appeared to be increased in these groups, however, these increases did not follow any consistent dose-related pattern.

**Study number 9:**

**Title and study number:** 8-Day Intravenous Toxicity Study of MPL™ in Rats. Study number: 3262.2.

**Performing laboratory:** (b) (4)

**Study initiation date:** 10/16/1991

**Final report date:** 07/23/1992

**Route of administration:** Intravenous

**Experimental design:**

Daily intravenous injection of MPL™ was administered at levels of 0, 0.1, 1.0 or 5.0 mg/kg/day. Due to excessive treatment-related toxicity, on study day 2, the high dose level was decreased from 5.0 to 2.5 mg/kg/day. All doses were given at a constant dose volume of 5.0 ml/kg. Control rats were administered the vehicle, (b) (4) in water for injection, USP, at an equivalent dose volume. The rats were observed daily and weighed on days 1, 2 and 8. Individual food consumption was measured daily. On all study animals on the day of scheduled sacrifice, clinical pathology determinations were performed. At the time of death or sacrifice, all study animals were subjected to a complete gross necropsy. From each rat, a complete set of tissues and organs was preserved and were processed for microscopic examination.

**Results:**

Two high-dose female rats died after receiving a single 5.0 mg/kg dose of MPL™. A third high-dose male rat was sacrificed moribund on day 4 due to severe debility. These deaths were considered treatment related. One control female rat died shortly after dosing on day 8. The specific cause of this death was not determined.

At the 2.5 mg/kg/day level, the most severe changes were recognized and included decreased activity, prostration, soft stools, few feces, mucoid stools, rough coat, unkempt appearance, piloerection, fecal and urine (b) (4), dehydration, hypothermia, reddened pinna, 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 0.1 and 1.0 mg/kg/day rats. At the 1.0 mg/kg/day level, MPL™-related clinical signs included rough coat, urine (b) (4), 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 (b) (4), corneal opacity, decreased activity and lacrimation. Following dosing, both control and MPL™-treated rats exhibited a high incidence of wobbly gait. This change was associated with intravenous administration of the vehicle, 10% ethanol in 5% dextrose in water for injection, USP.

On study day 2, statistically significant, dose-dependent reductions in mean body weight gain were reported in the 0.1, 1.0 and 2.5 mg/kg/day males and females. A net loss in body weight between days 1 and 2 in all groups were reported. This is 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. On day 8, mean body weight of the 2.5 mg/kg/day males remained statistically lower than controls. During days 2-8 and in all groups, mean weight gain returned to normal levels or exceeded control values indicating that a recovery had occurred.

In males and females treated with 0.1, 1.0, and 2.5 mg/kg/day, statistically significant decreases in food consumption (g/animal/day and g/kg/day) were reported. These reductions were first reported in all groups for days 1-2 and followed a dose-related pattern in both magnitude and duration. 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 males. 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) in females. Daily food consumption returned to normal levels or exceeded control values indicating that a recovery had occurred after these periods.

## **Clinical Pathology**

### Hematology

**RBC Parameters:** In the 0.1, 1.0 and 2.5 mg/kg/day males and females, RBC count, hemoglobin and hematocrit were statistically decreased. 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 MCHC in the 2.5 mg/kg/day females.

**Platelets:** In 1.0 and 2.5 mg/kg/day males, statistically significant decreases in platelets were reported. 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:** In 2.5 mg/kg/day males, total leukocytes, segmented neutrophils, lymphocytes and monocytes were statistically increased. In 1.0 mg/kg/day males, segmented neutrophils were statistically increased. In 2.5 mg/kg/day females, total leukocytes, lymphocytes and monocytes increased but were not statistically different from the controls. In 1.0 and 2.5 mg/kg/day females, segmented neutrophils were statistically increased. Increases in slight to moderate polychromasia and anisocytosis were reported in the 2.5 mg/kg/day males and females. Similar changes in red cell morphology were also reported in the 1.0 mg/kg/day females.

### Coagulation

Fibrinogen: In 0.1, 1.0 and 2.5 mg/kg/day males and females, fibrinogen levels were slightly but statistically increased.

### Clinical Chemistry

BUN and Creatinine: In 2.5 mg/kg/day males and females, BUN was slightly but significantly increased. In the 2.5 mg/kg/day males, serum creatinine was statistically decreased as compared to controls.

Alkaline Phosphatase: In 0.1, 1.0 and 2.5 mg/kg/day males, alkaline phosphatase was statistically decreased.

Total Protein, Albumin and Globulin: In both males and females, dose-related trend toward decreased total protein was reported. 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 reported in MPL<sup>TM</sup>-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.

AST and ALT: In 1.0 and 2.5 mg/kg/day females, serum ALT levels were slightly but statistically decreased.

Glucose: In 0.1, 1.0 and 2.5 mg/kg/day females, glucose levels were statistically increased as compared to controls.

Amylase: In 2.5 mg/kg/day males, amylase was statistically decreased. 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

Enlarged spleens were reported 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).

### Organ Weights

Spleen: In 0.1, 1.0 and 2.5 mg/kg/day males and females, dose-dependent increases in absolute and relative spleen weights were reported. As compared to controls, the magnitude of the spleen weight increases ranged from approximately two-folds (0.1 mg/kg/day level) to approximately three-folds (2.5 mg/kg/day).

Liver: In 0.1, 1.0 and 2.5 mg/kg/day females, absolute and relative liver weights were increased in a dose-related fashion. In males, absolute and relative liver weights also appeared to be increased at all three MPL<sup>TM</sup> 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: In 2.5 mg/kg/day males, absolute and relative adrenal gland weights were statistically increased. In 2.5 mg/kg/day females, absolute and relative adrenal weights also appeared to be increased, however, they were not statistically different from the control group.

Thymus Gland: In 2.5 mg/kg/day females, absolute and relative thymus gland weights were slightly but statistically decreased as compared to controls.


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 increases followed no apparent dose-response pattern.



(b) (4)

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***Study number 10:***

**Title and study number:** 14-Day Intravenous Toxicity of MPL™ in Dogs. Study number: 3262.1

**Performing laboratory:** (b) (4)

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**Study initiation date:** 11/05/1991

**Final report date:** 07/23/1992

**Experimental design:**

The experimental design and dosage levels tested were as follows:

<u>Group</u>	<u>No. of Animals</u>		<u>Dosage Material</u>	<u>Dosage Level (<math>\mu\text{g/kg/day}</math>)</u>	<u>Dosage Cone. (<math>\mu\text{g/ml}</math>)</u>	<u>Dosage Volume (ml/kg)</u>
	<u>Males</u>	<u>Females</u>				
1	3	3	Vehicle	0	0.00	1.2
2	3	3	MPL"	6	5.00	1.2
3	3	3	MPL"	120	100.00	1.2
4	3	3	MPL"	1200	1000.00	1.2

Table 47: Experimental design (study # 10).

(b) (4)

(b) (4)

Table 48: Test article information (study # 10); sponsor provided

**Route of administration:** Intravenous

**Experimental design:**

Once a day for at least 14 days, animals were treated with test or control materials by slow intravenous injection via the cephalic veins. Intravenous injection of the test article was selected since it is a potential route of administration in humans.

A total of fifteen male and fifteen female beagle dogs were received at (b) (4) on August 21, 1991. This shipment of dogs was used for both the range-finding study and the 14-day study.

At study initiation, the animals were approximately eight months of age. The males weighed in the range of 8.1-12.0 kg and the females weighed in the range of 6.9-9.6 kg.

**Results:**

No test article-related effects on clinical signs, body weight, food consumption, cardiovascular changes, gross findings, and histopathological findings were reported.

Hematology

In the 120 and 1200 µg/kg/day males and females' groups, very slight to moderate reductions in platelets were reported on days 2, 8, and 15/16. However, only the day 8 platelet level of the 1200 µg/kg/day females was statistically different from the control group. The lowest platelet levels were reported in the 1200 µg/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  $\geq 170 \times 10^3/\text{cmm}$ .

In the 1200 µg/kg/day males (day 2) and females (days 2 and 8), marked increases in serum fibrinogen levels were reported. These increases were statistically significant in the female, but not the male dogs. On day 2, a possible trend toward increased fibrinogen levels was also reported in the 120 µg/kg/day males and females.

In the 1200 µg/kg/day males and females groups, marked increases in leukocytes were reported on day 2. For each sex, the leukocytosis was attributable to an increase in segmented neutrophils.

Decreased prothrombin time in the 1200 µg/kg/day males on day 3 and decreased segmented neutrophils in the 6 µg/kg/day females were reported on day 2.

Decreased phosphorus in the 1200 µg/kg/ day males on day 2, decreased AST in the 1200 µg/kg/ day females on day 8, decreased total bilirubin in the 1200 µg/kg/day females on day 16, decreased pH in the 120 µg/kg/day females on day 2, increased calcium in the 1200 µg/kg/day females on day 2, increased sodium in the 1200 µg/kg/ day females on day 16, increased pH level in the 1200 µg/kg/day females on day 8, increased albumin in the 1200 µg/kg/day females on day -7, and increased calcium in the 6 µg/kg/day females on day 2 were reported. These differences were not considered toxicologically important since they were relatively minor, did not occur in any dose-related pattern, and they did not correlate with abnormal histopathology.

#### Urinalysis

In the 120 and 1200 µg/kg/day females, urine specific gravity was statistically decreased on day 2. This change, coupled with increased urine volume in the 120 and 1200 µg/kg/ day females, was indicative of slight treatment-related hypo-osmolar diuresis.

#### Organ Weights

No test article-related effect on absolute or relative organ weights were reported. However, both absolute and relative spleen weight of the 1200 µg/kg/day males and females were increased when compared to the control group.

#### Histopathology findings

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

#### **Discussion**

No test article-related effects on clinical signs, body weight, food consumption, cardiovascular changes, gross findings, and histopathological findings were reported.

Very slight to moderate reductions in platelets were reported in the 120 and 1200 µg/kg/day males and females groups on days 2, 8 and 15/16. The lowest platelet levels were reported in the 1200 µg/kg/day males on day 8 ( $116.7 \times 10^3/\text{cmm}$ ) and day 15 ( $133.3 \times 10^3/\text{cmm}$ ). Marked increases in serum fibrinogen levels were reported in the 1200 µg/kg/day males (day 2) and females (days 2 and 8). These increases were statistically significant in the female, but not the male dogs. In the 120 µg/kg/day males and females, a possible trend toward increased fibrinogen levels was reported on day 2.

In the 1200 µg/kg/day males and females' groups, marked increases in leukocytes were reported on day 2. For both sexes, the leukocytosis was attributable to an increase in segmented neutrophils. In the 1200 µg/kg/day males and females' groups, increased absolute and relative spleen weights were also reported. In the 1200 µg/kg/day females, evaluation of urinalysis data revealed slight hypo-osmolar diuresis. In the MPL™-treated dogs, evaluation of ECG and blood pressure measurements taken on days 1 and 14 did not reveal any test article-related cardiovascular changes. Similarly, microscopic examination did not reveal any test article-related changes in the MPL™-treated dogs.

**Study number 11:**

**Title and study number:** The Acute Intraperitoneal Toxicity of Monophosphoryl Lipid A (MPLA) in Rats. Study number: (b) (4) DT127

**Performing laboratory:** (b) (4)

**Study initiation date:** 12/04/1987

**Final report date:** 07/12/1988

**Experimental design:**

The experimental design and dosage levels tested were as follows:

Treatment Group	Treatment <sup>1</sup>	Dosage <sup>2</sup> Volume (ml/kg)	Dosage (ug/kg)		TAID (Randomization) No. By Replicate	
					1	2
I	0.9% NaCl <sup>3</sup> Containing 0.2% TEA <sup>3</sup>	4	---	M	RA 71835 ( 7)	RA 71832 ( 4)
					RA 71851 (22)	RA 71849 (20)
					RA 71837 ( 9)	RA 71857 (28)
				F	RA 71870 (37)	RA 71867 (34)
					RA 71885 (52)	RA 71883 (50)
					RA 71872 (39)	RA 71891 (58)
II	0.9% NaCl Containing 0.2% TEA and 2.5 µg MPLA/ml	4	10	M	RA 71846 (17)	RA 71836 ( 8)
					RA 71840 (11)	RA 71844 (15)
					RA 71833 ( 5)	RA 71850 (21)
				F	RA 71880 (47)	RA 71871 (38)
					RA 71874 (41)	RA 71878 (45)
					RA 71868 (35)	RA 71884 (51)
III	0.9% NaCl Containing 0.2% TEA and 10 µg MPLA/ml	4	40	M	RA 71859 (30)	RA 71841 (12)
					RA 71845 (16)	RA 71842 (13)
					RA 71843 (14)	RA 71858 (29)
				F	RA 71893 (60)	RA 71875 (42)
					RA 71879 (46)	RA 71876 (43)
					RA 71877 (44)	RA 71892 (59)
IV	0.9% NaCl Containing 0.2% TEA and 100 µg MPLA/ml	4	400	M	RA 71830 ( 2)	RA 71848 (19)
					RA 71854 (25)	RA 71831 ( 3)
					RA 71838 (10)	RA 71834 ( 6)
				F	RA 71865 (32)	RA 71882 (49)
					RA 71888 (55)	RA 71866 (33)
					RA 71873 (40)	RA 71869 (36)
V	0.9% NaCl Containing 0.2% TEA and 1000 µg MPLA/ml	4	4000	M	RA 71847 (18)	RA 71856 (27)
					RA 71853 (24)	RA 71855 (26)
					RA 71829 ( 1)	RA 71852 (23)
				F	RA 71881 (48)	RA 71890 (57)
					RA 71887 (54)	RA 71889 (56)
					RA 71864 (31)	RA 71886 (53)

<sup>1</sup> Followed by 36 ml 1.5% dextrose Dianeal®/kg

<sup>2</sup> Total dosage volume of 40 ml/kg

<sup>3</sup> 0.9% NaCl is 0.9% Sodium Chloride Injection, USP and TEA is triethylamine males, F = females

Table 49: Experimental design and dosage levels (study # 11); sponsor provided

**Test article:**

Monophosphoryl Lipid A (MPLA) manufactured by (b) (4)

was used in this study. The test article was received on December 7, 1987, and

was identified with Lot No. 039-146 (no expiration date specified). The test article was kept refrigerated approximately 2-8°C; MGEC000140, Lab 1350) until prior to its reconstitution with 0.9% Sodium Chloride injection, USP containing 0.2% triethylamine.

This study was conducted using 30 male and 30 female (b) (4) rats supplied by (b) (4). Animals weighed between 212-286 g and were approximately 8-11 weeks of age at the initiation of dosing.

**Route of administration:** Intraperitoneal

**Experimental design:**

Using adult (b) (4) rats, the potential acute intraperitoneal toxicity of Monophosphoryl Lipid A (MPLA) was assessed. Single dosages of approximately 10, 40, 400, and 4000 ug per kg body weight were administered 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.

**Results:**

No test article-related effects on clinical signs, body weight, ophthalmic examination, urinalysis organ weight, and gross findings were reported.

**Hematology**

Hematology parameters didn't show toxicological significance differences. Fibrinogen, burr cells and MCH, showed random group differences but no log linear dosage-response effects were apparent.

For hemoglobin and hematocrit (both sexes), an increasing log linear dosage-response effect ( $\alpha=0.01$ ) and higher group means were reported in the test article groups relative to controls. No meaningful toxicological significance was attached to this observation because the magnitude of change was small (hgb = 5-7%; hct = 7-8%; control vs. greatest change) and not correlated with other biochemical or histopathological effects.

**Clinical Chemistry**

No toxicologically significant differences were reported for any of the clinical chemistry parameters.

There was dosage response effect ( $p < 0.01$ ) on SGPT when one extreme value (66 IU/L, RA 71890) was deleted from the group V females. Moreover, without this extreme value, group V exhibited significantly lower mean SGPT (across both sexes) than the control group and group II. The biological significance of decreasing (approximately 20%) SGPT values has not been determined. Also, no literature was found which suggested that this effect is characteristic of a pathologic condition. Thus, no toxicologic significance was attached to this observation.

For phosphorus and uric acid, additional random group differences were detected. However, there were no log linear dosage response effects apparent for these parameters.

**Histopathology**

Treatment-related changes were confined to the omentum and mesentery of males in groups IV and V, and females in group V. The changes consisted of a slight increase in the incidence and/or relative severity of interstitial infiltrations of mononuclear inflammatory cells (lymphocytes, plasma cells, macrophages) present in these tissues.

The chronic inflammatory changes in the omentum and/or mesentery for males was 1/6, 1/6, 1/6, 4/6, and 5/6 for experimental groups I through V, respectively. The incidence among females in these groups was 1/6, 1/6, 1/6, 2/6, and 4/6, respectively. Among both sexes of animals in groups IV and V, these findings revealed a dose related increase in the incidence of this lesion. The relative severity of this lesion was also higher among both sexes of the high dose group indicative of a dose-related increase in severity.

**Test article analysis**

Frozen aliquots of test article were analyzed by a (b) (4) method. Variability in sample assay results indicated that this method of analysis was inappropriate. It was concluded that no quantitative assay for MPLA existed.

**Conclusions**

The single intraperitoneal administration of MPLA, at dosages equal to the manufacturers recommended safe human dose (10 ug/kg) and the active human dose (40 ug/kg), did not produce any significant effects in treated rats. However, administration of 10X and 100X the active human dose resulted in an apparent dosage-related mononuclear cell infiltration of the omentum and mesentery. This slightly irritating effect of MPLA was not correlated with any other anatomical or clinical changes.

***Study number 12:***

**Title and study number:** Repeated Dose Toxicity and Local Tolerance Study with QS-21 and with DQ Administered Intramuscularly to Male and Female Rats. Study number: (b) (4) V20041. Performing laboratory: (b) (4)

Study initiation date: 7 June, 2011.

Final Report date: 18 October, 2011

Test article batch/lot:

<u>Test article</u>	<u>Batch number</u>	<u>Expiration date</u>
QS-21	(b) (4)	
DQ	(b) (4)	
Saline	(b) (4)	

Animal species and strain: (b) (4) rat; (b) (4):CD

Breeder/supplier: (b) (4)

Number of animals per group and sex: 6 males and 6 females

Age: Approximately 12 weeks upon arrival

Body weight range: 275-300 g for males and 225-240 g for females

Route and site of administration: Intramuscular

Volume of injection: 0.2 mL per animal (two injections of 0.1 mL per site)

Frequency of administration and study duration:

Injection sites subgroup 1: day 0, anterior thigh muscle (R and L)

Injection sites subgroup 2: day 0, posterior thigh muscle (R and L); day 4, calf muscle (R and L); day 8, posterior thigh muscle (R and L); day 12, anterior thigh muscle (R and L).

Study duration was 15 days.

Dose: See study design

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study.

Statistical analysis: Yes

GLP: Yes

Means of administration: Intramuscular

Report status: Final

## Methods:

### Experimental design:

The experimental design was as follows:

Groups	Number of animals <sup>1</sup>	Animal nos.		Treatment (intramuscular; 0.1 mL per site)	
		Males Even nos.	Females Odd nos.	Left leg	Right leg
1	6 males + 6 females	2-12	1-11	Saline	Saline
2	6 males + 6 females	14-24	13-23	QS21; 20 µg/mL	QS21; 20 µg/mL
3	6 males + 6 females	26-36	25-35	QS21; 100 µg/mL	QS21; 100 µg/mL
4	6 males + 6 females	38-48	37-47	QS21; 200 µg/mL	QS21; 200 µg/mL
5	6 males + 6 females	50-60	49-59	DQ; 20 µg/mL	DQ; 20 µg/mL
6	6 males + 6 females	62-72	61-71	DQ; 100 µg/mL	DQ; 100 µg/mL
7	6 males + 6 females	74-84	73-83	DQ; 200 µg/mL	DQ; 200 µg/mL

<sup>1</sup> Each group consisted of 2 subgroups of 3 males and 3 females each

Table 50: Experimental design (study # 12); sponsor provided

The animals were dosed intramuscularly in duplicate (right and left hind leg muscle) on a single occasion (subgroup 1) or on four occasions (subgroup 2). On each occasion, two 0.1 mL injections were given with the three test concentrations (20, 100 and 200 µg/mL) of QS-21 and with the three test concentrations (20, 100 and 200 µg/mL) of DQ. The actual dose given to the animals of the three dose groups was 4, 20 and 40 µg of QS-21 or 4, 20 and 40 µg of DQ per animal on each occasion.

The following parameters were evaluated: clinical signs (twice daily), skin reactions at the intramuscular site of injection (approximately at 3, 24 and 48 hours after each injection), body weights (subgroup 1; days -5, 1 and 3; subgroup 2; days -5, 1, 5, 9, 13, and 15), food consumption (subgroup 1; days -5 - 0, 0 - 3; subgroup 2; days -5 - 0, 0 - 7, 7 - 15), ophthalmoscopy (Not performed), body temperature (Not measured), hematology and clinical chemistry (by orbital puncture on days 1 and 13 [subgroups 1 and 2, respectively] and by



abdominal aorta on days 3 and 15 [subgroups 1 and 2, respectively]), pathology (subgroup 1 on day 3; subgroup 2 on day 15).

Tissues collected:

Tissue/organ sampled	Weighed	Examined
Adrenals	X	X
Bone and bone marrow (sternum, femur and joints)		X
Brain (3 levels, including hypothalamus) and meninges	X	X
Inguinal lymph nodes		X
Popliteal lymph nodes	X	X
Heart	X	X
Iliac lymph nodes		X
Kidneys	X	X
Liver	X	X
Lungs including larynx, trachea and bronchi	X	X
Muscle at injection sites		X
Muscle (skeletal) = triceps		X
Spleen	X	X
Thymus	X	X
Gross lesions		X
Tissue masses or tumours (if found)		X

Table 51: Tissues collected (study # 12); sponsor provided

With the preserved anterior thigh muscles three areas were processed, i.e. central and adjacent left and right areas. These areas were also examined macroscopically for gross findings. The three areas of the injection site were (b) (4), sectioned at 5 µm and (b) (4). All other tissue/organs not submitted to histopathological examination were kept in (b) (4).

### Results:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, or organ weight were reported.

### Clinical signs

Erythema reported at the injection site of one 20 µg/mL QS-21 female. Due to a punctured subcutaneous blood vessel upon injection a hematoma at the site of injection was reported in one 200 µg/mL QS-21 male and one 100 µg/mL DQ male.

### Clinical Pathology Examinations

#### Hematology

##### *Red blood cells-Males:*

Significantly lower red blood cells were reported in group 3 subgroup (S) 1 on day 1. Hemoglobin levels were significantly lower in groups 2 and 3 of subgroup 1 on day 1. Hemoglobin levels were significantly lower in groups 3 and 4 of subgroup 1 on day 3. Mean corpuscular hemoglobin (MCH) levels were significantly lower in groups 3 and 4 of subgroup 2 on day 3. Thrombocyte levels were significantly higher in group 3 of subgroup 1 on day 1. Significantly lower red blood cells were reported in group 7 subgroup 1 on day 1. Hemoglobin

levels were significantly lower in groups 6 and 7 of subgroup 1 on day 1. Packed cell volume (PCV) levels were significantly lower in group 7 of subgroups 1 on day 1. A longer APTT was reported in groups 6 and 7 of subgroup 2 on day 3 after the fourth injection.

Without a clear dose-response relationship and because the individual values were within or very close to the saline control range, the toxicological significance of these findings was considered negligible.

**Fibrinogen:** Significant, dose-dependent, increase in fibrinogen levels were reported in all QS-21 dose groups (except 20 µg/mL S2; day 3 after the fourth injection) on all sampling dates. A higher, dose-dependent, fibrinogen levels were reported in all DQ groups, but only statistically significant in group 7 of subgroups 1 on day 3 and in groups 6 and 7 of subgroup 2 on day 1 after the fourth injection.

*White blood cells-Male:*

**Absolute neutrophil count:** Dose dependent higher neutrophil levels were reported in groups 2, 3, and 4 of subgroup 1 on day 1. This increase was not significant in group 2. Dose dependent higher neutrophil levels were reported in group 4 of subgroups 2 on day 1 after the fourth injection. The neutrophil count tended to be higher in group 3 of S2 on day 1 and in groups 3 and 4 of S2 on day 3 after the fourth injection. Increases (not to significance) in neutrophil levels were reported in groups 5, 6, and 7 of S1 on days 1 and 3.

**Absolute monocyte count:** Significantly higher monocyte levels were reported in group 3 of S2 on day 1. A higher monocyte count was reported in group 7 of S1 on day 1 after injection.

The increases in neutrophils and monocytes levels were considered to be part of the inflammatory process.

*White blood cells-Females:*

**Absolute white blood cells (WBC) count:** Dose dependent higher WBC levels were reported in groups 3 and 4 of S1 on day 1 and in group 4 of S2 on day 3 after the fourth injection. Dose dependent higher WBC levels were reported in group 7 of S1 on day 1.

**Absolute eosinophil count:** A higher absolute eosinophil count was reported in group 4 of S2 on days 1 and 3 after the fourth injection. Dose dependent higher eosinophil levels were reported in groups 6 and 7 of S2 on day 1 after the fourth injection.

**Absolute neutrophil count:** Dose dependent higher neutrophil levels were reported in groups 3 and 4 of S1 and S2 on day 1 and in group 4 of S2 on day 3 after the fourth injection. Dose dependent higher neutrophil levels were reported in groups 6 and 7 of S1 on day 1. Dose dependent higher neutrophil levels were reported in groups 5 and 7 of S2 on day 1 after the fourth injection.

**Absolute monocyte count:** A higher absolute monocyte count was reported in group 4 of S2 on day 1 after the fourth injection.

The relative counts (expressed as a percentage of the total WBC count) also showed several statistically significant differences. Both absolute and relative counts indicate a dose-dependent WBC response in QS-21 males and females. The response consisted predominantly of an increase in neutrophils mainly on day 1 after the single injection (S1) and the fourth injection (S2), and in the S2 high-dose QS-21 females was accompanied by an increase in lymphocytes, eosinophils and monocytes.

In the DQ males and females the WBC response was distinctly less pronounced and mainly consisted of an increase in neutrophil and eosinophil counts in the S1 high-dose DQ females. In the DQ males only a tendency towards an increase of neutrophils was reported.

### Clinical Chemistry

#### Males:

Aspartate aminotransferase (ASAT): An increase in ASAT levels was reported in group 2 of S1 on day 1. An increase in ASAT levels was reported in groups 2 and 4 of S2 on day 1 after the fourth injection.

Gamma-glutamyl transferase (GGT): An increase in GGT levels was reported in group 3 of S1 on day 3. An increase in ASAT levels was reported in groups 2 and 4 of S2 on day 1 after the fourth injection.

Total protein (TP): Dose dependent increase in TP levels was reported in groups 3 and 4 of S1 on day 1. Dose dependent increase in TP levels was reported in groups 2, 3, and 4 of S2 on days 1 and 3 after the fourth injection. This increase was significant only in group 4 on day 3 after the fourth injection.

Albumin/Globulin ratio (A/G ratio): A lower dose-dependent A/G ratio was reported in groups 3 and 4 of S1 on day 1 after injection, and in groups 2, 3, and 4 of S2 dose groups on days 1 and 3 after the fourth injection. A lower dose-dependent A/G ratio was reported in groups 6 and 7 of S1 on day 1 after the fourth injection. A decrease in A/G ratio was reported in group 7 of S1 dose groups and in groups 5, 6, and 7 of S2.

Bilirubin: On day 1 after the injection, higher bilirubin concentration was reported in group 4 of S1. On day 1 after the injection, higher bilirubin concentration was reported in groups 5, 6, and 7 of S1.

Urea: On day 1 after the injection, lower urea concentration was reported in group 4 of S1.

Inorganic phosphate (PO<sub>4</sub>): A higher PO<sub>4</sub> concentration was reported in groups 5, 6, and 7 of S1 dose groups on day 1 after injection.

#### Females:

Aspartate aminotransferase (ALAT): An increase in ALAT levels was reported in groups 2 and 3 of S1 on day 1.

Aspartate aminotransferase (ASAT): An increase in ASAT levels was reported in groups 2, 3, and 4 of S1 and S2 on day 1.

Lactate dehydrogenase (LDH): A decrease in LDH levels was reported in group 7 of S1 on day 1.

Albumin: A decrease in albumin levels was reported in groups 2 and 4 of S1 on day 1. A decrease in albumin levels was reported in groups 2, 3, and 4 of S1 on day 3. A decrease in albumin levels was reported in groups 2, 3, and 4 of S2 on days 1 and 3 after the fourth injection. Albumin concentration tended to be lower in groups 6 and 7 of S1 and S2 on all sampling dates.

Albumin/Globulin ratio (A/G ratio): A lower dose-dependent A/G ratio was reported in groups 2, 3, and 4 of S1 on day 1 after injection and in groups 3 and 4 of S2 dose groups on day 1 after the fourth injection. The A/G ratio tended to be lower in groups 2, 3, and 4 of S1 dose groups on day 3 after injection, and in groups 2, 3, and 4 of S2 dose groups on day 3 after the fourth injection. The A/G ratio tended to be dose-dependently lower in groups 5, 6, and 7 of S1 and S2 dose groups on all sampling dates. This decrease was statistically significant in group 7 of S1 and S2 dose groups, respectively on days 1 and 3 after injection and on day 3 after the fourth injection.

Bilirubin: On day 1 after the injection, higher bilirubin concentration was reported in group 3 of S1. On day 1 after the fourth injection, higher bilirubin concentration was reported in group 2 of S2.

Urea: A higher urea concentration was reported in group 6 of S2 dose groups on day 3 after the fourth injection.

Calcium (Ca) and Sodium (Na): A lower Ca and Na concentrations were reported in group 6 of S1 dose groups on day 1 after injection.

Chloride (Cl): A higher Cl concentration was reported in group 6 of S1 on day 1 after injection. A higher Cl concentration was reported in groups 6 and 7 of S2 on day 1 after the fourth injection.

#### Organ weights

Subgroup 1 (sacrificed 3 days after single injection)

Males (QS-21 and DQ groups):

Absolute mean heart weight was increased in group 3 males. This increase was not toxicologically significant because the histopathology of the heart did not reveal any treatment-related abnormalities and no dose response relationship was present. No statistically significant changes were reported in groups 5, 6, and 7 males

QS-21 females (groups 2, 3, and 4):

Absolute and relative mean spleen weights were increased in groups 2 and 4 females. This increase was not toxicologically significant because the histopathology of the spleen did not reveal any treatment-related abnormalities.

DQ females (groups 5, 6, and 7):

Absolute mean spleen weight was increased in group 6 females. This increase was not toxicologically significant because the histopathology of the spleen did not reveal any treatment-related abnormalities.

Subgroup 2 (sacrificed 3 days after the fourth injection)

QS-21 males:

Relative mean liver weight was increased in group 2 males. This increase was not toxicologically significant because the histopathology of the liver did not reveal any treatment-related abnormalities and no dose response relationship was present.

Compared to the saline control group, no statistically significant changes were reported in the QS-21 (groups 2, 3, and 4) females, the DQ (groups 5, 6, and 7) males and the DQ (groups 5, 6, and 7) females.

#### Macroscopic examination

Subgroup 1, sacrificed on day 3, 3 days post single injection:

Brown/discolored area at the injection site was reported in most QS-21 males and females. This gross finding correlated with the microscopical findings. This finding was also reported in a single group 7 male, few DQ (groups 5, 6, and 7) females, and in most saline control females. No microscopical finding was correlated to this finding.

The gross changes reported in the other organs were unremarkable.

Subgroup 2, sacrificed on day 15, 3 days post fourth injection:

Brown/discolored area at the injection site was reported in most QS-21 males and females. This gross finding correlated with the microscopical findings. This finding was also reported in few DQ females and in a single saline control male. No microscopical finding was correlated to this finding. Discoloration of the popliteal space was reported in one group 7 male and in one group 5 female. This finding was correlated with microscopical findings. The gross changes reported in the other organs were unremarkable.

#### Microscopy

Subgroup 1, sacrificed on day 3, 3 days post single injection:

Left and right treated anterior thigh muscle (3 days post injection).

QS-21 (groups 2, 3, and 4):

Segmental muscular necrosis at the injection sites was reported in all QS-21 animals. The majority of cases were graded moderate; in a few animals the process was graded as slight or severe. The necrotic area in all animals was surrounded by a mononuclear cell infiltrate consisting of lymphocytes and small macrophages. The inflammatory process was called localized when it was confined to the necrotic area. The process will be called widespread if there was also inflammatory cell infiltration in other parts of the muscle. In most cases, the necrotic area of the muscle contained minimal hemorrhage(s) but virtually no inflammatory cells. Small group of polymorphonuclear cells in the center of the necrotic area (in addition to the mononuclear infiltrate surrounding the necrotic area) was reported in few animals. These cases were called mixed inflammatory cell infiltrates. The inflammatory reaction was relatively

mild, and it was graded slight in most cases. The inflammatory reaction was present at the endomysium, perimysium, epimysium as well as extramuscular in most animals. A dose-effect relationship was not established.

DQ:

Muscular necrosis and hemorrhage(s) at the injection sites of the DQ (groups 5, 6, and 7) animals was not reported. The histopathological changes were characterized by mononuclear cell infiltrates extending in between and along the muscle fibers and groups of muscle fibers. In most cases the infiltrates were graded slight. Dose-effect relationship was reported and shown by a shift in severity from very slight to slight as well as by a shift in distribution from localized to widespread.

The other histopathological changes reported were unremarkable.

Subgroup 2, sacrificed on day 15, 3 days post fourth injection:  
Left and right treated anterior thigh muscle (3 days post fourth injection).

QS-21 (groups 2, 3, and 4):

The histopathological changes reported at the injection sites of the S2 QS-21 animals were comparable to those reported in the S1 QS-21 animals.

DQ (groups 5, 6, and 7):

Few necrotic muscle fibers were reported in one group 6 male. No muscular necrosis and hemorrhage(s) were reported in any of the other DQ animals. The histopathological changes reported at the injection sites of the S2 DQ animals were comparable to those reported in the S1 DQ animals. Treatment related discoloration of the popliteal space, caused by a focal inflammatory infiltrate, was reported in two DQ animals.

The other histopathological changes reported were unremarkable.

Assessment

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, or organ weight were reported.

Groups 2, 3, and 4 (S1 and S2):

Dose-dependent increase in fibrinogen on days 1 and 3 after the injection, and a dose-dependent WBC response, (mainly consisting of an increase in neutrophils predominantly on day 1 after injection) was reported. The increase in fibrinogen levels and the WBC response was considered to be part of the inflammatory process following injection. Dose-dependent increase in TP concentration on day 1 after injection and a decreased A/G ratio predominantly on day 1 after injection was reported in groups 2, 3, and 4 males. Increase in ASAT activity on day 1 after injection, a lower albumin concentration and a dose-dependent decreased A/G ratio predominantly on day 1 after injection were reported in groups 2, 3, and 4 females. The increased ASAT was consecutive to the muscular necrosis since it is present in high concentrations in skeletal muscle and its level in blood can be elevated after muscle injury.

Microscopic examination of the injection sites revealed histopathological changes characterized by a distinct area of segmental muscular necrosis with minimal hemorrhages, surrounded by a relatively mild mononuclear inflammatory cell infiltrate. There was no clear dose-effect relationship.

Groups 5, 6, and 7 (S1 and S2):

Dose-dependent increase in fibrinogen and WBC response, predominantly on day 1 after injection, was reported in groups 5, 6, and 7 males and females. The WBC response was considered to be part of the inflammatory process following injection. The increased fibrinogen was also considered related to the inflammatory process since it is a marker for the acute phase of tissue damage and inflammation linked to the local reaction. Dose-dependent decrease in A/G ratio (on days 1 and 3 after injection) and decreased albumin concentration was reported in groups 5, 6, and 7 females.

Microscopic examination of the injection sites revealed histopathological changes characterized by a mild, mononuclear inflammatory cell infiltrate extending in between the muscle tissue. There was a clear dose-effect relationship shown by a shift in severity from very slight to slight as well as by a shift in distribution from localized to widespread.

In conclusion, QS-21 was distinctly more reactive than DQ when considering the response on fibrinogen, neutrophils, A/G ratio and especially ASAT. The same degree of local reactogenicity was shown after single or multiple injections of test materials.

### **Repeat-Dose Studies**

Repeated-Dose Toxicity Study with DQ administered Intramuscularly to Male and Female Rats: In this study, 15 animals/sex/group will receive either saline, 20 µg/mL DQ, 100 µg/mL DQ or 200 µg/mL DQ intramuscularly in the left and right legs at a volume of 0.1 mL. Each group will be divided into a subgroup of 10 males and 10 females. The saline control group and the high-dose DQ group will have a second subgroup of 5 males and 5 females. The animals of S1 will be sacrificed on study day 21 (3 days post-6th injection) and those of S2 on study day 46 (28 days post-6th injection). Animals will be injected on day 0, posterior thigh muscle; day 4, calf muscle; day 7, posterior thigh muscle; day 11, calf muscle; day 14, posterior thigh muscle; and day 18, anterior thigh muscle. Cage-side observations will be performed twice daily, and a full physical examination will be carried out in case of abnormal clinical signs. Injection sites will be observed approximately 3, 24 and 48 hours after injection and skin effects, if occurring, will be graded according to the method of Draize (erythema, eschar formation and edema formation). Ophthalmoscopy observations will be conducted on 10 animals/sex/group pre-dose and on days 19 and 42 of the study. All visible structures of the eyes (cornea, eye chambers, iris, lens, vitreous body and the fundus) will be examined. Body temperature will be recorded prior to and circa 4 and 24 hours after the first and the sixth injection (subgroup 1 only) via subcutaneous temperature.

**Reproductive Toxicology Studies Reviews:**

**Study #1: Zoster Candidate Vaccine (gE/AS01B): Study of Effects on Embryo-Fetal, Pre- and Post-natal Development in CD Rats by Intramuscular Administration (Including Pre-Mating Immunization Phase). Study number: (b) (4)**

Reviewer: Claudia Wrzesinski

**Summary:**

Female CD rats with were treated with either saline or the candidate vaccine gE/AS01B or adjuvant AS01B at 40% of the full human dose per occasion, on 28 and 14 days before pairing and then on days 3, 8, 11 and 15 of gestation and on day 7 of lactation. The vaccine was well tolerated by the F0 females with effects restricted to slight, transient swelling at the injection site and did not adversely affect embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to day 25 of age.

**Study no.:** (b) (4)

**Conducting laboratory and location:** GlaxoSmithKline Biologicals S A

**Date of study initiation:** 18 May 2010

**GLP compliance:** yes

**QA reports:** yes

**Drug, lot #, and % purity:**

**gE:** Batch number: (b) (4) (Monodose vial containing 62.5 µg of freeze-dried gE antigen per cake)

**AS01B:** Batch number: (b) (4) (700 µl monodose vial containing 50 µg QS-21 and 50 µg MPL in a liposome-based formulation per 500 µl)

**Doses:**

**AS01B:** 200µl (2 x 100 µl) was injected per rat, equivalent to two fifths of a human dose.

**gE/AS01B:** 200 µl (2 x 100 µl) was injected per rat, equivalent to two fifths of a human dose.

Group	Treatment	Dose (volume/occasion)	Treatment day	Number of females ‡	Animal numbers
1	Saline	200 µl	Day -28 and -14 before pairing, then Days 3, 8, 11, 15 after mating and Day 7 of lactation	44	1-44
2	AS01B	200 µl	Day -28 and -14 before pairing, then Days 3, 8, 11, 15 after mating and Day 7 of lactation	44	45-88
3	gE/AS01B	200 µl	Day -28 and -14 before pairing, then Days 3, 8, 11, 15 after mating and Day 7 of lactation	44	89-132

‡ 44 animals per group were allocated and treated, in order to obtain 40 females with a positive indication of mating. 40 females per group were treated during gestation and 20 females per group were treated during lactation.

Table 52: Experimental design in study (b) (4) (repro tox study # 1); sponsor provided

**Species/strain:** (b) (4):CD (b) (4) rats

**Number/sex/group:** 40 females per dosage group



**Route, formulation, volume, and infusion rate:** Intramuscular

**Study design:** Three groups of 44 female rats were allocated initially to study and treated on days -28 and -14 (before pairing). The females were paired with stock males of the same strain. Forty females in each group with a positive indication of mating were treated on days 3, 8, 11 and 15 after mating and the excess animals were killed on day 6 or 7 after mating. For each group, 20 animals were killed on day 20 after mating (embryo-fetal phase) and the remaining 20 animals were allowed to litter and rear their young to day 25 of age (postnatal phase). Females in the postnatal phase were then treated on day 7 of lactation. The F1 offspring received no direct administration of the test substances: any exposure was in utero or via the milk.

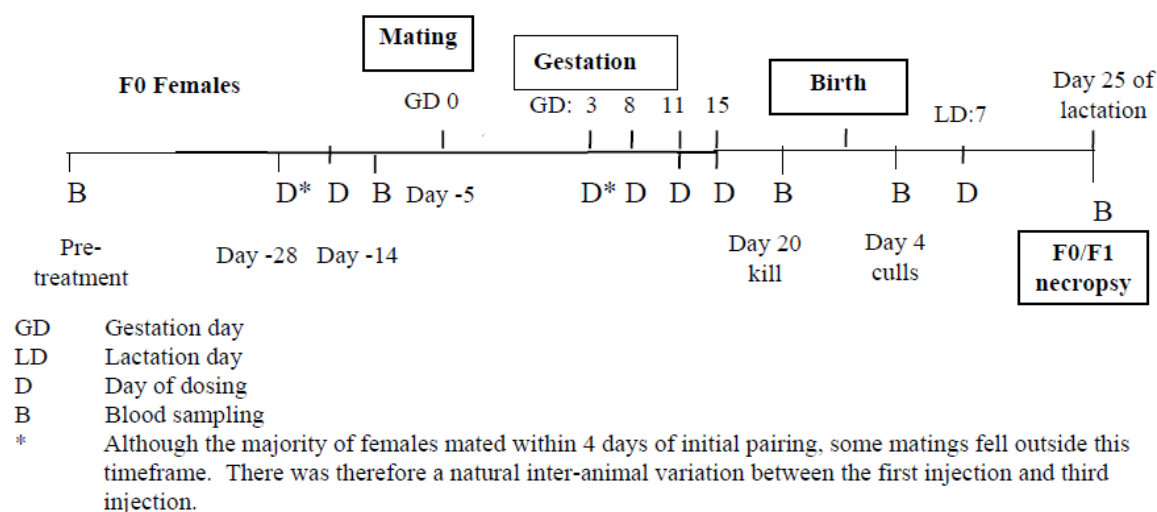


Figure 8: Study design study (b) (4) (repro tox study # 1); sponsor provided

**Parameters and endpoints evaluated:**

**Clinical observations:** Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. At each treatment, detailed observations were recorded at the following times in relation to dose administration:

- Immediately before dosing including injection sites
- Immediately after dosing on return of the animal to its cage
- On completion of dosing of each group
- Between one and two hours after completion of dosing of all groups
- As late as possible in the working day

Injection sites were examined on each day of dosing, until 4 days after the injection and weekly thereafter and at termination. In addition, a more detailed physical examination was performed on each F0 animal weekly before pairing, on days 0, 6, 13 and 20 after mating and days 1, 7, 14, 21 and 25 of lactation to monitor general health.

**Body weight:** The weight of each F0 female was recorded on the day of treatment and weekly until mating was detected, on Days 0, 3, 6, 8, 11, 15, 17 and 20 after mating, daily until parturition, and on days 1, 4, 7, 11, 14, 18, 21 and 25 of lactation.

**Food consumption:** For each F0 female was recorded weekly before pairing, for the periods days 0-2, 3-5, 6-7, 8-10, 11-14, 15-16 and 17-19 after mating and on days 1-3, 4-6, 7-10, 11-13, 14-17, 18-20 and 21-24 of lactation. From these records the mean weekly consumption (g/animal/week) before pairing and daily consumption (g/animal/day) after mating and during lactation was calculated for each animal.

**Parturition observations and gestation length:** From day 20 after mating, females were inspected three times daily for evidence of parturition. The progress and completion of parturition was monitored, numbers of live and dead offspring were recorded, and any difficulties observed were noted. The duration of gestation was calculated as the time elapsing between the detection of mating and commencement of parturition.

**Records made during littering phase:** All litters were examined at approximately 24 hours after birth (day 1 of age) and then daily thereafter. The records maintained were as follows:

- **Clinical signs:** Daily records were maintained for evidence of ill health or reaction to treatment; these were on an individual offspring basis or for the litter as a whole, as appropriate.
- **Litter size:** Daily records were maintained of mortality and consequent changes in litter size from days 1-25 of age. On day 4 of age, litters containing more than ten offspring were reduced to ten by random culling, leaving, whenever possible, five male and five female offspring in each litter.
- **Sex ratio:** The sex ratio of each litter was recorded on days 1, 4 (before and after culling) and on day 25 of age.
- **Bodyweight:** Individual offspring bodyweights were recorded on days 1, 4 (before culling), 7, 11, 14, 18, 21 and 25 of age.

**Pre-weaning examination:**

The following pre-weaning reflex developmental tests were performed on each offspring:

- **Surface righting:** Assessed daily from day 2 of age until achieved.
- **Air righting:** Assessed daily from day 16 of age until achieved but not beyond day 21 of age.
- **Auditory function:** The startle response to a sudden sharp sound was assessed on day 20 of age.
- **Visual function:** The pupil closure response of dark-adapted eyes to a bright point source of light was assessed on day 20 of age.

**Biosampling (antibody assay):**

- Blood samples were obtained from the F0 females pretreatment, on day -5 before pairing, day 20 after mating (embryo-fetal phase) and day 25 of lactation (postnatal phase).
- Umbilical cord blood samples were obtained from the fetuses in 10 litters per group on day 20 of gestation.
- Blood samples were obtained from all offspring killed on day 4 of age and up to 3 male and 3 female offspring per litter on day 25 of age.

**Necropsy and fetal processing:**

- F0 animals allocated to the embryo-fetal and postnatal phases of the study were subject to a detailed necropsy.
- **Animals allocated to the embryo fetal phase:** 20 per group were killed on day 20 after mating. For each animal, the number of corpora lutea in each ovary and the number of implantation sites, the number and distribution of resorption sites (classified as early or late) and live and dead fetuses were recorded for each uterine horn. For apparently non-pregnant animals, and for apparently empty uterine horns, the number of uterine implantation sites was checked after (b) (4)

Fetal examination and processing: All fetuses and placentae were dissected from the uterus and weighed individually. Fetuses were individually identified within the litter, using a coding system based on their position in the uterus. Each fetus and placenta were externally examined, and any abnormalities were recorded. Free-hand serial sections were prepared from the Bouin's fixed fetuses and were examined under the microscope for visceral abnormalities.

- **Animals allocated to the postnatal phase:** 20 were allowed to litter and rear their young to day 25 of age. The F0 females and remaining F1 offspring were killed on day 25 of lactation.
- **F0 females:** For F0 females, the numbers of implantation sites in each uterine horn were counted. For females failing to produce a viable litter, the number of uterine implantation sites was checked after (b) (4).
- **F1 offspring:** Offspring culled on day 4 of age which were externally abnormal were examined at necropsy. The offspring considered to be externally normal were discarded without examination

**Reproductive assessment:**

Individual values are presented for the numbers of corpora lutea, implantations, resorptions (early, late and total), live fetuses (male, female, total) and sex ratio (percentage male) for litters at day 20 of gestation.

Pre-natal losses are separated into pre- and post-implantation phases. Pre-implantation loss includes losses due to non-fertilization of ova, in addition to post-fertilization losses before implantation. It was calculated from the formula:

Pre-implantation loss (%) = (Number of corpora lutea – Number of implantations)/Number of corpora lutea x 100

Where the number of implantations exceeded the number of corpora lutea observed, pre-implantation loss was assumed to be zero (i.e. no pre-implantation loss was considered to have occurred).

Post-implantation loss was considered to exclude the first two to three days post-implantation as deaths occurring at this stage are considered to leave no remains visible at day 20 of gestation. It was calculated from the formula:

Post-implantation loss (%) = (Number of implantations – Number of live fetuses)/ Number of implantations x 100

All group values and SD (as appropriate) were calculated from the individual litter values

**Fetal, litter and placental weights:** Mean fetal weights were calculated for each litter. Values were presented for male, female, and overall fetal weight. Litter weight was calculated as the sum of all fetal weights. Mean placental weight was also calculated for each litter.

**Detailed fetal examination:**

Findings from external, visceral and skeletal examination of fetuses are presented on an individual basis for affected litters and fetuses, linking the results of initial external examinations with subsequent visceral and/or skeletal examinations and fetal weight. Group incidences of observations on fetuses and litters are summarized in terms of major or minor abnormalities or as skeletal variants. The incidence of structural changes is presented as numeric fetal and litter incidences.

**Findings observed were classified, according to severity and incidence, as:**

**Major abnormalities:** Normally rare, definitely detrimental to normal subsequent development, possibly lethal, e.g. ventricular septal defect

**Minor abnormalities:** Minor differences from normal that are detected relatively frequently considered to have little detrimental effect and may be a transient stage in development e.g. bipartite centrum, dilated ureter.

**Variants:** Alternative structures or stages of development occurring regularly in the control population, e.g. number of ribs, incomplete ossification of 5<sup>th</sup> and 6<sup>th</sup> sternbrae.

**Pre-coital interval:** Individual intervals were tabulated for females only, for the time elapsing between initial pairing and mating. Percentage of females with pre-coital intervals were calculated for durations of 1-4, 5-8, 9-12 or 13-14 days of pairing.

**Gestation length:**

Gestation length was calculated as the number of gestation days up to and including the day on which offspring were first observed, with day 1 = day of mating for calculation purposes.

**Gestation index:**

Gestation index (%) = Number of live litters born/Number pregnant x 100

**Mating performance and fertility:**

Individual data was tabulated. Group values were calculated for males and females separately for the following:

Percentage mating: Number of animals mating/ Animals paired x100

Conception rate (%): Number of animals achieving pregnancy/ Animals mated x100

Fertility index (%): Number of animals achieving pregnancy/ Animals paired x100

**Litter size:** Individual litter values were tabulated for the number of implantation sites, total at day 1 after littering (live and dead) and live at days 1, 4 (before and after culling), 7, 11, 14, 18, 21 and 25 of age. Group mean litter size and SD were calculated from the individual litter values.

**Survival indices:**

The following were calculated for each litter:

Post-implantation survival index (%) = Total number of offspring born/ Total number of uterine implantation sites x 100

Post-implantation survival index was expressed as 100% where the number of offspring exceeded the number of implantation sites recorded.

Live birth index (%) = Number of live offspring on day 1 after littering/ Total number of offspring born x 100

Viability index (%) = Number of live offspring on day 4 before culling/Number live offspring on day 1 after littering x 100

Lactation index (%) = Number of live offspring on day of examination/Number live offspring on day 4 (after culling) x 100

**Sex ratio:**

The percentage of male offspring in each litter was calculated at day 1 after littering (live and dead), and for live offspring on days 1, 4 (before and after culling) and 25 of age.

Percentage males = Number of males in litter/ Total number of offspring in litter x 100

**Pre-weaning examinations:** Surface and righting reflexes were presented as the age that the reflexes were observed. Auditory and visual functions were presented as percentage passing the test. Group mean values were calculated from the individual values presented.

**Macroscopic findings:**

The individual findings for F0 animals have been presented in an Appendix (only females/litters with findings are presented). Findings from examination of offspring are presented in an Appendix on an individual basis for affected litters and offspring before and at scheduled termination.

**Statistical methods**

Statistical analyses were performed on the majority of data presented and results of these tests, whether significant or non-significant, are presented on the relevant tables. For some parameters, including mating performance, the similarity of the data was such that analyses were not considered to be necessary.

All statistical analyses were carried out separately for males and females. Data relating to

food consumption was analyzed on a cage basis. For all other adult parameters, the analyses were carried out using the individual animal as the basic experimental unit. For litter findings the litter was taken as the treated unit and the basis for statistical analysis.

The following data types were analyzed at each time point separately:

Bodyweight, using absolute weights and gains over appropriate study periods

Food consumption, using means over appropriate study periods

Litter size and survival indices

Fetal, placental and litter weight

Pre-weaning examinations

Mating performance and fertility

The following sequence of statistical tests was used for bodyweight, food consumption, litter size and survival indices, fetal, placental and litter weight and pre-weaning examinations data:

A parametric analysis was performed if Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level. Groups were compared using t-tests.

A non-parametric analysis was performed if Bartlett's test was still significant at the 1% level following both logarithmic and square-root transformations. Groups were compared using Wilcoxon rank sum test.

For survival indices and live fetuses, if 75% of the data (across all groups) were the same value, for example c, Fisher's Exact tests were performed. Treatment groups were compared using pairwise comparisons of each dose group against the control both for i) values  $<c$  versus values  $\geq c$ , and for ii) values  $\leq c$  versus values  $>c$ , as applicable.

Pre- and post-implantation losses were analyzed by generalized mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test. For resorptions, each treated group was compared to control by exact Wilcoxon rank sum test.

Sex ratio were analyzed by generalized mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test. The numerator was Number of males, the denominator was Number of live fetuses.

Significant differences between Control and treated groups were expressed at the 5% ( $p<0.05$ ) or 1% ( $p<0.01$ ) level.

## Results:

### F0 Generation

Mortality/Clinical signs:

Parameter		F0 generation		
		Control	AS01B	gE/AS01B
Injection site observation (summary of all events)	Swelling-both	0	13	34
	Swelling-left	0	16	25

Parameter	F0 generation		
	Control	AS01B	gE/AS01B
Swelling right	0	11	28
Right hind limb elevated	0	3	0
Appearance	unaffected	unaffected	unaffected
Abnormal Stool	ND	ND	ND
Mortality (fetuses dying before scheduled termination)	4	0	4
Neurotoxicity	ND	ND	ND

ND: not determined

Table 53: Mortality and clinical signs (study **b) (4)** ) (repro tox study # 1).

During the pre-pairing phase, after administration on day -28 and day -14, there were no signs that could be related to dosing. During gestation animals were dosed on 4 occasions (days 3, 8, 11 and 15 after mating), signs observed in association with dosing were limited to transient swelling at the injection sites for animals receiving the adjuvant and the whole vaccine and the incidence of signs was slightly higher for animals receiving gE/AS01B. Two animals receiving AS01B had limited use of the right hindlimb on days 5 and/or 6 of gestation after dose administration on day 3 but this sign did not persist. Following the final dose on day 7 of lactation there was a very low incidence of swelling at the injection site on day 8 of lactation for animals that received AS01B or gE/AS01B.

#### **Body weight:**

***Observations during pre-pairing period (female):*** Overall there was no effect on bodyweight gain during the 4-week pre-pairing period.

***Observations during Gestation (female):*** Bodyweight change during gestation showed no clear effect of treatment with either AS01B or gE/AS01B. Overall (days 0-20 of gestation) the mean bodyweight gain for females receiving AS01B was slightly but significantly lower when compared with the controls and bodyweight gain for females receiving gE/AS01B was slightly but significantly higher when compared with females receiving AS01B.

***Observations during Lactation (female):*** Bodyweight gain during lactation was unaffected by treatment with either AS01B or gE/AS01B.

**Food consumption:** Food consumption measurement during the four-week pre-pairing phase, during gestation and during lactation showed no adverse effect of treatment with either AS01B or gE/AS01B.

There were incidences of statistical differences relative to the control group and the adjuvant group, however the differences were slight. During the pre-pairing phase and the gestation phase animals receiving AS01B tended to show slightly lower food consumption compared to the controls; however, the group receiving gE/AS01B had mean food consumption that was similar to the controls and slightly higher when compared with females receiving AS01B. These differences were not considered to be of toxicological significance

During lactation food consumption was essentially similar amongst the groups until late lactation (days 21-25) when both treated groups had food consumption that was significantly higher when compared with the controls. At this stage of lactation, the majority of the consumption can be attributed to the offspring and as such this is unlikely to relate to administration of either the adjuvant or vaccine.

### **Necropsy:**

### **Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):**

#### **Reproductive parameters examined (in F<sub>0</sub> animals), natural birth group:**

Mating performance and fertility: Mating performance and fertility was unaffected by treatment with either AS01B or gE/AS01B.

Parameter	F0 generation		
	Control	AS01B	gE/AS01B
Female Fertility Index (both groups)	98%	100%	98%
Gestation Index (natural birth group)	100%	100%	100%
Gestation Length 22 days	55%	53%	55%
(natural birth group) 22.5 days	25%	20%	26%
23 days	20%	25%	21%
Post Implantation Survival Index	93.7%	89.0%	95.3%
Live Birth Index (natural birth group)	99.0%	98.8%	98.7%
Viability Index	100%	100%	99.5%
Lactation Index day 7	100%	100%	99.5%
day 14	99.5%	100%	99.5%
day 21	99.5%	100%	99.5%
Number (Total and Per Liter) of Stillbirths at Day 0	ND	ND	ND
Natural Birth: Number (Total and Per Liter) of Live Births at Day 0	(294/14.7)	(253/12.7**)	(275/14.5)
Implantation Loss pre	4.7%	5.8%	3.4%
	post	4.6%	5.0%
Sex Ration (5M)	44.0%	50.8%	53.9%*
Number of Corpora Lutea	15.8%	16.1%	15.7%
Number of Implantation Sites	15.7	14.2**	15.2
Number of Resorptions Early	0.6	0.8	0.4
Late	0.1	0	0.1
Total	0.7	0.8	0.5

Table 54: Reproductive parameters (study (b) (4) ), natural birth group (repro tox study # 1); sponsor provided. ND: Not determined; \*p<0.05; \*\*p<0.01

The gestation length was within the normal range of 22 to 23½ days and there was no evidence for a treatment-related shift in the distribution of gestation lengths. The gestation index was unaffected by treatment, with all pregnant females producing live litters.



**Results F1 generation (natural birth group)****Reproductive parameters:**

GENERATION		F <sub>1</sub> LITTER		
		Control	AS01B	gE/AS01B
LITTER SIZE				
Number Born Day 1– Total <sup>1</sup> Per Litter	N MEAN (S.D.)	14.7 (1.5)	12.7** (1.9)	14.5 (1.5)
Day 1 – Total Per Litter	N MEAN (S.D.)	14.6 (1.6)	12.5** (1.9)	14.3 (1.7)
Day 4 (before culling) – Total Per Litter	N MEAN (S.D.)	14.6 (1.5)	12.5** (1.9)	14.3 (1.7)
Day 7 – Total <sup>2</sup> Per Litter	N MEAN (S.D.)	10.0 (0.0)	9.9 (0.4)	9.9 (10.0)
Day 14 – Total <sup>2</sup> Per Litter	N MEAN (S.D.)	10.0 (0.4)	9.9 (0.2)	9.9 (0.2)
Day 21 – Total <sup>2</sup> Per Litter	N MEAN (S.D.)	10.0 (0.2)	9.9 (0.4)	9.90 (0.2)
LITTER WEIGHT IN G (♂/♀)				
Day 1	N MEAN [S.D.]	(6.7/6.4) [0.5/0.6]	(7.1/6.7) [0.7/0.7]	(6.9/6.6) [0.5/0.5]
Day 4 (before cull)	N MEAN [S.D.]	(9.6/9.0) [0.8/0.8]	(10.3*/9.9) [1.1/1.1]	(9.8/9.4) [1.0/0.9]
Day 7	N MEAN [S.D.]	(15.9/15.0) [1.2/1.2]	(16.5/15.8) [1.4/1.5]	(16.1/15.3) [1.2/1.8]
Day 14	N MEAN [S.D.]	(32.1/30.7) [2.0/2.0]	(32.5/31.4) [2.0/2.2]	(32.1/30.7) [1.5/2.0]
Day 21	N MEAN [S.D.]	(50.6/48.3) [3.4/3.5]	(52.7/50.3) [4.5/4.4]	(50.9/48.6) [3.8/4.1]
VIABILITY INDICES				
Day 1-4		99.7%	100%	100%
Day 4-25		99.5%	100%	99.5%
WEANING INDEX	N MEAN S.D.	ND	ND	ND
SEX RATIO (M%)	Day 1	44.0%	50.8%	53.9%*

Table 55: Fertility parameter (study (b) (4) ) (repro tox study # 1); sponsor provided  
Includes: 1) Offspring that died prior to the designated day 1 of age, 2) Culling: On day 4 of age, litters containing more than ten offspring were reduced to ten by random culling, leaving, whenever possible, five male and five female offspring in each litter; ND: Not determined;  
\*p<0.05; \*\*p<0.01

The number of implantations, and litter size was slightly but significantly lower for the group receiving adjuvant AS01B alone compared to the control group. However, animals that received the complete vaccine gE/AS01B were similar to the control group. Further, the study report stated that this difference in implantation counts in the adjuvant alone group was not observed in females allocated to the embryo-fetal phase. When the number of implantations for both phases

are combined there is no significant difference of implantation sites between the treatment groups (mean for control group: 15.4, for AS01B group: 14.6; for gE/AS01B group: 15.1).

Number of implantations	Number of animals			Percentage of animals		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
	Control	AS01B	gE/AS01B	Control	AS01B	gE/AS01B
12	0	4	1	0	10	3
13	2	8	3	5	20	8
14	7	7	10	18	18	26
15	12	9	11	31	23	28
16	11	5	7	28	13	18
17	5	5	4	13	13	10
18	1	2	2	3	5	5
19	1	0	1	3	0	3

Table 56: Overall number of implantation sites for both combined embryo-fetal and post-natal phase (repro tox study # 1); sponsor provided

The sex ratio (% male pups) in the gE/AS01B group was significantly higher than the saline control group, but neither sex ratio was grossly different from the expected 50% male and the apparent effect was partially due to a low control value and was considered to be of no toxicological importance.

Offspring bodyweight on day 1 of age and subsequent bodyweight gain up to day 25 of age showed no adverse effects of maternal treatment with either AS01B or gE/AS01B.

#### Fetal alterations: F<sub>1</sub> generation

			Control	AS01B	gE/AS01B
Litters evaluated		N	20	20	20
Fetuses evaluated		N	139	144	146
<b>Minor skeletal abnormalities</b>					
Cranial: structural bone/additional suture	Litter incidence	N	1	1	1
	Fetal incidence	N	1	1	1
Cranial: interparietal fissure	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Cranial: unossified areas	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Vertebral abnormality (thoracic)	Litter incidence	N	0	0	1
	Fetal incidence	N	0	0	1
Sternebrae misaligned ossification site	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Cervical rib short supernumerary	Litter incidence	N	1	2	5
	Fetal incidence	N	1	2	2
13 <sup>th</sup> rib short	Litter incidence	N	1	2	2
	Fetal incidence	N	1	2	2

			Control	AS01B	gE/AS01B
Litters evaluated		N	20	20	20
Fetuses evaluated		N	139	144	146
Number of ribs 13/14 or 14/14	Litter incidence	N	12	6	<b>20</b>
	Fetal incidence	N	7	3	9
14 <sup>th</sup> rib full supernumerary	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Thoracolumbar vertebrae 18	Litter incidence	N	0	0	1
	Fetal incidence	N	0	0	1
Thoracolumbar vertebrae 20	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
<b>Incomplete ossification/unossified</b>					
Head/neck: cranial centers	Litter incidence	N	8	9	17
	Fetal incidence	N	5	6	8
Head/neck: hyoid	Litter incidence	N	11	12	15
	Fetal incidence	N	5	8	7
Head/neck: presphenoid	Litter incidence	N	0	0	2
	Fetal incidence	N	0	0	1
Vertebrae: cervical	Litter incidence	N	0	2	4
	Fetal incidence	N	0	1	2
Vertebrae: thoracic	Litter incidence	N	8	7	6
	Fetal incidence	N	4	6	4
Vertebrae: lumbar	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Vertebrae: sacrocaudal	Litter incidence	N	8	8	15
	Fetal incidence	N	5	3	5
Sternebrae: 5 <sup>th</sup> and/or 6 <sup>th</sup>	Litter incidence	N	74	87	86
	Fetal incidence	N	18	18	19
Sternebrae total	Litter incidence	N	74	90	87
	Fetal incidence	N	18	19	19
Sternebrae other	Litter incidence	N	4	15	9
	Fetal incidence	N	4	8	5
Gridles pelvic bones	Litter incidence	N	6	5	12
	Fetal incidence	N	5	3	5
Limbs metacarpal	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs metatarsal	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Limbs long bones	Litter incidence	N	0	1	3
	Fetal incidence	N	0	1	2
Precocious ossification cervical vertebral centra more than 4 ossified	Litter incidence	N	1	2	0
	Fetal incidence	N	1	2	0
<b>Minor visceral abnormality</b>					
Lens variation in shape	Litter incidence	N	2	1	0
	Fetal incidence	N	1	1	0
Thyroid absent lobe	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Thymus partially undescended lobe	Litter incidence	N	1	2	2
	Fetal incidence	N	1	2	2
Thinning of diaphragm with liver protrusion	Litter incidence	N	0	0	2
	Fetal incidence	N	0	0	2
	Litter incidence	N	0	0	4

			Control	AS01B	gE/AS01B
Litters evaluated		N	20	20	20
Fetuses evaluated		N	139	144	146
Liver bilobed/fissured posterior caudate lobe folded posterior caudate lobe,	Fetal incidence	N	0	0	3
Kidney small renal papilla	Litter incidence	N	0	2	1
	Fetal incidence	N	0	1	1
Ureter dilated	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Testis displaced	Litter incidence	N	2	5	3
	Fetal incidence	N	2	3	3
Umbilical artery left	Litter incidence	N	1	0	0
	Fetal incidence	N	1	0	0
Hemorrhages head brain	Litter incidence	N	4	7	7
	Fetal incidence	N	4	6	5
Hemorrhages head aqueous/vitreous humor eye	Litter incidence	N	4	7	7
	Fetal incidence	N	4	6	5
Hemorrhages neck/thorax intra-thoracic	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Hemorrhage abdominal cavity	Litter incidence	N	11	13	7
	Fetal incidence	N	7	8	7
Hemorrhage liver lobes	Litter incidence	N	4	3	2
	Fetal incidence	N	3	3	2
Hemorrhage general subcutaneous	Litter incidence	N	0	4	0
	Fetal incidence	N	0	2	0
<b>Major abnormality findings</b>					
Heart and Major vessels	Litter incidence	N	0	1	0
Membranous ventricular septal defect	Fetal incidence	N	0	1	0
Limbs and Girdles: Short scapula(e)	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short pelvic bones	Litter incidence	N	0	2	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short/thickened long bones	Litter incidence	N	0	1	0
	Fetal incidence	N	0	1	0
Limbs and Girdles: Short long bones	Litter incidence	N	0	2	0
	Fetal incidence	N	0	1	0

Table 57: Fetal alterations (study (b) (4) ) (repro tox study # 1); sponsor provided Caesarian delivered live fetuses; F1 generation; <sup>a</sup> Excludes values for fetus #10398-7 and #10398-12 only the heads had been examined at the tissue examination (appeared normal)

The incidence of major and minor abnormalities and skeletal variants did not show any relationship to treatment. In the group that received AS01B there were three fetuses in two litters with shortened/thickened/bent scapula(e), long bones and associated short pelvic bones but this was within historical control data range. In the group that received gE/ AS01B there appeared to be a slightly higher incidence of fetuses with 14 ribs, compared with concurrent Control, this was within historical control data range, although on the high end of it. The group receiving gE/ AS01B also showed a slightly higher incidence of unossified gridles pelvic bones, head/neck cranial centers and sacrocaudal vertebrae, but these changes were not considered to be of toxicological relevance.

**Pre-weaning examination:**

The following pre-weaning reflex developmental tests were performed on each offspring:

**Surface righting:** Assessed daily from day 2 of age until achieved.

**Air righting:** Assessed daily from day 16 of age until achieved but not beyond day 21 of age.

**Auditory function:** The startle response to a sudden sharp sound was assessed on day 20 of age.

**Visual function:** The pupil closure response of dark-adapted eyes to a bright point source of light was assessed on day 20 of age.

Pre-weaning examinations F1				
		Control	AS01B	gE/AS01B
Surface righting	Days of age	3.5	3.5	3.8
Air righting	Days of age	17.0	16.9	17.2
Pupil reflex	% passed	100%	100%	100%
Startle response	% passed	100%	100%	100%

Table 58: Pre-weaning examinations -group values for offspring (F1) (study (b) (4)) (repro tox study # 1); sponsor provided

Age of attainment for air and surface righting, the pupil reflex and startle response were unaffected by maternal treatment.

Macropathology of offspring killed/dying before scheduled termination		
group	Days of age	Macroscopic observations
Control	9	No abnormalities
	<1	No abnormalities
	<1	No milk in stomach
	<1	No milk in stomach
3	6	No abnormalities
	<1	No milk in stomach
	<1	No milk in stomach
	<1	No milk in stomach

Table 59: Macropathology -individual findings for offspring killed or dying before scheduled termination (F1) (study (b) (4)) (repro tox study # 1); sponsor provided

Macroscopic examination of offspring that died before scheduled termination and examination of offspring at scheduled termination on day 25 of age did not reveal any findings that could be related to maternal treatment.

**Serology:**

The study report mentioned that serology was evaluated by the sponsor for this study, but the serology report was not included in the BLA submission. An information request was submitted to the sponsor and the sponsor submitted the serology report under amendment 23 to the original BLA submission. The anti-gE antibody responses measured using an (b) (4) assay.

The serological analysis showed that anti-gE antibody response was observed in 100% of dams after 7 intra-muscular administrations of the gE/AS01B VZV candidate vaccine. Anti-gE antibody response was observed in 100% of fetuses, as well as in 100% of the pups from dams receiving 7 administrations of VZV vaccine candidate. No anti gE antibodies were detected in

the sera collected from dams or fetuses in AS01B adjuvant-treated control group animals. Weak anti-gE antibody response was observed in 10% of dams receiving 7 injections of saline. These weak responses were at least 2000-fold lower than those observed for dams injected with the VZV candidate vaccine. As consequence, some seroconversion was also observed in the related fetuses and pups. Investigations are ongoing at (b) (4) and GSK to find a plausible explanation to that observation.

Overall, the serological data confirm the exposure to, and vaccine take of dams prior and/or during pregnancy. The results also demonstrate the transfer of antibodies from vaccinated dams to fetuses and offspring during in utero development and lactation.

### **Conclusions:**

Treatment of female CD rats with the candidate vaccine gE/AS01B or adjuvant AS01B at 40% of the full human dose per occasion, on 28 and 14 days before pairing and then on days 3, 8, 11 and 15 of gestation and on day 7 of lactation was well tolerated by the F0 females and did not adversely affect embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to day 25 of age.

***Study # 2: Zoster Candidate Vaccine: Study of Effects on the Fertility of Male CD Rats by Intramuscular Administration. Study number: (b) (4) .***

Reviewer: Claudia Wrzesinski

### **Summary:**

Male rats received either gE/AS01B (100 µl/occasion, equals 1/5<sup>th</sup> of a human dose), AS01B (100 µl/occasion, equals 1/5<sup>th</sup> of a human dose) or saline by intramuscular injection on days -42, -28 and -14 prior to pairing. Forty-two days after the first dose administration, treated males were paired with untreated females for assessment of potential effects on fertility and early embryonic development. Treatment with either gE/AS01B or AS01B was well tolerated. Local swelling/edema was observed at the injection site in groups treated with vaccine and adjuvant, the day following each injection. Mating performance and fertility were unaffected by treatment with gE/AS01B or AS01B. No adverse effects on sperm motility, or morphology were apparent after treatment with the adjuvant AS01B or the Zoster candidate vaccine gE/AS01B. However, values for the epididymal sperm concentration, testicular spermatid concentration and total testicular spermatid were statistically significantly lower in the vaccine group compared to saline control, while no significant differences were observed for the adjuvant group. Historical Control Data (HCD) ranges indicated that the concurrent control values for total testicular spermatid, testicular spermatid count and epididymal sperm count were near the maximum range or slightly higher than expected and that all the statistically low values obtained for the animals treated with the gE/AS04B vaccine were within the HCD range. Microscopic examination did not highlight any treatment related changes affecting the right testis, right epididymis, prostate and seminal vesicles. Mating performance and fertility, as assessed by percentage mating, conception rate and fertility index, were unaffected by treatment with gE/AS01B or AS01; 100% was reached for

each group in each assessed variable. There was no evidence of an effect of treatment of the males with gE/AS01B or AS01B on early embryonic development.

In conclusion, the treatment of male CD rats with the Zoster candidate vaccine gE/AS01B or adjuvant AS01B alone on three occasions prior to pairing (on days -42, -28, -14) did not affect male mating performance, fertility or early embryonic development.

Study no.: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: 1 Feb 2010

GLP compliance: yes

QA reports: yes

Drug, lot #: gE: batch number: (b) (4)

AS01B: batch number: (b) (4)

### Methods:

#### Doses:

Group 1: 0.9% saline,

Group 2: AS01B adjuvant formulation: 100 µl (10 µg QS-21 and 10 µg MPL, equals 1/5<sup>th</sup> of a human dose) was injected per rat,

Group 3: gE/AS01B candidate vaccine: 100 µl (10 µg gE, 10 µg QS-21 and 10 µg MPL, equals 1/5<sup>th</sup> of a human dose) was injected per rat

Frequency of dosing: Days -42, -28 and -14 prior to pairing

Dose volume: 100 µl

Route of administration: Intramuscular injection

Species/Strain: (b) (4):CD® (b) (4) rats

Number/Sex/Group: 22

Study design:

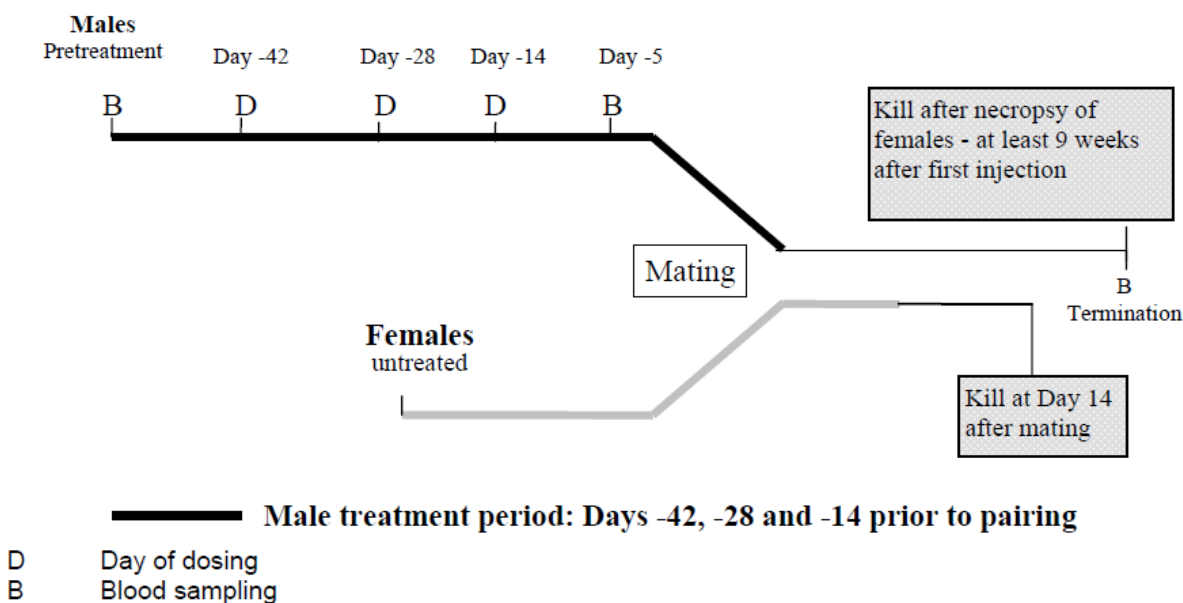


Figure 9: Study design (study (b) (4)) (repro tox study # 2); sponsor provided

Group	Treatment	Dose	Volume	Number of males
1	Saline	0.9% saline	100 µl	22
2	AS01B	10 µg QS-21, 10 µg MPL	100 µl	22
3	gE/AS01B	10 µg gE, 10 µg QS-21, 10 µg MPL	100 µl	22

Table 60: Study design and dosing (study (b) (4) ) (repro tox study # 2).

### Observations and Results:

**Mortality:** There were no deaths.

### Clinical Signs:

Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. Cages and cage-trays were inspected daily for evidence of ill-health amongst the occupant(s). A detailed physical examination was performed weekly on each male and for females on days 0, 7 and 14 after mating, to monitor general health. Injection sites were examined daily on each day of dosing, until two days subsequent and then approximately weekly thereafter and at termination, for signs of reaction (e.g. local swelling and reddening). If signs were still apparent two days after injection, daily monitoring continued until the signs had resolved.

Injection site observation of local swelling/edema was observed the day following the first, second and final injections and this sign generally resolved in 1-2 days. The sign was observed in both animals treated with vaccine gE/AS01B and adjuvant AS01B, and it was observed at the highest incidence following the second injection. This sign was no longer apparent after day 33 of study (day -10 prior to pairing). No swelling or edema was observed at the injection site of the control animals.

### Body Weight

Males were weighed on the day that treatment commenced (day -42), at twice-weekly intervals to termination. Females were weighed on days 0, 4, 7, 11 and 14 after mating.

There were no adverse effects on bodyweight. Bodyweight gain for the vaccine (gE/AS01B) and adjuvant (AS01B) treatment groups was slightly lower during the first three days following the first dose administration, the difference attained statistical significance for the adjuvant treatment group. Bodyweight gain was comparable with the control group for the remainder of the study.

### Feed Consumption

The weight of food supplied to each cage of males, that remaining and an estimate of any spilled was recorded on a twice-weekly basis from the start of treatment until the animals were paired



for mating. From these records the mean daily consumption per animal (g/rat/day) was calculated for each phase, for each cage.

There were no adverse effects on food consumption prior to pairing from either vaccine gE/AS01B or adjuvant AS01B administration. Mean food consumption was marginally but statistically significantly lower than in controls during the first three days after the first and second doses of the adjuvant (days 1-3 and 15-17 of study). Mean food consumption was also marginally low during the first three days after the first dose of the vaccine (days 1-3 of study).

### **Mating procedure:**

Following the scheduled period of treatment (42 days after first pre-pairing injection), males and females were paired on a one-to-one basis for a period of up to 2 weeks. If there was no positive indication of mating after seven days, the female partner was replaced by a spare female.

Once mating occurred, the males and females were separated, and smearing was discontinued. The pre-coital interval was calculated for each male as the time elapsing between initial pairing and detection of mating.

### **Biosampling (antibody assay):**

Blood samples were obtained from the males at pre-treatment and on day -5 (prior to pairing) and at termination. Each 0.8 mL sample collected during the in-life phase was taken from the sublingual vein and collected into plain tubes. Samples collected at termination were taken from the retro-orbital sinus without recovery into plain tubes. Each animal was held under (b) (4) anesthesia during the sampling procedure.

### **Necropsy**

Females were killed on day 14 after mating. Males were killed after at least nine weeks after the first injection on day -42 prior to pairing, following completion of the necropsy of the females and assessment of the day 14 litter parameters. All adult animals were subject to a detailed necropsy, which involved the following: After a review of the history of each animal, a full macroscopic examination of the tissues was performed including injection sites. All external features and orifices were examined visually. For males, samples for sperm analysis (as detailed below) were taken as soon as possible after death. After ventral mid-line incision, the neck and associated tissues and the thoracic, abdominal and pelvic cavities and their viscera were exposed and examined in situ. Any abnormal position, morphology or interaction was recorded.

The requisite organs were weighed and external and cut surfaces of the organs and tissues were examined as appropriate. Any abnormality in the appearance or size of any organ and tissue was recorded and the required tissue samples preserved in appropriate fixative.

The following reproductive assessment was made for all females: The number of corpora lutea in each ovary and the number of implantation sites, the number and distribution of resorption sites (classified as early or late) and live embryos.

The following organs were taken and weight from each male: epididymitis, prostate, seminal vesicles, testes.

Groups	Epididymis (g)	Prostate (g)	Seminal Vesicles (g)	Testes (g)
Saline	1.269	1.289	2.233	3.60
AS01B	1.332	1.278	2.247	3.73
gE/AS01B	1.336	1.194	2.222	3.69

Table 61: Organ weight - mean group values (study (b) (4) ) (repro tox study # 2); sponsor provided

The weights of the testes, prostate and seminal vesicles were not affected by treatment with either the vaccine gE/AS01B or adjuvant AS01B.

#### Histopathology:

	Saline	AS01B	gE/AS01B
<b>Prostate (22 animals examined)</b>			
Lymphoid Aggregates	1	2	3
Oedema	0	1	0
Reduced Colloid/ Colloid Alteration	0	1	1
<b>Rt. Epididymis (22 animals examined)</b>			
Degenerate Spermatogenic Cells in Duct(s)	0	0	1
Lymphoid Aggregates	0	1	1
<b>Rt. Testis (22 animals examined)</b>			
Eosinophilic Globules	0	0	1

	Saline	AS01B	gE/AS01B
Multinucleate Giant Cells	0	2	1
Seminiferous Tubular Vacuolation	0	1	0
Seminal Vesicles (22 animals examined)			

Table 62: Histopathologic evaluation of prostate and testis (study (b) (4) ) (repro tox study # 2); sponsor provided

In the testis multinucleate giant cells involving single tubules within the parenchyma were observed in 2 males from the adjuvant AS01B treatment group and one male from the vaccine gE/AS01B treatment group.

### Sperm analysis:

Immediately after scheduled sacrifice of each male, the left vas deferens, epididymis and testis were removed, and the epididymis and testis were weighed. The following tests were performed: sperm motility, sperm morphology, sperm count, homogenization-resistant spermatids count.

Groups	Motility sperm (%)	Progressively motility sperm	Cauda epididymis			Testis		
			Weight	Sperm count (millions/g)	Total (millions)	Weight	Sperm count (millions/g)	Total (millions)
Saline	92	54	0.239	1076	257	1.80	199	357
AS01B	92	50	0.251	1028	258	1.86	183	343
gE/AS01B	90	51	0.254	970**	246	1.84	167**	306**

Table 63: Sperm analysis - group mean values (study (b) (4) ) (repro tox study # 2); sponsor provided

Groups	Normal sperm morphology (%)	Abnormal sperm morphology (%)
Saline	97.4	2.6
AS01B	97.5	2.5
gE/AS01B	97.9	2.1

Table 64: Sperm morphology analysis - group mean value (study (b) (4) ) (repro tox study # 2); sponsor provided

No adverse effects on sperm motility, concentration or morphology were apparent after treatment with the adjuvant AS01B. No adverse effects on sperm motility, or morphology were apparent after treatment with the Zoster candidate vaccine gE/AS01B. However, values for the epididymal sperm concentration, testicular spermatid concentration and total testicular spermatid were statistically significantly lower in the vaccine group compared to saline control, while no significant differences were observed for the adjuvant group. Historical Control Data (HCD) ranges indicated that the concurrent control values for total testicular spermatid, testicular spermatid count and epididymal sperm count were near the maximum range or slightly higher than expected and that all the statistically low values obtained for the animals treated with the gE/AS04B vaccine were within the HCD range. Therefore, it was concluded that these differences were not related to treatment with the vaccine.

### **Mating performance and fertility:**

#### **Reproductive assessment:**

Pre-implantation loss (%):  $(\text{number of corpora lutea} - \text{number of implantation}) / \text{number of corpora lutea} \times 100$

Post-implantation loss (%):  $(\text{number of implantation} - \text{number of live embryos}) / \text{number of implantations} \times 100$

Groups	Corpora lutea	Implantations	Live embryos	Resorption (mean)		Implantation loss (%)	
				Early	Late	Pre-	Post-
Saline	17.2	16.6	15.5	1.1	0	4.1	6.5
AS01B	16.5	16.4	14.7	1.7	0	1.8	10.6
gE/AS01B	16.6	16.2	15.1	1.0	0	3.6	6.5

Table 65: Reproductive assessment - group mean values (study (b) (4) ) (repro tox study # 2); sponsor provided

Vaccine treatment of the males showed no effect on litter data, as assessed by the mean number of corpora lutea, implantations, resorptions, live embryos and pre- and post-implantation losses. All females were pregnant with live embryos on day 14 of gestation.

A slightly higher post-implantation loss was seen in the adjuvant only group. This was mainly due to one litter with an atypically high number of resorptions. The vaccine group did not show higher post-implantation loss compared to the saline control group.

#### **Statistical analyses:**

All statistical analyses were carried out separately for males and females. Data relating to food consumption was analyzed on a cage basis. For all other adult parameters, the analyses were carried out using the individual animal as the basic experimental unit. For litter findings the litter was taken as the treated unit and the basis for statistical analysis.

The following data types were analyzed at each timepoint separately: Bodyweight, using absolute weights and gains over appropriate study periods, Food consumption, using means over appropriate study periods, litter size and survival indices, organ weights, absolute and adjusted for terminal bodyweight, sperm analysis, motility and count.

The following sequence of statistical tests was used for bodyweight, food consumption, litter size and survival indices, organ weights and sperm analysis, motility and count: A parametric analysis was performed if Bartlett's test for variance homogeneity was not significant at the 1% level. Groups were compared using *t*-tests. A non-parametric analysis was performed if Bartlett's test was still significant at the 1% level following both logarithmic and square-root transformations. Groups were compared using Wilcoxon rank sum tests.

For corpora lutea, implantations, live embryos and sperm analysis, motility and count data, if 75% of the data (across all groups) were the same value, for example *c*, Fisher's Exact tests were performed. Treatment groups were compared using pairwise comparisons of each dose group against the control both for i) values  $<c$  versus values  $\geq c$ , and for ii) values  $\leq c$  versus values  $>c$ , as applicable.

Pre- and post-implantation losses were analyzed by generalized mixed linear model with binomial errors, a logit link function and litter as a random effect. Each treated group was compared to control using a Wald chi-square test. For resorptions, each treated group was compared to control by exact Wilcoxon rank sum test. For organ weight data, analysis of covariance was performed using terminal bodyweight as covariate. The treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.

#### **Antibody assay report:**

Blood samples were taken from male rats at day and antibodies against gE were measured by (b) (4). Anti-gE antibody response was observed in 100% of male rats after three IM administrations of gE/AS01B. No anti-gE antibodies were detected in sera collected from rats receiving either saline or AS01B. The serological data confirm the exposure to the vaccine prior to pairing.

**Conclusions:**

Treatment of male CD rats with the Zoster candidate vaccine gE/AS01B or adjuvant AS01B alone at 20% of the full human dose on three occasions prior to pairing (on days -42, -28, and -14) did not affect male mating performance, fertility or early embryonic development. At termination, no treatment-related differences were detected in males in respect to reproductive organ weights, seminology parameters or macroscopic and microscopic appearance of the reproductive tissues.

***Study number 3: A Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) of RSVPreF3 or AS01B by Intramuscular Injection in Rabbits. Study number: 20152506.***

**Performing laboratory:** (b) (4)

**Study initiation date:** November 08, 2018

**Final report date:** September 17, 2019

**Test article batch/lot:**

Test Article <sup>a</sup>	Storage	Batch Number	Expiration Date
RSVPreF3 vaccine	In a refrigerator, set to maintain 4°C	TRSVA004A	31 May 2019

(b) (4)

**Adjuvant**

Adjuvant	Storage	Batch Number	Expiration Date
AS01 <sub>B</sub>	In a refrigerator, set to maintain 4°C	AA1BA009A	31 December 2019

**Saline Control Article**

Saline Control Article	Manufacturer	Storage	Batch Number	Expiration Date
0.9% Sodium Chloride	GSK HBRIX	Refrigerated	AD02B782AR	30 November 2019

**Animal species and strain:** (b) (4) rabbits

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

**Number of animals per group and sex:** 48 rabbits per group (and assigned to replicates 1 through 5 due to staggered start), based on a computer-generated randomization scheme balanced by body weights and allocated to either the Cesarean section cohort (n=24 per group) or the natural delivery cohort (n=24 per group).

**Age:** 5-6 months

**Body weight range:** 2.6-3.7 kg

**Route and site of administration:** Intramuscular (IM)

**Volume of injection:** 0.5 mL

**Frequency of administration and study duration:** Study days (DS) 1 (28 days prior to mating) and 15 (14 days prior to mating), on gestation days (GD) 3, 11, 16, and 24, and after natural delivery on lactation day (LD) 7 (as applicable).

**Dose:** 120 µg RSVPreF3/dose.

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. Stability for (b) (4) was reported in the following table:

(b) (4)

(b) (4)

(b) (4)

**Means of administration:** Intramuscular (IM)**Report status:** Final**Experimental design:**

Animals were randomized and assigned to 3 different groups. Each group consisted of 48 animals. Animals were dosed by intramuscular (IM) route on study days (DS) 1 (28 days prior to mating) and 15 (14 days prior to mating), on gestation days (GD) 3, 11, 16, and 24, and after natural delivery on lactation day (LD) 7 (as applicable). The details of the study design are listed in the following table:

Table of the experimental design

Group	Test Material	Dose (µg/injection)	Number of Females	
			Assigned to Cesarean-Sectioning	Assigned to Natural Delivery
1	Control	0 (Control article)	24	24
2	RSVPreF3	120 <sup>a</sup>	24	24
3	AS01 <sub>B</sub>	50 <sup>b</sup>	24	24

<sup>a</sup> This dose was expected to give an approximate 20X dose multiple assuming a 3kg average rabbit weight and a 60 kg average human weight.

<sup>b</sup> The dose of AS01B [50 µg of a saponin molecule (QS-21) and 50 µg of 3-O-desacyl-4'-monophosphoryl lipid A (MPL)] was selected because it is the highest clinical dose used for the licensed Shingrix vaccine and other vaccine candidates in development.

Table 67: Experimental design (repro tox study # 3).

Figure of the study design

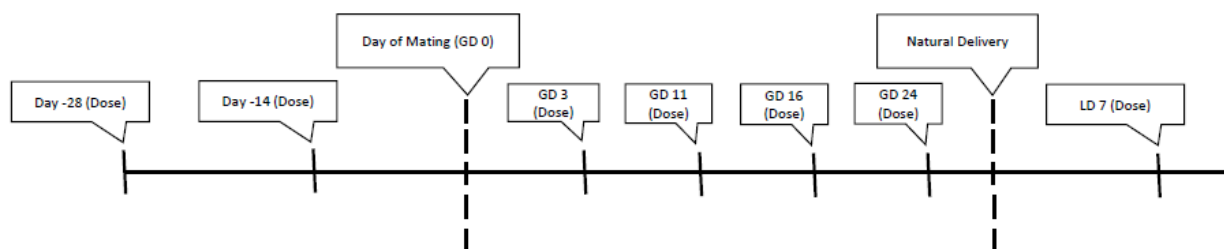


Figure 10: Experimental design (repro tox study # 3); sponsor provided

**Methods:****Randomization procedure:** Yes**Statistical analysis plan:** Yes.**The following parameters were evaluated:**Measurements and observations – F0 generation

During the pre-dose period, female rabbits were observed for viability at least twice daily and for general appearance twice during acclimation. Body weights were recorded on the day of arrival at the test facility, and on the day of randomization. Food consumption was also recorded during the pre-dose period daily beginning the day after arrival at the test facility.

Cage side observations (twice daily), clinical observations (weekly and daily during lactation), body weights (once weekly during the premating phase, including on each day of dose administration [DS 1 and DS 15], and then on GDs 0, 3, 8, 11, 16, 20, 24, 29, and 35 [where required for does that did not deliver], and on LD 4, 7, 10, 14, 17, 20, 24, 29 and 35), food consumption (daily), and mating performance (study day 28).

Female rabbits were evaluated for the following natural delivery observations: clinical signs observed, duration of gestation (GD 0 to the day the first kit was observed), litter size (defined as the number of kits present on day 4 and any removed kits), and kit viability on PND 4. The day that delivery occurred was designated as LD 1 for adult females and postnatal day (PND) 1 for F1 kits. Maternal behavior was recorded daily between LD 4 and 34.

Unscheduled deaths, females that did not naturally deliver a litter, and females with non-viable litters were recorded. Embryo-fetal survival, fetal weight, and gravid uterine weight were recorded. Surviving mated females were euthanized on GD 29 (Cesarean section cohort) or LD 35 (natural delivery cohort), and a gross necropsy of the thoracic, abdominal (stomachs rinsed with saline), pelvic viscera and injection sites was performed.

For the natural delivery cohort, the number of implantation scars was recorded. For the Cesarean-sectioning cohort, the gravid uterus (with evidence of at least apparently one live fetus) was weighed. The ovaries and uterus of pregnant females were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, or shape), early and late



resorptions, and live and dead fetuses. Placentae were removed from the uterine wall so that all surfaces could be examined. Live fetuses were weighed individually and euthanized prior to performing visceral examinations or fresh eye and brain examinations.

Fetal examinations: Rabbits assigned to Cesarean-sectioning; Fetal evaluations (including fetal weights, and external, visceral and skeletal examinations) were conducted. Live fetuses were examined for external abnormalities with a dissecting microscope. Dead fetuses and any late resorptions were examined to the extent possible and then discarded.

Ovaries were weighed at necropsy for all female rabbits, including all nonpregnant rabbits, or rabbits euthanized before scheduled termination. The gravid uterus (with at least one apparently live fetus) was weighed for all females assigned to Cesarean sectioning at scheduled termination.

Measurements and observations – F1 generation – Natural delivery cohort:

Clinical observations:

LD1 through 3-twice daily.

LD4 through 35-kits observed twice daily

PND4-twice daily

Body weight: Kits were individually weighed on PNDs 4, 7, 11, 14, 17, 21, 28, and 35.

Development landmarks:

To evaluate the development of the kits, litters were evaluated for:

Parameters	Day Initiated
Hair Growth	PND 5
Eye Opening	PND 9
Air Righting Reflex	PND 10
Acoustic (Auditory) Startle <sup>a</sup>	PND 14
Pupil Constriction	Once on PND 22

a. The auditory startle stimulus was produced using a clipboard.

Table 68: Development landmarks (repro tox study # 3); sponsor provided

Unscheduled deaths and early euthanasia:

One kit died on PND1. For kits that were found dead or were euthanized on or after postnatal day 4, a necropsy of the thoracic, abdominal, and pelvic viscera was performed to determine the cause of death or condition. The kits were sexed and evaluated for gross lesions and were examined externally and for visceral defects using the Staples method.

Scheduled euthanasia, necropsy, and organ weights: PND 35

Immunogenicity analysis:

Maternal sample collection: DS 1 and 24 (28 and 5 days before mating) and on GD 29 (Cesarean section cohort), or LD 35 (natural delivery cohort). On DS 1 and 24, blood was collected from

the medial auricular artery and on GD 29 and LD 35 blood samples were collected via the vena cava following euthanasia.

Fetal sample collection: GD 29 from groups 1 and 2 only. Blood samples were collected from the umbilical cord.

Kits sample collection: PND 35 from groups 1 and 2 only. Blood samples (2.0 mL per kit) were collected from the vena cava (following euthanasia) for each individual kit.

### **Results:**

#### F0 generation dam's morbidity and/or mortality:

No test article-related morbidity and/or mortality were reported.

Three animals in group 2 were found dead on GD31, LD28, and LD30. No apparent cause for the death but the incidence of mortality during the gestation phase (1 female out of 24 died on GD 31) is within the historical control data (HCD) range for this type of study. Rosell, 2016 (8) reported that female rabbits in the last week of pregnancy and the first week of lactation have higher mortality rates than nonpregnant females. Body weight losses and food consumption decreases were reported in the deaths of rabbits on LD's 28 and 30. Incidental maternal toxicity has occurred at a range of 0- 2 does/group in the HCD collected during the prenatal and embryofetal development stages (HCD for the post-partum lactation period is not available). During the later postpartum, no literature was identified describing the incidence of maternal mortality phase. However, mortality in conjunction with reduced food intake and indigestion has been reported (6, 7). Other conditions in periparturient does that are linked to anorexia and mortality (8) has also been reported. These deaths were considered incidental and unlikely related to AS01B because of the inconsistency between the previous dose and the time of death (7-23 days).

#### Clinical observations, body weight, body weight changes, and food consumption:

No test article-related clinical observations, body weight, body weight changes, and food consumption during the premating, gestation, or lactation periods were reported.

#### Reproductive performance:

No test article-related effects on the number of rabbits mated (91.7% to 95.8%), fertility index (88.6% to 97.8%), or number of rabbits pregnant/number of rabbits paired (81.2% to 93.8%) were reported.

#### Necropsy observations:

No test article-related necropsy observations were reported in the rabbits at the end of the gestation or lactation periods.

#### Organ weights:

No test article-related effects on ovary weights or the ratio of ovary weight to terminal body weights at the end of the gestation or lactation periods were reported.

#### Embryo-fetal survival, fetal weight, and gravid uterine weight:

No test article-related effects on numbers of corpora lutea, implantations, resorptions, live and dead fetuses per litter, sex ratio, fetal body weight, gravid uterine weight or placental morphology were reported.

In group 2, the pre-implantation loss was slightly higher than group 1. This loss was not considered test article-related because it was not statistically significant, and it is primarily out of range due to 1 rabbit (9774) that had a value of 71.4 % (14 corpora lutea and 4 implants). Without the reading from this animal, the mean value will be 22.0% which is within the historical control ranges for this laboratory. Additionally, there is literature supporting that laboratory animals have shown a correlation between high numbers of corpora lutea and pre-implant loss (9).

In group 2, the mean number of live fetuses/litters was slightly lower (7.7) when compared to group 1 (8.3). This decrease was not considered treatment related as it was not statistically significant and within the historical control range (mean 8.8, range 7.0-10.1).

In groups 2 and 3, the post-implantation loss was slightly higher when compared to group 1 (4.27, 4.23 vs 2.02, respectively). This was not considered treatment related as neither were statistically significant and both were within the historical control ranges for this laboratory (mean of 3.5, range of 0.8-22.9).

Sex: Female		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
Day(s) Relative to Mating (Litter: A)				
Group Size - Females		24	24	24
Number of Females Pregnant [f]	N+ve	22	20	23
	%	91.7	83.3	95.8
Female with Live Fetuses [f]	N+ve	22	20	23
	%	100.0	100.0	100.0
Female with all Nonviable [f]	N+ve	0	0	0
	%	0.0	0.0	0.0
Female with Resorptions [f]	N+ve	4	5	7
	%	18.2	25.0	30.4
Terminal Euthanasia [f]	N+ve	24	24	24
	%	100.0	100.0	100.0
Fem. Euthanized Preterminally [f]	N+ve	0	0	0
	%	0.0	0.0	0.0
Found Dead [f]	N+ve	0	0	0
	%	0.0	0.0	0.0
Unscheduled Euthanasia [f]	N+ve	0	0	0
	%	0.0	0.0	0.0
Aborted [f]	N+ve	0	0	0
	%	0.0	0.0	0.0
Delivered [f]	N+ve	0	0	0
	%	0.0	0.0	0.0

Table 69: Summary of maternal performance and mortality - Cesarean section cohort (repro tox study # 3); sponsor provided

Sex: Female		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
Day(s) Relative to Mating (Litter: A)				
Female with Live Fetuses [f]	N+ve	22	20	23
	%	100.0	100.0	100.0
Number of Corpora Lutea [k]	Mean	10.2	11.5	9.2
	SD	2.4	1.9	2.5
	N	22	20	23
Number of Implantations [k]	Mean	8.5	8.6	8.1
	SD	1.4	2.4	1.6
	N	22	20	23
Pre-implantation Loss (%) [k]	Mean	14.54	24.48	9.73
	SD	13.76	18.38	12.44
	N	22	20	23
Total Number of Resorptions [k]	Mean	0.2	0.3	0.3
	SD	0.4	0.6	0.6
	N	22	20	23
Number of Early Resorptions [k]	Mean	0.2	0.3	0.2
	SD	0.4	0.6	0.4
	N	22	20	23
Number of Late Resorptions [k]	Mean	0.0	0.0	0.1
	SD	0.0	0.0	0.3
	N	22	20	23
Total Number of Fetuses [k]	Mean	8.3	8.2	7.7
	SD	1.3	2.4	1.7
	N	22	20	23
Number of Live Fetuses [k]	Mean	8.3	8.2	7.7
	SD	1.3	2.4	1.7
	N	22	20	23
Number of Live Male Fetuses [k]	Mean	4.0	4.2	3.8
	SD	1.8	1.4	1.5
	N	22	20	23
Number of Dead Fetuses [k]	Mean	0.0	0.1	0.0
	SD	0.0	0.2	0.0
	N	22	20	23
Post-implantation Loss (%) [k]	Mean	2.02	4.27	4.23
	SD	4.47	7.58	6.71
	N	22	20	23
Live Male Fetus/Litter (%) [k]	Mean	46.44	51.69	48.52
	SD	15.62	12.36	18.47
	N	22	20	23
Number of Live Female Fetuses [k]	Mean	4.4	4.0	4.0
	SD	1.3	1.5	1.6
	N	22	20	23
Mean Fetal Weight (both) (g) [G]	Mean	42.98	42.78	43.72
	SD	3.36	4.29	3.99
	N	22	20	23
	Mean	43.89	43.57	43.94

Mean Fetal Weight (M) (g) [G]	SD	3.88	5.04	4.55
	N	22	20	22
Mean Fetal Weight (F) (g) [G]	Mean	42.24	41.92	43.49
	SD	3.71	4.25	4.27
	N	22	20	23
Gravid Uterus Weight (g) [G1]	Mean	520.46	505.93	497.02
	SD	75.41	116.60	91.11
	N	22	20	23

Table 70: Summary of ovarian and uterine examinations and litter observations – Cesarean section cohort (repro tox study # 3); sponsor provided

#### Fetal examination:

No treatment-related effects on fetal malformations or variations at any dose were reported.

Nontreatment-related malformations are described in the following table and text:

Test Material	Dam Number	Fetus Number	Malformation(s)
Control Article	9726	3	Branched Ribs; Fused Thoracic Centra
		8	Fused Thoracic Arches; Fused Thoracic
	9747	1	Large Adrenal Glands
		3	Supernumerary Lumbar Vertebrae
		4	Large Adrenal Glands
		7	Large Adrenal Glands
		8	Supernumerary Lumbar Vertebrae
RSVPreF3	9764	1	Absent Lumbar Vertebrae
	9767	10	Supernumerary Lumbar Vertebrae
		11	Supernumerary Lumbar Vertebrae
AS01 <sub>B</sub> adjuvant	9822	6	Small Eye
	9841	5	Domed Head; Hindlimbs Malrotated; Protruding Tongue; Distended Abdomen; Misshapen Brain, Frontals, Mandibles, Supraoccipital; Small Lung; Bent Long Bones (Humeri, Radii, Ulnae, Femurs; Fibulas, Tibias); Bent Metatarsal

Table 71: Nontreatment-related malformations (repro tox study # 3); sponsor provided

An absent lumbar vertebra in one fetus of group 2 was reported. Also, 2 fetuses in a second litter had supernumerary lumbar vertebrae was reported in the same group.

In group 3, one fetus had a small eye and one multiply malformed fetus (domed head, hindlimbs malrotated, protruding tongue, distended abdomen, brain misshapen, small lungs, bent long bones [humerus/radius/ulna/femur/tibia/fibula/metatarsal, and frontal/mandible/supraoccipital misshapen]) was reported in a different litter.

In group 1, there were 3 fetuses in 1 litter with large adrenal glands (also 2 other fetuses in this same litter had supernumerary lumbar vertebrae) and 2 fetuses in 1 litter with fused thoracic centrum (one of these fetuses also had a branched rib, the other fetus had a fused thoracic arch).

All malformations and variations reported in groups 2 and 3 were considered unrelated to RSVPref3 and AS01<sub>B</sub> because: 1) the abnormality was limited to a single fetus; 2) the abnormality occurred at a similar incidence in the control group; and/or 3) the litter and/or fetal incidence was within the range of the historical control data for the testing facility.

<b><u>Exam Type: External</u></b>		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
	Number of Fetuses Examined:	183	164	178
	Number of Fetuses Evaluated:	183	165	178
	Number of Litters Examined:	22	20	23
	Number of Litters Evaluated:	22	20	23
<b>Incidental</b>				
	Number of Fetuses	0	0	1
	Litter % of Fetuses [k]	0.00	0.00	0.72
	Number of Litters	0	0	1
<b>Malformation</b>				
	Number of Fetuses	0	0	1
	Litter % of Fetuses [k]	0.00	0.00	0.72
	Number of Litters	0	0	1
<b>All classifications</b>				
	Number of Fetuses	0	0	1
	Litter % of Fetuses [k]	0.00	0.00	0.72
	Number of Litters	0	0	1
<b><u>Exam Type: FreshVis</u></b>				
<b>Variation-</b>				
	Number of Fetuses	3	5	4
	Litter % of Fetuses [k]	1.67	2.37	2.48
	Number of Litters	3	3	3
<b>Malformation</b>				
	Number of Fetuses	3	0	2
	Litter % of Fetuses [k]	1.52	0.00	1.27
	Number of Litters	1	0	2
<b>All classifications</b>				
	Number of Fetuses	6	5	5
	Litter % of Fetuses [k]	3.19	2.37	3.02
	Number of Litters	4	3	4
<b><u>Exam Type: Skeletal</u></b>				
<b>Variation</b>				
	Number of Fetuses	135	96	123
	Litter % of Fetuses [k]	73.85	57.99	68.56
	Number of Litters	22	20	23
<b>Malformation</b>				
	Number of Fetuses	4	3	1
	Litter % of Fetuses [k]	2.02	1.67	0.72
	Number of Litters	2	2	1
<b>All classifications</b>				
	Number of Fetuses	135	97	123
	Litter % of Fetuses [k]	73.85	58.83	68.56

<b><u>Exam Type: External</u></b>		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
Number of Fetuses Examined:		183	164	178
Number of Fetuses Evaluated:		183	165	178
Number of Litters Examined:		22	20	23
Number of Litters Evaluated:		22	20	23
Number of Litters		22	20	23
<b><u>Exam Type: External</u></b>				
<b>General</b>				
Skin, Discolored - Incidental	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Head/neck</b>				
Head, Domed - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Limb</b>				
Hindlimb, Malrotated - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Mouth</b>				
Tongue, Protruding - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Trunk</b>				
Abdomen, Distended - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)

Table 72: Summary of fetal abnormalities by classification: Gestation – Cesarean section cohort (repro tox study # 3); sponsor provided

<b><u>Exam Type: FreshVis</u></b>		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
Number of Fetuses Examined:		183	164	178
Number of Fetuses Evaluated:		183	165	178
Number of Litters Examined:		22	20	23
Number of Litters Evaluated:		22	20	23
<b>Adrenal gland</b>				
Adrenal gland, Large - Malformation	Fetuses N(%)	3(1.52)	0(0.00)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
<b>Brain</b>				
Brain, Misshapen - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Eye</b>				
Eye, Small - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.54)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Gallbladder/bile duct</b>				
Gallbladder, Small - Variation	Fetuses N(%)	1(0.45)	0(0.00)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
Gallbladder, Supernumerary - Variation	Fetuses N(%)	0(0.00)	1(0.45)	0(0.00)
	Litters N(%)	0(0.0)	1(5.0)	0(0.0)
<b>General</b>				
Abdomen, Fluid filled - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)

<b><u>Exam Type: FreshVis</u></b>		0 ug/ injection	120 ug/ injection Group 2	50 ug/ injection Group 3
Number of Fetuses Examined:		183	164	178
Number of Fetuses Evaluated:		183	165	178
Number of Litters Examined:		22	20	23
Number of Litters Evaluated:		22	20	23
<b>Liver</b>	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Lobe, Misshapen - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.48)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Lung</b>				
Lobe, Absent - Variation	Fetuses N(%)	2(1.22)	4(1.91)	2(1.27)
	Litters N(%)	2(9.1)	3(15.0)	2(8.7)
Lung, Small - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b><u>Exam Type: Skeletal</u></b>				
<b>Clavicle</b>				
Clavicle, Misshapen - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Forelimb</b>				
Forepaw phalanges, Unossified - Variation	Fetuses N(%)	12(6.57)	5(2.86)	6(3.29)
	Litters N(%)	6(27.3)	4(20.0)	6(26.1)
Humerus, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Radius, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Ulna, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Hindlimb</b>				
Femur, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Fibula, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Fibula, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Hindpaw phalanges, Unossified - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Metatarsal, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Tibia, Bent - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Pelvic girdle</b>				
Ilium, Misshapen - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Pubis, Incomplete ossification - Variation	Fetuses N(%)	2(1.07)	2(0.83)	0(0.00)
	Litters N(%)	2(9.1)	1(5.0)	0(0.0)
<b>Rib</b>				
Rib, Branched - Malformation	Fetuses N(%)	1(0.51)	0(0.00)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
Rib, Nodule - Variation	Fetuses N(%)	1(0.45)	0(0.00)	2(1.35)



<b><u>Exam Type: FreshVis</u></b>		0 ug/ injection	120 ug/ injection Group 2	50 ug/ injection Group 3
Number of Fetuses Examined:		183	164	178
Number of Fetuses Evaluated:		183	165	178
Number of Litters Examined:		22	20	23
Number of Litters Evaluated:		22	20	23
Rib, Wavy rib - Variation	Litters N(%)	1(4.5)	0(0.0)	2(8.7)
	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
<b><u>Exam Type: Skeletal</u></b>				
<b>Rib (Continued...)</b>				
Rib, Wavy rib - Variation	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Rib, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Scapula</b>				
Scapula, Misshapen - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
<b>Skull</b>				
Frontal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Frontal, Misshapen - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Hyoid ala, Bent - Variation	Fetuses N(%)	5(2.60)	0(0.00)	4(2.37)
	Litters N(%)	5(22.7)	0(0.0)	4(17.4)
Hyoid ala, Malpositioned - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Hyoid body, Incomplete ossification - Variation	Fetuses N(%)	1(0.51)	1(0.42)	0(0.00)
	Litters N(%)	1(4.5)	1(5.0)	0(0.0)
Interparietal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.54)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Interparietal, Unossified - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Mandible, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Mandible, Misshapen - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Maxilla, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	2(1.27)
	Litters N(%)	0(0.0)	0(0.0)	2(8.7)
Nasal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Parietal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Premaxilla, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Squamosal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Supraoccipital, Misshapen - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
<b><u>Exam Type: Skeletal</u></b>				
<b>Skull (Continued...)</b>				
Supraoccipital, Misshapen - Malformation	Litters N(%)	0(0.0)	0(0.0)	1(4.3)

<b><u>Exam Type: FreshVis</u></b>		0 ug/ injection	120 ug/ injection Group 2	50 ug/ injection Group 3
Number of Fetuses Examined:		183	164	178
Number of Fetuses Evaluated:		183	165	178
Number of Litters Examined:		22	20	23
Number of Litters Evaluated:		22	20	23
Suture bone, Supernumerary site - Variation	Fetuses N(%)	1(0.45)	0(0.00)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
Tympanic annulus, Unossified - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Zygomatic arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	0(0.00)	2(1.27)
	Litters N(%)	0(0.0)	0(0.0)	2(8.7)
<b>Sternebra</b>				
Sternebra, Fused - Variation	Fetuses N(%)	4(2.12)	1(1.00)	4(1.93)
	Litters N(%)	3(13.6)	1(5.0)	2(8.7)
Sternebra, Misshapen - Variation	Fetuses N(%)	1(0.76)	2(1.00)	2(1.45)
	Litters N(%)	1(4.5)	1(5.0)	1(4.3)
Sternebra, Unossified - Variation	Fetuses N(%)	11(5.60)	7(4.33)	3(1.93)
	Litters N(%)	6(27.3)	4(20.0)	2(8.7)
Sternebra, Incomplete ossification - Variation	Fetuses N(%)	7(3.82)	11(6.22)	3(2.03)
	Litters N(%)	6(27.3)	5(25.0)	3(13.0)
Sternebra, Isolated ossification site - Variation	Fetuses N(%)	1(0.45)	3(1.50)	2(1.10)
	Litters N(%)	1(4.5)	1(5.0)	2(8.7)
<b>Supernumerary rib</b>				
Cervical, Full - Variation	Fetuses N(%)	0(0.00)	1(0.45)	0(0.00)
	Litters N(%)	0(0.0)	1(5.0)	0(0.0)
Cervical, Short - Variation	Fetuses N(%)	1(0.57)	2(0.87)	0(0.00)
	Litters N(%)	1(4.5)	2(10.0)	0(0.0)
Thoracolumbar, Full - Variation	Fetuses N(%)	85(46.86)	45(27.25)	81(44.64)
	Litters N(%)	19(86.4)	14(70.0)	21(91.3)
Thoracolumbar, Short - Variation	Fetuses N(%)	49(27.74)	51(30.79)	50(28.50)
	Litters N(%)	19(86.4)	17(85.0)	21(91.3)
<b>Vertebra</b>				
Caudal vertebra, Misaligned - Variation	Fetuses N(%)	2(0.96)	1(0.42)	1(0.72)
	Litters N(%)	2(9.1)	1(5.0)	1(4.3)
Caudal vertebra, Unossified - Variation	Fetuses N(%)	1(0.45)	0(0.00)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
Caudal vertebra, Incomplete ossification - Variation	Fetuses N(%)	3(1.41)	0(0.00)	2(1.45)
	Litters N(%)	3(13.6)	0(0.0)	2(8.7)
Cervical arch, Misshapen - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
<b><u>Exam Type: Skeletal</u></b>				
<b>Vertebra (Continued...)</b>				
Cervical arch, Misshapen - Variation	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
Cervical arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(1.25)	1(0.72)
	Litters N(%)	0(0.0)	1(5.0)	1(4.3)
Cervical centrum, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.45)	0(0.00)
	Litters N(%)	0(0.0)	1(5.0)	0(0.0)

<b>Exam Type: FreshVis</b>		0 ug/ injection	120 ug/ injection Group 2	50 ug/ injection Group 3
Number of Fetuses Examined:		183	164	178
Number of Fetuses Evaluated:		183	165	178
Number of Litters Examined:		22	20	23
Number of Litters Evaluated:		22	20	23
Cervical centrum, Isolated ossification site - Variation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
Lumbar arch, Incomplete ossification - Variation	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
Lumbar vertebra, Absent - Malformation	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
	Fetuses N(%)	0(0.00)	1(0.83)	0(0.00)
Lumbar vertebra, Supernumerary - Malformation	Litters N(%)	0(0.0)	1(5.0)	0(0.0)
	Fetuses N(%)	2(1.01)	2(0.83)	0(0.00)
Sacral arch, Incomplete ossification - Variation	Litters N(%)	1(4.5)	1(5.0)	0(0.0)
	Fetuses N(%)	0(0.00)	0(0.00)	1(0.72)
Thoracic arch, Fused - Malformation	Litters N(%)	0(0.0)	0(0.0)	1(4.3)
	Fetuses N(%)	1(0.51)	0(0.00)	0(0.00)
Thoracic arch, Incomplete ossification - Variation	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
	Fetuses N(%)	1(0.51)	0(0.00)	1(0.72)
Thoracic centrum, Fused - Malformation	Litters N(%)	1(4.5)	0(0.0)	1(4.3)
	Fetuses N(%)	2(1.01)	0(0.00)	0(0.00)
Thoracic centrum, Incomplete ossification - Variation	Litters N(%)	1(4.5)	0(0.0)	0(0.0)
	Fetuses N(%)	1(0.51)	0(0.00)	0(0.00)
	Litters N(%)	1(4.5)	0(0.0)	0(0.0)

Table 73: Summary of fetal abnormalities by finding: Gestation - Cesarean section cohort (repro tox study # 3); sponsor provided

Sex: Female		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
Day(s) Relative to Mating (Litter: A)				
Terminal Body Weight (g) [G]	Mean	3804.2	3829.2	3807.8
	SD	276.9	253.5	281.6
	N	24	24	24
Ovary Weight (g) [I]	Mean	1.0129	0.9568	0.9499
	SD	0.2208	0.1737	0.2328
	N	24	24	24

Table 74: Summary of absolute organ weights: Gestation - Cesarean section cohort (repro tox study # 3); sponsor provided

Sex: Female		0 ug/ injection Group 1	120 ug/ injection Group 2	50 ug/ injection Group 3
Day(s) Relative to Mating (Litter: A)				
Ovary (%) [G]	Mean	0.02666	0.02495	0.02502
	SD	0.00562	0.00407	0.00623
	N	24	24	24

Table 75: Summary of organ weights relative to body weight: Gestation - Cesarean section cohort (repro tox study # 3); sponsor provided

#### Natural delivery and litter observations

No treatment-related effects on natural delivery or litter observations were reported. Among the three groups, the followings are all comparable; values for the numbers of does delivering litters, the duration of gestation, averages for implantation sites per delivered litter, does with stillborn and liveborn kits, the gestation index (number of does with one or more liveborn kits/number of pregnant rabbits), dams with all kits dying, kits found dead or presumed cannibalized, viability and lactation indices, surviving kits per litter, percentage of male kits per litter, live litter size at weighing, and kit weights per litter. Because the magnitude of the differences was minor, all differences in these parameters, including those of statistical significance, were considered unrelated to the test article or adjuvant.

In group 3, there was a slight statistically significant decrease in gestation length. This was not considered treatment related as the difference was minor (32.2 days vs 32.7 days in controls). In group 2, there was a slight increase in the total number of liveborn kits that was statistically significant (145, 98.6% vs 126, 91.3% in controls). This was not considered test article-related because the magnitude of the difference is minor and shows an increase in liveborn.

RABBITS ASSIGNED TO NATURAL DELIVERY:				
GROUP		1	2	3
TEST MATERIAL		CONTROL	RSVPref3	AS01-B ADJUVANT
DOSE LEVEL (UG/INJECTION)a		0	120	50
RABBITS TESTED	N	24	24	24
PREGNANT	N(%)	17( 70.8)	21( 87.5)	22( 91.7)
DELIVERED LITTERS	N(%)	17(100.0)	21(100.0)	21( 95.4)b
DURATION OF GESTATION c	MEAN±S.D.	32.7 ± 0.7	32.7 ± 0.5	32.2 ± 0.5*
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	144 8.5 ± 2.1	147 7.4 ± 2.1 [ 20]e	174 8.3 ± 2.8
DOES WITH STILLBORN KITS	N(%)	0( 0.0)	0( 0.0)	1( 4.8)
DOES WITH NO LIVEBORN KITS	N(%)	0( 0.0)	0( 0.0)	1( 4.8)
GESTATION INDEX d	% N/N	100.0 17/ 17	100.0 21/ 21	95.4 21/ 22
DOES WITH ALL KITS DYING DAYS 1-4 POSTPARTUM	N(%)	0( 0.0)	0( 0.0)	0( 0.0)
DOES WITH ALL KITS DYING DAYS 5-35 POSTPARTUM	N(%)	1( 5.9)	0( 0.0)	0( 0.0)f

a. Dose administration occurred on Day 1 and 15 of study, Gestation Day 3, 11, 16 and 24 and Lactation Day 7 (rabbits that delivered a litter).

b. Excludes values for rabbit 9797, which was found dead on Gestation Day 31.

c. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as Day 0 of gestation) and the day the first pup was delivered.

d. Number of rats with live offspring/number of pregnant rats.

e. Excludes a value for doe 9750, which appeared incorrectly recorded.

f. Excludes values for does that were found dead.

\* Significantly different from the control group value (p<0.05).

Table 76: Summary of natural delivery observations: F0 generation female rabbits (repro tox study # 3); sponsor provided

RABBITS ASSIGNED TO NATURAL DELIVERY:					
GROUP		1	2	3	
TEST MATERIAL		CONTROL	RSVPreF3	AS01-B ADJUVANT	
DOSE LEVEL (UG/INJECTION) <sup>a</sup>		0	120	50	
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN KITS	N	17	21	20	
KITS DELIVERED (TOTAL)	N	138	147	150	
	MEAN±S.D.	8.1 ± 2.0	7.0 ± 2.0	7.5 ± 1.7	
LIVEBORN	MEAN±S.D.	7.4 ± 2.4	6.9 ± 2.0	7.2 ± 1.7	
	N(%)	126( 91.3)	145( 98.6)**	144( 96.0)	
STILLBORN	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	
	N(%)	0( 0.0)	0( 0.0)	1( 0.7)	
UNKNOWN VITAL STATUS <sup>b</sup>	N	12	2	5	
KITS FOUND DEAD OR EUTHANIZED DUE TO ADVERSE CLINICAL OBSERVATIONS					
DAYS 4- 7	N/N(%)	6/126( 4.8)	4/145( 2.8)	7/144( 4.9)	
DAYS 8-11	N/N(%)	7/120( 5.8)	1/141( 0.7)*	4/137( 2.9)	
DAYS 12-14	N/N(%)	0/113( 0.0)	0/140( 0.0)	0/133( 0.0)	
DAYS 15-17	N/N(%)	0/113( 0.0)	0/140( 0.0)	1/133( 0.8)	
DAYS 18-21	N/N(%)	1/113( 0.9)	2/140( 1.4)	0/132( 0.0)	
DAYS 22-28	N/N(%)	1/112( 0.9)	1/138( 0.7)	0/132( 0.0) <sup>c</sup>	
DAYS 29-35	N/N(%)	0/111( 0.0)	3/137( 2.2)	3/119( 2.5) <sup>d</sup>	
LACTATION INDEX <sup>e</sup>	%	88.1	92.4	80.6	
	N/N	111/126	134/145	116/144 <sup>c,d</sup>	

DAY(S) = DAY(S) POSTPARTUM

a. Dose administration occurred on Day 1 and 15 of study, Gestation Day 3, 11, 16 and 24 and Lactation Day 7 (rabbits that delivered a litter).

b. Includes kits that did not have viability statuses recorded on Day 4 postpartum.

c. Excludes 7 kits in litter 9837 that were euthanized on Day 28 postpartum due to death of doe.

d. Excludes 6 kits in litter 9839 that were euthanized on Day 30 postpartum due to death of doe.

e. Number of live kits on Day 35 postpartum/number of live kits on Day 4 postpartum.

\* Significantly different from the control group value (p<=0.05).

\*\* Significantly different from the control group value (p<=0.01).

Table 77: Summary of litter observations (naturally delivered kits): F1 generation litters (repro tox study # 3); sponsor provided

### F1 Generation

No treatment-related effects on clinical signs, reflex and physical development, or macroscopic findings were reported in the F1 generation.

All clinical signs that were observed were considered unrelated to RSVPreF3 or AS01<sub>B</sub> because: 1) they occurred at similar incidences in the control group; 2) they were limited to a single kit in a given dose group; and/or 3) they were considered a common finding in this species and strain of laboratory animal.

No significant differences in the mean number of litters achieving criterion for hair growth, eye opening, air righting, acoustic (auditory) startle, or pupil constriction among the three dose groups were reported. Transient but statistically significant differences in eye opening and air righting at specific postnatal days were reported.

### Necropsy findings

Necropsy findings were considered unrelated to RSVPreF3 or AS01<sub>B</sub> because: 1) the observation was noted in similar incidences in the control group; and/or 2) the observation was limited to a small number of kits (≤3.1% in a given dose group).

Not test article-related early kits deaths and kit brain weights (combined, male, or female) were reported.

**Serology:**

To measure the IgG antibodies directed against the preF3 antigen in rabbit serum, anti-PreF3 (b) (4) were used. Blood was also collected for possible future anti- GRP78 analysis (not performed).

On adult female rabbit sera, specific antibody analysis for antibodies against the RSVPreF3 vaccine was performed to assess the development of antibodies. It is also performed in fetuses to assess the placental transfer of maternal antibodies, and in kits to verify neonatal exposure to maternal antibodies that are produced.

In all group 2 animals, 100% seroconversion (anti-RSVPreF3 IgG antibody titers above the LOD (0.42 EU/mL)) were reported. In group 2, median titers on DS 24 were 11237 EU/mL (range 97.57 to 194091 EU/mL), on GD 29 were 128037 EU/mL (range 26724 to 802027 EU/ml), and on LD 35 were 34341 EU/mL (range 7031 to 292303 EU/mL). These were 9, 5, and 28 days after the previous vaccination, respectively. Anti-RSVPreF3 IgG antibody titers were measured to be below the LOD in all pre-dose samples. Throughout critical periods of ovulation, fertilization, implantation, pregnancy period, parturition, and lactation, the anti-RSVPreF3 IgG titers in group 2 animals were detectable.

At GD 29, the mean titer values of the pooled fetal samples and the corresponding F0 females were comparable (geometric mean- female GD 29: 145873 EU/mL, fetus GD 29: 159228 EU/mL). This indicated the passive transfer of immunity to the fetus. When compared to the F0 females, mean titer values for the kits were lower (geometric mean –female LD 35: 39405 EU/mL, Kit PND 35: 5481 EU/mL) but was indicative of passive transfer of maternal antibodies to F1 animals.

In the control group, 3 out of 48 samples collected from DS 24 females and 1 out of 15 samples collected from LD 35 females showed titer values greater than the LOD. On DS 24 and LD 35, the IgG titer values were lower compared to the corresponding samples collected from group 2 females. The measurable IgG titers in the control animals were transient.

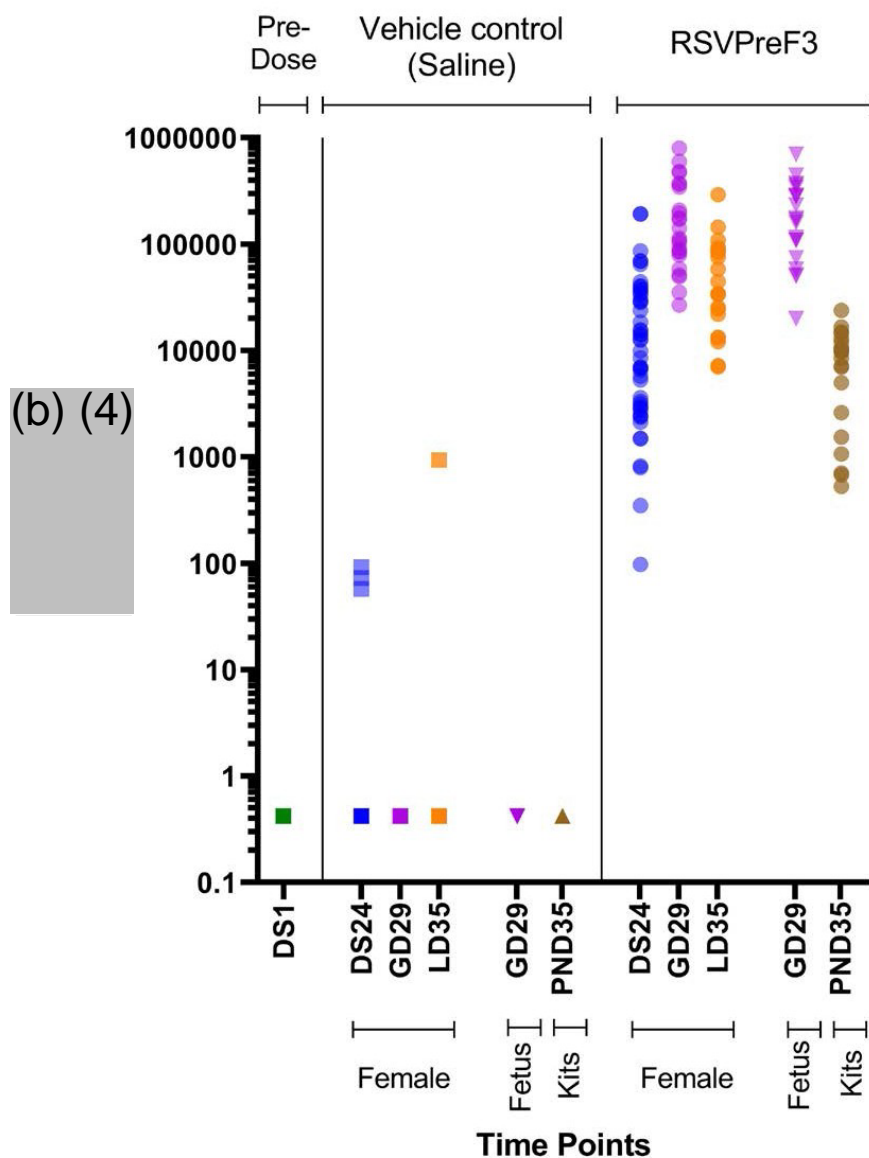


Figure 11: IgG antibodies directed against the preF3 antigen in rabbit serum (repro tox study # 3); sponsor provided

### Conclusions

No RSVPreF3 or AS01<sub>B</sub>-related effects on clinical signs, dermal observations, body weights, food consumption, organ weights, or necropsy observations in the does were reported. No RSVPreF3 or AS01<sub>B</sub>-related effects on mating and fertility, ovarian and uterine parameters, fetal examinations, or natural delivery and litter parameters were reported. Also, no effects on growth or development of the F1 kits were reported. Under the defined experimental conditions, RSVPreF3 and AS01<sub>B</sub> did not adversely affect female fertility, embryo-fetal or pre- and post-natal survival, growth, or development of the offspring up to day 35 of age. Immune responses due test article treatments were reported.

***Study number 4: A Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) of RSVPreF3 by Intramuscular Injection in Rats. Study number: 20152507.***  
**Performing laboratory:** (b) (4)

**Study initiation date:** October 29, 2018

**Final report date:** September 26, 2019

**Test article batch/lot:**

Test Article <sup>a</sup>	Storage	Batch Number	Expiration Date
RSVPreF3 vaccine	In a refrigerator, set to maintain 4°C	TRSVA004A	31 May 2019

(b) (4)

**Saline Control Article**

Saline Control Article	Manufacturer	Storage	Batch Number	Expiration Date
0.9% Sodium Chloride	GSK HBRIX	Refrigerated	AD02B782AR	30 November 2019

**Animal species and strain:** (b) (4) rats

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

**Number of animals per group and sex:** 48 rats per group, based on a computer-generated randomization scheme balanced by body weights and allocated to either the Cesarean section cohort (n=24 per group) or the natural delivery cohort (n=24 per group)

**Age:** 67 days

**Body weight range:** 168 and 196 grams

**Route and site of administration:** Intramuscular (IM)

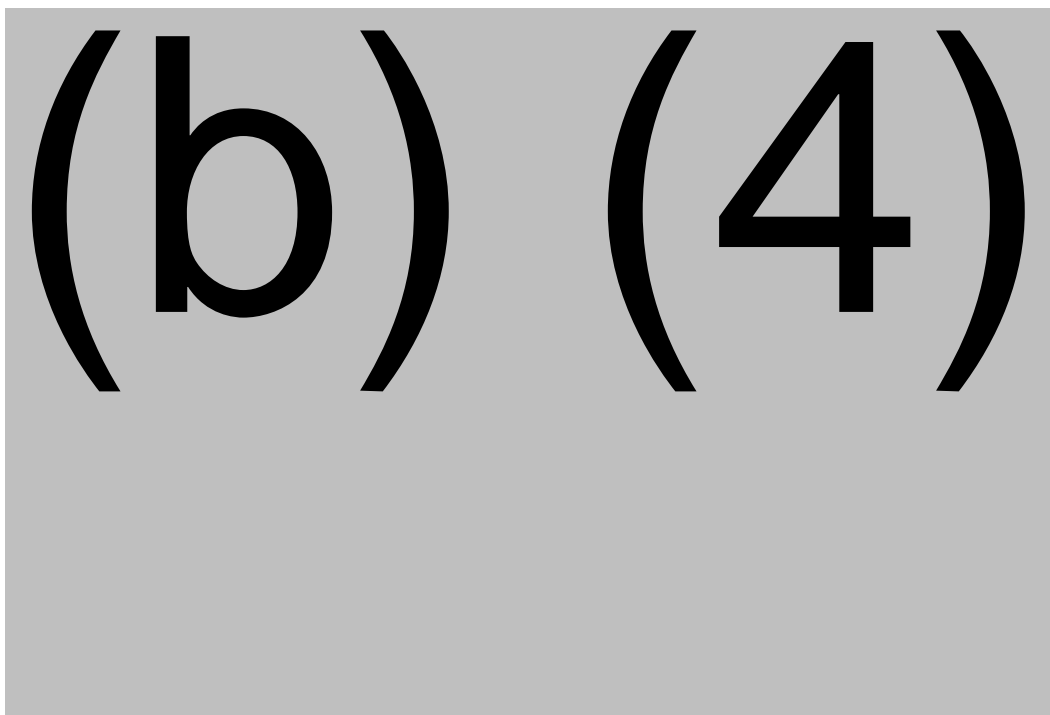
**Volume of injection:** 0.2 mL's

**Frequency of administration and study duration:** Study days (DS) 1 (28 days prior to mating) and 15 (14 days prior to mating), on gestation days (GDs) 3, 9, and 15, and after natural delivery on lactation day (LD) 7.

**Dose:** 120 µg RSVPreF3/dose.

**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. Stability for (b) (4) was reported in the following table:





(b) (4)

**Means of administration:** Intramuscular (IM) injection into the hindlimb (gastrocnemius muscle)

**Report status:** Final

**Experimental design:**

Animals were randomized and assigned to 2 different groups. Each group consisted of 48 animals allocated to either the Cesarean section cohort (n=24 per group) or the natural delivery cohort (n=24 per group). Animals were dosed by intramuscular (IM) route on study days (DS) 1 (28 days prior to mating) and 15 (14 days prior to mating), on gestation days (GDs) 3, 9, and 15 and after natural delivery on lactation day (LD) 7. The dose volume was a constant 0.2 mL per injection and was not adjusted for body weight. The details of the study design are listed in the following table:

Group	Test Material	Dose (µg/injection)	Number of Females	
			Assigned to Cesarean- Sectioning	Assigned to Natural Delivery
1	Control	0 (Control Article)	24	24
2	RSVPreF3	48 <sup>a</sup>	24	24

a. Due to the size of the rat, 2/5<sup>th</sup> of the full human dose of 120 µg (i.e., 48 µg) was administered. This dose was expected to give an approximate 80X dose multiple based on body weight (assuming a 300 gram average rat weight and a 60 kg average human weight).

Table 79: Experimental design (Study number: 20152507) (repro tox study # 4).

Figure of the study design

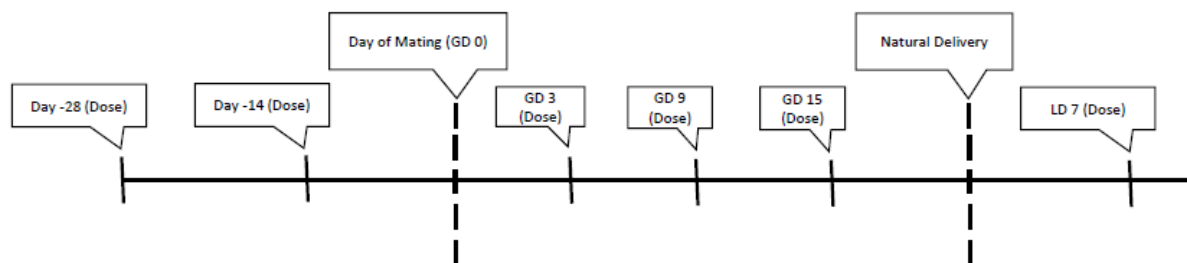


Figure 12: Study design (repro tox study # 4); sponsor provided

**Methods:****Randomization procedure:** Yes**Statistical analysis plan:** Yes.**The following parameters were evaluated:**Measurements and observations – F0 generation

Viability observations (twice daily), clinical observations (weekly during the dosing period, including on each day of dosing prior to dose administration, on GDs 0, 3, 9, 11, 15, 18, and 21 (and 24 and 25 where required for dams that did not deliver), and on LDs 1, 4, 7, 10, 14, 17, and 21), dermal scoring (before each dose and 1 to 3 hours after each dose), body weights (once weekly during the premating phase, including on each day of dose administration (DS 1 and DS 15) and then on GDs 0, 3, 9, 11, 15, 18, 21 (and 24 and 25 where required for dams that did not deliver), and on LDs 1, 4, 7, 10, 14, 17, and 21), food consumption (weekly during the premating period, and on GDs 0, 3, 9, 11, 15, 18, and 21 and GD 24 (if necessary) and on LDs 1, 4, 7, 10, 14, 17, and 21), and mating performance (study day 28).

Female rats were evaluated for the following natural delivery observations: clinical signs observed, duration of gestation (GD 0 to the day the first pup was observed), litter size (defined as pups delivered), and pup viability at birth. Natural delivery was performed hourly on GD20. Completed deliveries were considered lactation day (LD) 1 (F0 females) or postnatal day (PND) 1 for F1 offspring. Maternal behavior was recorded daily during lactation period.

F0 female rats were exsanguinated from the abdominal aorta after blood collection on GD 21 (Cesarean section cohort) or LD 21 (natural delivery cohort) and a gross necropsy of the thoracic, abdominal (stomachs rinsed with saline), pelvic viscera and injection sites was performed

Embryo-fetal survival, fetal weight, and gravid uterine weight: The ovaries and uterus of pregnant females were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color or shape), early and late resorptions, and live and dead fetuses.

Fetal examinations: Each live fetus was examined externally for sex and external abnormalities with a dissecting microscope. Dead fetuses and late resorptions were externally examined to the extent possible then discarded without further examination. Approximately one-half of the

fetuses (live and dead) in each litter were examined, using a dissecting microscope, for abdominal and thoracic visceral abnormalities. The remaining (euthanized) fetuses (approximately one-half of the fetuses in each litter) were decapitated, and the heads were individually identified by permanent marker with the fetus number and fixed in Bouin's solution for subsequent examination using freehand sectioning technique of Wilson J.G. 1965 (10).

The paired ovaries were weighed at necropsy for all female rats in the Cesarean section and natural delivery cohorts, including all nonpregnant rats.

#### Measurements and observations – F1 generation – Natural delivery cohort:

##### Clinical observations:

Litters were observed for dead pups at least twice daily, once in the morning and once near the end of the normal working day. The pups in each litter were counted and the clinical observations were recorded once daily. On PND 1, clinical observations and sex determinations were performed for all F1 pups. Pups were examined daily for presence or absence of milk in stomach until precluded by presence of fur. Only findings of absent milk in stomach were recorded.

Body weight: Pups were individually weighed on PNDs 1 (day of birth), 4, 7, 14, and 21. Body weights were not recorded for pups that were found dead or partially cannibalized.

##### Development landmarks and reflex measurements:

To evaluate the development of the F1 pups, litters were evaluated for:

Parameters	Day Initiated
Surface Righting Reflex (Ability to Right in 5 Seconds)	PND 1
Pinna Unfolding	PND 2
Incisor Eruption	PND 9
Eye Opening	PND 12
Acoustic (Auditory) Startle Reflex (Clipboard)	PND 13
Pupil Constriction	Once on PND 21
Forelimb Grip Reflex	Once on PND 21
Tibia Measurement (Left)	PNDs 7, 14, and 21

Table 80: Evaluation of the development of F1 pups (litters evaluation) (repro tox study # 4).

#### Postweaning measurements and observations – F1 generation

Rats were observed for viability at least twice daily and clinical observations were recorded at least once weekly, and on days of body weight measurements. Rats were individually weighed at least once weekly, and a terminal weight was recorded on the day of scheduled euthanasia. F1 male and female offspring were evaluated for attainment of physical development indicators of sexual maturation; females were examined daily for the occurrence of vaginal opening from PND 27 until evident and males were examined daily for the occurrence of balano-preputial skin

fold separation from PND 39 until evident. Body weight was recorded on the day criterion was met.

One male and one female rat from each litter were tested for effects on sensorimotor and functional neurobehavioral development following procedures described for FOB (11-14).

One rat that died on PND 47 and 1 rat that was euthanized on PND 29 for humane reasons (due to severe eye injury) were necropsied. The rats were examined for gross lesions, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.

After completion of all behavioral testing (PND 71 to 74), F1 generation rats were euthanized by carbon dioxide asphyxiation and a necropsy of the thoracic, abdominal and pelvic viscera was performed (stomachs rinsed with saline).

#### Immunogenicity analysis:

Maternal sample collection: Blood was collected prior to dosing (DS 1), on DS 24 (5 days before mating), and on GD 21 (Cesarean section cohort), or LD 21 (natural delivery cohort). Blood samples (2.0 mL each) were also collected on GD 25 for 3 females that were assigned to natural delivery cohort but showed no positive signs of mating during the cohabitation period. Blood was collected on DS 1 and 24 from conscious rats via the jugular vein. Also, blood was collected on GDs 21 and 25, and LD 21 via the vena cava following euthanasia.

Fetal sample collection: Blood was collected from the umbilical cord on GD 21 from all viable fetuses in each litter assigned to the Cesarean subgroup.

Pups sample collection: Blood was collected from the vena cava (following euthanasia) from all PND 21 culled F1 pups that were not selected for study continuation.

### **Results:**

#### F0 generation dams' morbidity and/or mortality:

No test article-related mortality, clinical observations, mean body weights, or food consumption were reported during the premating, gestation, or lactation periods.

#### Reproductive performance:

No RSVPreF3-related effects on the mean number of days in cohabitation until mating (2.5 days in each group), the number of rats mated (91.7% to 93.8%), fertility index (90.9% to 97.8%), or number of rats pregnant/number of rats paired (83.3% to 91.7%) were reported.

GROUP TEST MATERIAL		1 CONTROL	2 RSVPreF3
NOMINAL DOSE LEVEL (µg/INJECTION) <sup>a</sup>		0	48
RATS IN COHABITATION	N	48	48
DAYS IN COHABITATION <sup>b</sup>	MEAN±S.D.	2.5 ± 1.0	2.5 ± 1.1
RATS THAT MATED	N(%)	45(93.8)	44(91.7)
FERTILITY INDEX <sup>c</sup>	N/N	44/ 45	40/ 44
	(%)	(97.8)	(90.9)
RATS WITH CONFIRMED MATING DATES	N	45	44
RATS PREGNANT/RATS IN COHABITATION	N/N	44/ 48	40/ 48
	(%)	(91.7)	(83.3)

- a. Dose administration occurred on days 1 and 15 of study, gestation days 3, 9, and 15, and lactation day 7 (rats that delivered a litter).
- b. Restricted to rats with a confirmed mating date and rats that did not mate.
- c. Number of pregnancies/number of rats that mated.

Table 81: Summary of mating and fertility: F0 generation female rats (repro tox study # 4); sponsor provided

#### Necropsy observations:

No test article-related necropsy observations were reported in the rats at the end of the gestation or the lactation periods.

#### Organ weights:

No test article-related effects on absolute paired ovarian weights compared to the control at the end of the gestation (0.1290 g versus 0.1346 g, control) or lactation (0.105 g versus 0.103 g, control) periods were reported.

#### Embryo-fetal survival, fetal weight, and gravid uterine weight:

No RSVPreF3-related effect on numbers of corpora lutea, implantations, resorptions, percent pre- and post-implantation loss, live and dead fetuses per litter, sex ratio, fetal body weight, gravid uterine weight or placental morphology (group mean values were similar to control values) were reported.

The slightly higher post-implantation loss in the RSVPreF3 group was not considered vaccine related as the overall group mean value of 9.03% is within historical ranges (1.2% to 11.4%) reported by the test facility.

Sex: Female		0 ug/ Injection Group 1	48 ug/ Injection Group 2
Day(s) Relative to Mating (Litter: A)			
Female with Live Fetuses [f]	N+ve	21	18
	%	100.0	100.0
	N	21	18
Number of Corpora Lutea [k]	Mean	13.7	13.2
	SD	2.3	1.3
	N	20	18
Number of Implantations [k]	Mean	12.8	12.8
	SD	2.8	1.3
	N	21	18
Pre-implantation Loss (%) [k1]	Mean	6.93	3.28
	SD	13.22	3.79
	N	20	18
Total Number of Resorptions [k]	Mean	0.7	1.1
	SD	0.8	1.5
	N	21	18
Number of Early Resorptions [k]	Mean	0.7	1.1
	SD	0.7	1.5
	N	21	18
Number of Late Resorptions [k]	Mean	0.0	0.0
	SD	0.2	0.0
	N	21	18
Total Number of Fetuses [k]	Mean	12.1	11.7
	SD	2.9	2.2
	N	21	18
Number of Live Fetuses [k]	Mean	12.0	11.7
	SD	2.8	2.2
	N	21	18
Number of Live Male Fetuses [k]	Mean	6.7	6.1
	SD	2.4	2.2
	N	21	18
Number of Dead Fetuses [k]	Mean	0.0	0.0
	SD	0.2	0.0
	N	21	18
Post-implantation Loss (%) [k]	Mean	6.41	9.03
	SD	6.95	12.68
	N	21	18
Live Male Fetus/Litter (%) [k]	Mean	55.58	51.33
	SD	13.82	17.72
	N	21	18
Number of Live Female Fetuses [k]	Mean	5.4	5.6
	SD	2.2	2.1
	N	21	18
Mean Fetal Weight (both) (g) [G]	Mean	5.164	5.268
	SD	0.397	0.223
	N	21	18
Mean Fetal Weight (M) (g) [G1]	Mean	5.295	5.380
	SD	0.426	0.287
	N	21	18

Sex: Female		0 ug/ Injection Group 1	48 ug/ Injection Group 2
Day(s) Relative to Mating (Litter: A)			
Mean Fetal Weight (F) (g) [G]	Mean	5.003	5.171
	SD	0.349	0.244
	N	21	18
Gravid Uterus Weight (g) [G2]	Mean	83.78	78.46
	SD	17.15	16.08
	N	21	18

[f] - Fisher's Exact [k] - Dunn

[k1] - Dunn

[k] - Dunn

[G] - Kruskal-Wallis & Dunn

[G1] - Anova & Dunnett

[G2] - Anova & Dunnett

Table 82: Summary of ovarian and uterine examinations and litter observations: Gestation - Cesarean section cohort (repro tox study # 4); sponsor provided

#### Fetal examination:

No treatment-related effects on fetal malformations or variations at any dose were reported.

Nontreatment-related malformations are described in the following table and text:

Test Material	Dam Number	Fetus Number	Malformation(s)
Control Article	8025	9	Fused Rib; Absent Cervical Arch; Absent Thoracic Arch
RSVPreF3	8050	8	Supernumerary Lumbar Vertebrae

Table 83: Nontreatment-related malformations (repro tox study # 4); sponsor provided

In group 2, a single malformed fetus that had 7 lumbar vertebrae (supernumerary) were reported. No other external, visceral, or skeletal abnormalities were reported. This finding was not considered test article-related because this is a single finding and because the fetal incidence (0.79%) is within the historical control range (up to 1.2%) reported by the test facility. In a single fetus of group 1, malformations of the axial skeleton were also reported (fused rib, absent cervical, or thoracic arches).

When compared to control group, the following skeletal variations reported at a higher frequency:

- 1- Nodulated rib (15 fetuses in 8 litters [13.15%] versus 4 fetuses in 3 litters [2.66%], control);
- 2- Statistically significant ( $p \leq 0.05$ ), wavy rib (3 fetuses in 2 litters [2.96%] versus 0 in control);
- 3- Incomplete ossification of interparietal skull bone (11 fetuses in 9 litters [12.01%] versus 4 fetuses in 4 litters, control [3.06%]);
- 4- Statistically significant ( $p \leq 0.05$ ), misshapen sternebrae (4 fetuses in 3 litters [3.34%] versus 1 control fetus [0.68%]);

- 5- Shortened cervical rib (6 fetuses in 3 litters [8.25%] versus 3 fetuses in 3 litters, control [1.96%]);
- 6- And incomplete ossification of cervical arch (2 fetuses in 2 litters [2.04%] versus 0, control).

All the reported variations, including those of statistical significance, were considered unrelated to test article treatment because these variations were within or only slightly above the normal background occurrence in this species and strain of rat based on the historical control data reported by the testing facility.

Exam Type: Skeletal		0 ug/ Injection Group 1	48 ug/ Injection Group 2
Number of Fetuses Examined:		131	111
Number of Fetuses Evaluated:		254	210
Number of Litters Examined:		21	18
Number of Litters Evaluated:		21	18
<b>Rib</b>			
Rib, Fused - Malformation	Fetuses N(%)	1(0.79)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)
Rib, Nodule - Variation	Fetuses N(%)	4(2.66)	15(13.15)*
	Litters N(%)	3(14.3)	8(44.4)
Rib, Wavy rib - Variation	Fetuses N(%)	0(0.00)	3(2.96)
	Litters N(%)	0(0.0)	2(11.1)
<b>Skull</b>			
Frontal, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(1.11)
	Litters N(%)	0(0.0)	1(5.6)
Interparietal, Incomplete ossification - Variation	Fetuses N(%)	4(3.06)	11(12.01)*
	Litters N(%)	4(19.0)	9(50.0)
Parietal, Incomplete ossification - Variation	Fetuses N(%)	12(9.10)	13(11.32)
	Litters N(%)	7(33.3)	9(50.0)
Squamosal, Incomplete ossification - Variation	Fetuses N(%)	4(2.95)	4(5.00)
	Litters N(%)	4(19.0)	4(22.2)
Supraoccipital, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	2(2.04)
	Litters N(%)	0(0.0)	2(11.1)
Zygomatic arch, Incomplete ossification - Variation	Fetuses N(%)	6(4.42)	6(6.62)
	Litters N(%)	4(19.0)	5(27.8)
Zygomatic arch, Fused - Variation	Fetuses N(%)	1(0.68)	2(1.72)
	Litters N(%)	1(4.8)	2(11.1)
<b>Sternebra</b>			
Sternebra, Fused - Variation	Fetuses N(%)	1(0.68)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)
Sternebra, Misshapen - Variation	Fetuses N(%)	1(0.68)	4(3.34)
	Litters N(%)	1(4.8)	3(16.7)
Sternebra, Incomplete ossification - Variation	Fetuses N(%)	1(0.68)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)
<b>Supernumerary rib</b>			
Cervical, Full - Variation	Fetuses N(%)	1(0.68)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)
Cervical, Short - Variation	Fetuses N(%)	3(1.96)	6(8.25)
	Litters N(%)	3(14.3)	3(16.7)



Exam Type: Skeletal		0 ug/ Injection Group 1	48 ug/ Injection Group 2
Number of Fetuses Examined:		131	111
Number of Fetuses Evaluated:		254	210
Number of Litters Examined:		21	18
Number of Litters Evaluated:		21	18
Thoracolumbar, Full - Variation	Fetuses N(%)	4(2.83)	1(0.79)
	Litters N(%)	4(19.0)	1(5.6)
<b>Supernumerary rib (Continued...)</b>			
Thoracolumbar, Short - Variation	Fetuses N(%)	70(51.55)	51(44.78)
	Litters N(%)	19(90.5)	16(88.9)
<b>Vertebra</b>			
Cervical arch, Absent - Malformation	Fetuses N(%)	1(0.79)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)
Cervical arch, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	2(2.04)
	Litters N(%)	0(0.0)	2(11.1)
Lumbar vertebra, Supernumerary - Malformation	Fetuses N(%)	0(0.00)	1(0.79)
	Litters N(%)	0(0.0)	1(5.6)
Thoracic arch, Absent - Malformation	Fetuses N(%)	1(0.79)	0(0.00)
	Litters N(%)	1(4.8)	0(0.0)
Thoracic centrum, Incomplete ossification - Variation	Fetuses N(%)	2(1.59)	2(1.90)
	Litters N(%)	2(9.5)	2(11.1)

[Fetuses %] - Dunn: \* =  $p \leq 0.05$

Fetuses N (%) N=Group Fetal Incidence; (%) = Mean Litter % of Fetuses with the Abnormality

Table 84: Summary of fetal abnormalities by classification: Gestation – Cesarean section cohort (repro tox study # 4); sponsor provided

#### Natural delivery and litter observations

No RSVPreF3-related effects on F0 gestation, natural delivery, litter observations, or lactation were reported.

All 23 group 1 and 22 mated group 2 animals assigned to the natural delivery cohort were pregnant and delivered liveborn litters. In both groups, the mean values for the numbers of mated dams that delivered live litters were 100%. The duration of gestation in group 2 was 22.6 days versus 22.5 days in group 1. In group 2, parturition time, averages for implantation sites per delivered litter, number of stillborn, and number of liveborn pups per litter were 11.3 minutes/pup, 13.0, 0, and 12.3 pups versus 13.9 minutes/pup, 12.0, 6 stillbirths, and 11.6 pups in group 1, respectively.

No RSVPreF3 vaccine-related effects on F1 survival (only 2 pup deaths in RSVPreF3 group versus 5 deaths in control between PND 2 and PND 21) during the preweaning period were reported. No RSVPreF3 vaccine-related effects reported at necropsy (visceral examination) for the decedent pups were reported.

RATS ASSIGNED TO NATURAL DELIVERY:				
MATERNAL GROUP TEST MATERIAL MATERNAL NOMINAL DOSE LEVEL (µg/INJECTION) <sup>a</sup>		1 CONTROL 0	2 RSVPreF3 48	
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	23	22	
PUPS DELIVERED (TOTAL)	N	273	271	
	MEAN±S.D.	11.9 ± 2.5	12.3 ± 2.4	
LIVEBORN	MEAN±S.D. N(%)	11.6 ± 2.5 267 ( 97.8)	12.3 ± 2.4 271(100.0)*	
STILLBORN	MEAN±S.D. N(%)	0.3 ± 0.9 6( 2.2)	0.0 ± 0.0 0( 0.0)*	
PUPS FOUND DEAD OR MISSING (PRESUMED CANNIBALIZED)				
DAY 1	N/N(%)	5/267( 1.9)	0/271( 0.0)	
DAYS 2- 4	N/N(%)	4/262( 1.5)	2/271( 0.7)	
DAYS 5- 7	N/N(%)	1/258( 0.4)	0/269( 0.0)	
DAYS 8-14	N/N(%)	0/257( 0.0)	0/269( 0.0)	
DAYS 15-21	N/N(%)	0/257( 0.0)	0/269( 0.0)	
VIABILITY INDEX <sup>b</sup>	% N/N	96.6 258/267	99.3 269/271	
LACTATION INDEX <sup>c</sup>	% N/N	99.6 257/258	100.0 269/269	
DAY = DAY POSTPARTUM				
a. Dose administration occurred on Days 1 and 15 of study, Gestation Days 3, 9, and 15, and Lactation Day 7 (rats that delivered a litter).				
b. Number of live pups on Day 4 postpartum/number of liveborn pups on Day 1 postpartum.				
c. Number of live pups on Day 21 postpartum (weaning)/number of live pups on Day 4 postpartum.				
* Significantly different from the control group value (p≤0.05).				

Table 85: Summary of litter observations: F1 generation delivered pups (repro tox study # 4); sponsor provided

RATS ASSIGNED TO NATURAL DELIVERY:				
MATERNAL GROUP TEST MATERIAL MATERNAL NOMINAL DOSE LEVEL (µg/INJECTION) a		1 CONTROL 0	2 RSVPreF3 48	
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS		N	23	22
SURVIVING PUPS/LITTER b				
DAY 1c	MEAN±S.D.		11.6 ± 2.5	12.3 ± 2.4
DAY 4	MEAN±S.D.		11.2 ± 2.4	12.2 ± 2.3
DAY 7	MEAN±S.D.		11.2 ± 2.4	12.2 ± 2.3
DAY 14	MEAN±S.D.		11.2 ± 2.4	12.2 ± 2.3
DAY 21	MEAN±S.D.		11.2 ± 2.4	12.2 ± 2.3
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED				
DAY 1c	MEAN±S.D.		49.7 ± 14.2	47.9 ± 14.4
DAY 4	MEAN±S.D.		49.7 ± 13.6	48.3 ± 14.4
DAY 7	MEAN±S.D.		49.9 ± 13.6	48.3 ± 14.4
DAY 14	MEAN±S.D.		49.9 ± 13.6	48.3 ± 14.4
DAY 21	MEAN±S.D.		49.9 ± 13.6	48.3 ± 14.4
DAY = DAY POSTPARTUM				
a. Dose administration occurred on Days 1 and 15 of study, Gestation Days 3, 9, and 15, and Lactation Day 7 (rats that delivered a litter).				
b. Average number of live pups per litter, including litters with no surviving pups.				
c. Includes liveborn pups and pups that died before weighing on Day 1 postpartum.				

Table 86: Summary of litter observations: F1 generation delivered pups (repro tox study # 4); sponsor provided

RATS ASSIGNED TO NATURAL DELIVERY:				
MATERNAL GROUP		1	2	
TEST MATERIAL		CONTROL	RSVPreF3	
MATERNAL NOMINAL DOSE LEVEL (µg/INJECTION) <sup>a</sup>		0	48	
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS		N	23	22
LIVE LITTER SIZE AT WEIGHING				
DAY 1	MEAN±S.D.	11.4 ± 2.5	12.3 ± 2.4	
DAY 4	MEAN±S.D.	11.2 ± 2.4	12.2 ± 2.3	
DAY 7	MEAN±S.D.	11.2 ± 2.4	12.2 ± 2.3	
DAY 14	MEAN±S.D.	11.2 ± 2.4	12.2 ± 2.3	
DAY 21	MEAN±S.D.	11.2 ± 2.4	12.2 ± 2.3	
PUP WEIGHT/LITTER (GRAMS)				
DAY 1	MEAN±S.D.	5.8 ± 0.4	5.9 ± 0.3	
DAY 4	MEAN±S.D.	8.8 ± 1.0	8.5 ± 0.8	
DAY 7	MEAN±S.D.	13.5 ± 1.8	12.9 ± 1.5	
DAY 14	MEAN±S.D.	25.9 ± 3.9	24.8 ± 3.9	
DAY 21	MEAN±S.D.	38.0 ± 6.0	37.2 ± 6.3	
DAY = DAY POSTPARTUM				
a. Dose administration occurred on Days 1 and 15 of study, Gestation Days 3, 9, and 15, and Lactation Day 7 (rats that delivered a litter).				

Table 87: Summary of litter observations: F1 generation delivered pups (repro tox study # 4); sponsor provided

#### Necropsy observation and ovarian weights:

No RSVPreF3-related effects on necropsy observations or ovarian weight were reported at the end of the gestation or lactation periods.

#### F1 Generation; preweaning

##### Clinical observations

No RSVPreF3-related clinical signs reported in the F1 generation prior to weaning. In group 2, clinical signs included 1 pup with abnormal respiration (gasping), decreased activity, and whole-body purple discoloration on PND 2; 1 pup from a different litter that had digit abnormalities (constricted, swollen, and absent) of right hind paw from PND 9 to 21; and 1 litter of 15 pups that had ungroomed haircoats on PND 20 only. Because clinical signs were limited to a single pup, only occurred on a single day, and were considered common findings in this species and strain of laboratory animal, they were not considered test article related.

##### Body weights

No RSVPreF3-related effects on body weights in the F1 generation prior to weaning were reported.

##### Reflex and physical development

No RSVPreF3-related effects on reflex and physical development were reported. No significant differences in the mean number of litters or in the days needed for 50% of pups in each group to achieve criterion for surface righting (2.9 days), pinna unfolding (3.4 days), eye opening (approximately 15 days), acoustic (auditory) startle response (100% present on PND 13), forelimb grip reflex (near 100% present on PND 21), pupil constriction response (100% present on PND 21), or tibia length (0.99X to 0.99X control on PND 7 to 21, respectively) between the 2 dose groups were reported.

In group 2, incisor eruption among F1 pups occurred earlier than group 1 as 66.2% of litters achieved criterion on PND 11 compared to only 21.0% of group 1 litters by this time. This finding was statistically significant ( $p \leq 0.01$ ) but it was not considered related to test article treatment because the response of the pups in the remaining litters was on par with the developmental timelines for the majority of group 1 pups with 91.5% of group 1 and 90.7% of group 2 litters reaching criterion by PND 13 and all 100% of litters by PND 14.

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RATS ASSIGNED TO NATURAL DELIVERY:

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GROUP TEST MATERIAL NOMINAL DOSE LEVEL ( $\mu\text{g}/\text{INJECTION}$ ) <sup>a</sup>		1 CONTROL 0	2 RSVPreF3 48
LITTERS TESTED	N	23	22
<u>SURFACE RIGHTING</u>			
DAY 1	MEAN $\pm$ S.D.	26.1 $\pm$ 21.0	26.9 $\pm$ 16.7
DAY 2	MEAN $\pm$ S.D.	40.8 $\pm$ 16.7	35.0 $\pm$ 17.2
DAY 3	MEAN $\pm$ S.D.	57.2 $\pm$ 31.1	51.3 $\pm$ 24.4
DAY 4	MEAN $\pm$ S.D.	79.6 $\pm$ 25.9	72.8 $\pm$ 26.3
DAY 5	MEAN $\pm$ S.D.	95.4 $\pm$ 9.2	91.4 $\pm$ 12.8
DAY 6	MEAN $\pm$ S.D.	98.5 $\pm$ 4.2	97.4 $\pm$ 7.4
DAY 7	MEAN $\pm$ S.D.	99.2 $\pm$ 2.6	97.0 $\pm$ 12.8
DAY 8	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	2.9 $\pm$ 1.4	2.9 $\pm$ 1.3
<u>PINNA UNFOLDING</u>			
DAY 2	MEAN $\pm$ S.D.	0.0 $\pm$ 0.0	0.3 $\pm$ 1.3
DAY 3	MEAN $\pm$ S.D.	60.6 $\pm$ 43.4	58.9 $\pm$ 43.8
DAY 4	MEAN $\pm$ S.D.	98.3 $\pm$ 5.7	98.9 $\pm$ 3.5
DAY 5	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	3.4 $\pm$ 0.5	3.4 $\pm$ 0.5
<u>INCISOR ERUPTION</u>			
DAY 9	MEAN $\pm$ S.D.	1.6 $\pm$ 3.6	1.8 $\pm$ 4.7
DAY 10	MEAN $\pm$ S.D.	5.6 $\pm$ 10.4	16.4 $\pm$ 26.6
DAY 11	MEAN $\pm$ S.D.	21.0 $\pm$ 19.9	66.2 $\pm$ 31.4**
DAY 12	MEAN $\pm$ S.D.	67.6 $\pm$ 38.9	85.1 $\pm$ 31.3
DAY 13	MEAN $\pm$ S.D.	91.5 $\pm$ 18.5	90.7 $\pm$ 20.0
DAY 14	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	12.2 $\pm$ 0.7	11.4 $\pm$ 1.0**

DAY = DAY POSTPARTUM

a. Dose administration occurred on Days 1 and 15 of study, Gestation Days 3, 9, and 15, and Lactation Day 7 (rats that delivered a litter).

b. The average day postpartum that at least 50% of the pups had the developmental measure present.

\*\* Significantly different from the control group value ( $p \leq 0.01$ ).

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RATS ASSIGNED TO NATURAL DELIVERY:

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GROUP TEST MATERIAL NOMINAL DOSE LEVEL ( $\mu\text{g}/\text{INJECTION}$ ) <sup>a</sup>		1 CONTROL 0	2 RSVPreF3 48
LITTERS TESTED	N	23	22
<u>EYE OPENING</u>			
DAY 12	MEAN $\pm$ S.D.	0.4 $\pm$ 1.7	0.9 $\pm$ 2.8
DAY 13	MEAN $\pm$ S.D.	2.7 $\pm$ 5.8	7.9 $\pm$ 13.5
DAY 14	MEAN $\pm$ S.D.	35.4 $\pm$ 36.4	37.8 $\pm$ 31.0
DAY 15	MEAN $\pm$ S.D.	73.9 $\pm$ 31.8	82.3 $\pm$ 30.3
DAY 16	MEAN $\pm$ S.D.	98.8 $\pm$ 4.0	100.0 $\pm$ 0.0
DAY 17	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	15.0 $\pm$ 0.8	14.7 $\pm$ 0.7
<u>ACOUSTIC (AUDITORY) STARTLE REFLEX (CLIPBOARD)</u>			
DAY 13	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	13.0 $\pm$ 0.0	13.0 $\pm$ 0.0
<u>PUPIL CONSTRICTION</u>			
DAY 21	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	21.0 $\pm$ 0.0	21.0 $\pm$ 0.0
<u>FORELIMB GRIP REFLEX</u>			
DAY 21	MEAN $\pm$ S.D.	100.0 $\pm$ 0.0	98.0 $\pm$ 6.7
CRITERION DAY <sup>b</sup>	MEAN $\pm$ S.D.	21.0 $\pm$ 0.0	21.0 $\pm$ 0.0

DAY = DAY POSTPARTUM

a. Dose administration occurred on Days 1 and 15 of study, Gestation Days 3, 9, and 15, and Lactation Day 7 (rats that delivered a litter).

b. The average day postpartum that at least 50% of the pups had the developmental measure present.

RATS ASSIGNED TO NATURAL DELIVERY:			
GROUP		1	2
TEST MATERIAL		CONTROL	RSVPreF3
NOMINAL DOSE LEVEL (µg/INJECTION) <sup>a</sup>		0	48
LITTERS TESTED	N	23	22
TIBIA MEASUREMENT (LEFT) (MILLIMETERS)			
DAY 7 POSTPARTUM:			
MALE PUPS	MEAN±S.D.	16.71 ± 0.72	16.55 ± 0.52
FEMALE PUPS	MEAN±S.D.	16.44 ± 0.80	16.31 ± 0.67
MALE AND FEMALE PUPS	MEAN±S.D.	16.58 ± 0.72	16.43 ± 0.54
DAY 14 POSTPARTUM:			
MALE PUPS	MEAN±S.D.	22.57 ± 1.14	22.40 ± 0.82
FEMALE PUPS	MEAN±S.D.	22.35 ± 1.05	22.18 ± 0.79
MALE AND FEMALE PUPS	MEAN±S.D.	22.46 ± 1.07	22.29 ± 0.77
DAY 21 POSTPARTUM:			
MALE PUPS	MEAN±S.D.	28.66 ± 1.45	28.47 ± 1.71
FEMALE PUPS	MEAN±S.D.	28.25 ± 1.44	28.05 ± 1.66
MALE AND FEMALE PUPS	MEAN±S.D.	28.49 ± 1.42	28.25 ± 1.66
DAY = DAY POSTPARTUM			
a. Dose administration occurred on Days 1 and 15 of study, Gestation Days 3, 9, and 15, and Lactation Day 7 (rats that delivered a litter).			

Table 88: Summary of developmental landmarks and reflex measures: F1 generation delivered litters (repro tox study # 4); sponsor provided

### Postweaning

#### Mortality

No RSVPreF3-related mortality, clinical signs, or body weight in the F1 males or females were reported.

In group 2 F1 male offspring and following weaning, a statistically significant decrease in mean body weight was reported during PND 50 to 57 (0.94X of control;  $p \leq 0.05$ ). However, a similar finding was not evident among F1 female offspring during this time. This finding was not associated with any delays in overall growth and development of F1 males as there was no difference from control in tibia lengths in the preweaning period and no difference in age or body weight at time of attainment of puberty. This finding was not considered related to test article treatment because the difference from control in mean body weight gains among F1 male rats in the RSVPreF3 group were minimal and there were no other statistically significant changes reported at any other time during the postweaning period.

#### Sexual Maturation

No RSVPreF3-related effects on the age of attainment for balano-preputial separation for the males (44.7 and 44.2 days in group 1 and 2 animals, respectively) or vaginal patency of the females (30.4 and 30.6 days in group 1 and 2 animals, respectively) were reported.

Three females' group 1 pups and one female group 2 pup were reported as having patent vaginal openings on PND 27, the first day of examination.

MATERNAL GROUP TEST MATERIAL MATERNAL NOMINAL DOSE LEVEL (µg/INJECTION)		1 CONTROL 0	2 RSVPreF3 48
MALE RATS	N	24	23a
PREPUTIAL SEPARATION b	MEAN±S.D.	44.7 ± 2.5	44.2 ± 2.4
BODY WEIGHT AT SEPARATION (G) c	MEAN±S.D.	183.9 ± 18.5	175.4 ± 17.5
FEMALE RATS	N	24	24
VAGINAL PATENCY d	MEAN±S.D.	30.4 ± 1.6 [ 21] f	30.6 ± 1.6 [ 23] f
BODY WEIGHT AT VAGINAL PATENCY (G) e	MEAN±S.D.	81.0 ± 11.5 [ 21] f	82.0 ± 11.8 [ 23] f

a. Excludes values for rat 2839, which was euthanized on Day 29 due to adverse clinical observations.  
b. Average day postpartum that the prepuce was observed to be separated.  
c. Average body weight on day prepuce was first observed to be separated.  
d. Average day postpartum that the vagina was observed to be patent.  
e. Average body weight on day vagina was first observed to be patent.  
f. Excludes values for rats 2856, 2866, 2871 and 2873; the vagina was patent on the first day of observation, Day 27 postpartum, therefore the exact day of maturity could not be determined.

Table 89: Summary of physical developmental landmarks of sexual maturation: F1 generation rats (repro tox study # 4); sponsor provided

### Functional Observational Battery

No RSVPreF3-related effects on any of the parameters evaluated as part of the functional observational battery in the F1 males or females when tested on PND 70±2 was reported. As reported values for home cage and open field behaviors as well as response to routine handling, tactile, auditory and visual stimulation were all normal. Also, the animals were well groomed with no abnormal eye or oral secretions and no alterations in body temperature, respiration or mobility. And no abnormalities in various reflexes or grip strength when compared to controls were reported.

DAY 70 ± 2 POSTPARTUM			
MATERNAL GROUP TEST MATERIAL MATERNAL NOMINAL DOSE LEVEL (µg/Injection)		1 CONTROL 0	2 RSVPreF3 48
MALE RATS	N	23	21a
HOME CAGE BEHAVIOR			
1: Immobile, Sleeping	N	5	4
2: Immobile, Awake, No Alterations	N	12	9
3: Mobile, No Alterations	N	6	8
4: Immobile, with Alterations	N	0	0
5: Mobile, with Alterations	N	0	0
ALTERATIONS (HOME CAGE)			
1: None	N	23	21
2: Stereotyped behavior	N	0	0
3: Bizarre behavior	N	0	0
4: Limb twitches/tremor	N	0	0
5: Whole body tremor/spasm	N	0	0
6: Unusual posture	N	0	0
7: Tonic-clonic seizure	N	0	0
REACTION TO REMOVAL			
(1) Sits quietly	N	22	17
(2) Vocalization	N	1	4
(3) Runs or freezes	N	0	0
(4) Tail or throat rattles	N	0	0
	MEAN SCORE	1.0	1.2
REACTION TO HANDLING			
(1) No resistance	N	23	21
(2) Vocalization	N	0	0
(3) Tense	N	0	0
(4) Squirming	N	0	0
	MEAN SCORE	1.0	1.0

n: = Category number for descriptive test item.

(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats.

a. Excludes values for rat 2839, which was euthanized on Day 29 postpartum due to adverse clinical observations.

DAY 70 ± 2 POSTPARTUM				
MATERNAL GROUP		1	2	
TEST MATERIAL		CONTROL	RSVPreF3	
MATERNAL NOMINAL DOSE LEVEL (µg/Injection)		0	48	
MALE RATS		23	21a	
REARS IN OPEN FIELD	MEAN±S.D.	11.4 ± 4.1	10.6 ± 3.9	
DEFECATION IN OPEN FIELD				
1: None	N	23	21	
2: Feces normal	N	0	0	
3: Soft or liquid feces	N	0	0	
URINATION IN OPEN FIELD				
(1) None	N	16	13	
(2) Normal urination	N	7	8	
(3) Excess urination	N	0	0	
	MEAN SCORE	1.3	1.4	
LEVEL OF AROUSAL				
(1) Stuporous	N	0	0	
(2) Sluggish	N	0	0	
(3) Apparently normal	N	23	21	
(4) Sudden darting	N	0	0	
(5) Freezing, vocalization	N	0	0	
	MEAN SCORE	3.0	3.0	
ALTERATIONS (OPEN FIELD)				
1: None	N	23	21	
2: Stereotyped behavior	N	0	0	
3: Bizarre behavior	N	0	0	
4: Limb twitches/tremor	N	0	0	
5: Whole body tremor/spasm	N	0	0	
6: Unusual posture	N	0	0	
7: Tonic-clonic seizure	N	0	0	
n: = Category number for descriptive test item.				
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats.				
a. Excludes values for rat 2839, which was euthanized on Day 29 postpartum due to adverse clinical observations.				

DAY 70 ± 2 POSTPARTUM				
MATERNAL GROUP		1	2	
TEST MATERIAL		CONTROL	RSVPreF3	
MATERNAL NOMINAL DOSE LEVEL (µg/Injection)		0	48	
MALE RATS		23	21a	
GAIT PATTERN				
1: Apparently normal	N	23	21	
2: Ataxic	N	0	0	
3: Limbs splay or drag	N	0	0	
4: Spastic, tip-toe	N	0	0	
5: Duck-walk	N	0	0	
6: Scissors gait	N	0	0	
GAIT ABNORMALITY, SEVERITY				
(1) Normal gait	N	23	21	
(2) Slight	N	0	0	
(3) Moderate	N	0	0	
(4) Extreme	N	0	0	
	MEAN SCORE	1.0	1.0	
PALPEBRAL CLOSURE				
(1) Wide open	N	23	21	
(2) Slightly drooping	N	0	0	
(3) Half-closed	N	0	0	
(4) Completely shut	N	0	0	
	MEAN SCORE	1.0	1.0	
PROMINENCE OF THE EYE				
1: Normal	N	22	21	
2: Exophthalmos	N	1	0	
3: Enophthalmos	N	0	0	
n: = Category number for descriptive test item.				
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats.				
a. Excludes values for rat 2839, which was euthanized on Day 29 postpartum due to adverse clinical observations.				

DAY 70 ± 2 POSTPARTUM			
MATERNAL GROUP		1	2
TEST MATERIAL		CONTROL	RSVPreF3
MATERNAL NOMINAL DOSE LEVEL (µg/Injection)		0	48
MALE RATS		23	21a
LACRIMATION			
(1) No excess	N	23	21
(2) Excess at eyelid margin	N	0	0
(3) Margin persistently damp	N	0	0
(4) Extends beyond margin	N	0	0
	MEAN SCORE	1.0	1.0
SALIVATION			
(1) No excess	N	23	21
(2) Margin of mouth wet	N	0	0
(3) 1/4 to 1/2 submandibular	N	0	0
(4) Entire submandibular	N	0	0
	MEAN SCORE	1.0	1.0
PILOERECTION	N	0	0
ABNORMAL RESPIRATION	N	0	1
APPEARANCE			
(1) Clean and groomed	N	23	21
(2) Unkempt	N	0	0
(3) Urine and/or fecal stain	N	0	0
	MEAN SCORE	1.0	1.0
VISUAL REACTION			
(1) None	N	0	0
(2) Orienting	N	23	21
(3) Startle	N	0	0
(4) More energetic reaction	N	0	0
(5) Attacks	N	0	0
	MEAN SCORE	2.0	2.0

n: = Category number for descriptive test item.  
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats.  
a. Excludes values for rat 2839, which was euthanized on Day 29 postpartum due to adverse clinical observations.

Table 90: Summary of functional observational battery data: F1 generation male rats (repro tox study # 4); sponsor provided

### Necropsy Observations

No abnormalities detected at necropsy examinations in the F1 males or females at scheduled euthanasia.

### Brain Weights

No RSVPreF3-related effects on absolute brain weights (male: 1.89 g versus 1.91 g, control; female: 1.82 g versus 1.85 g control) or the ratio of brain weights to terminal body weights in the F1 generation males or females were reported.

MATERNAL GROUP		1	2
TEST MATERIAL		CONTROL	RSVPreF3
MATERNAL NOMINAL DOSE LEVEL (µg/INJECTION)		0	48
RATS TESTED		24	23a
TERMINAL BODY WEIGHT	MEAN±S.D.	336.2 ± 26.1	322.0 ± 34.4
BRAIN	MEAN±S.D.	1.91 ± 0.12 [ 21]b	1.89 ± 0.12 [ 21]b
BRAIN (%)	MEAN±S.D.	0.573 ± 0.043 [ 21]b	0.596 ± 0.068 [ 21]b

ALL WEIGHTS WERE RECORDED IN GRAMS (G).  
[ ] = NUMBER OF VALUES AVERAGED  
a. Excludes values for rat 2839, which was euthanized on Day 29 postpartum due to adverse clinical observations.  
b. Excludes values for rats that had organs damaged (weight affected).  
RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

Table 91: Summary of terminal body weights, brain weights and ratios (%) of brain weight to terminal body weight: F1 generation male rats (repro tox study # 4); sponsor provided



MATERNAL GROUP TEST MATERIAL MATERNAL NOMINAL DOSE LEVEL (µg/INJECTION)		1 CONTROL 0	2 RSVPreF3 48
RATS TESTED		23 <sup>a</sup>	24
TERMINAL BODY WEIGHT	MEAN±S.D.	208.7 ± 13.3	210.0 ± 14.3
BRAIN	MEAN±S.D.	1.85 ± 0.15 [ 20] <sup>b</sup>	1.82 ± 0.12 [ 21] <sup>b</sup>
BRAIN (%)	MEAN±S.D.	0.888 ± 0.085 [ 20] <sup>b</sup>	0.870 ± 0.077 [ 21] <sup>b</sup>

ALL WEIGHTS WERE RECORDED IN GRAMS (G).  
[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rat 2865, which was found dead on Day 47 postpartum.  
b. Excludes values for rats that had organs damaged (weight affected).

RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

Table 92: Summary of terminal body weights, brain weights and ratios (%) of brain weight to terminal body weight: F1 generation female rats (repro tox study # 4); sponsor provided

### Serology:

Anti-PreF3 (b) (4) method was used to measure the IgG antibodies directed against the preF3 antigen in rat serum. Also, blood was also collected for possible future anti-GRP78 analysis (not performed). Exposure in fetuses to assess the placental transfer of maternal antibodies, and in pups to verify neonatal exposure to maternal antibodies that were produced was also measured. Pup samples were pooled by litter for analysis.

In all pre-dose samples (control and RSVPreF3 groups) and in all control group animals (females, fetuses, and pups) at all sample collection timepoints, anti-RSVPreF3 IgG antibody titers were below the limit of detection (LOD = 0.25 EU/ml).

After the first 2 injections [5 days before mating (DS24)], the majority of the RSVPreF3 treated rats had detectable IgG titers (range 26.2 to 18465.0 EU/mL). No IgG titers were reported in 16 female rats at this same time point and 12 females had no RSVPreF3 IgG titers at later time points (GD21 or LD21). A mean IgG titer of 2302.90 EU/ml, 1827.51 EU/ml, and 2449.90 EU/ml were reported at DS24, GD21 and LD21, respectively.

Transfer of anti-RSVPreF3 IgG antibody from F0 to F1 animals were reported. The majority of the pooled samples in the offspring had detectable levels of IgG antibodies at GD 21 (10 of 18 litters; range 61.3 to 2952.0 EU/ml) or PND 21 (17 of 22 litters; range 81.3 to 44086.0 EU/ml). For the corresponding F0 generation, the pooled samples from the F1 progeny with no seroconversion also showed no seroconversion. As an indication of passive transfer of immunity from the F0 animals to the F1 progeny, mean titer values for fetuses and pups that had IgG titers was 679.96 EU/ml and 4268.89 EU/ml, respectively.

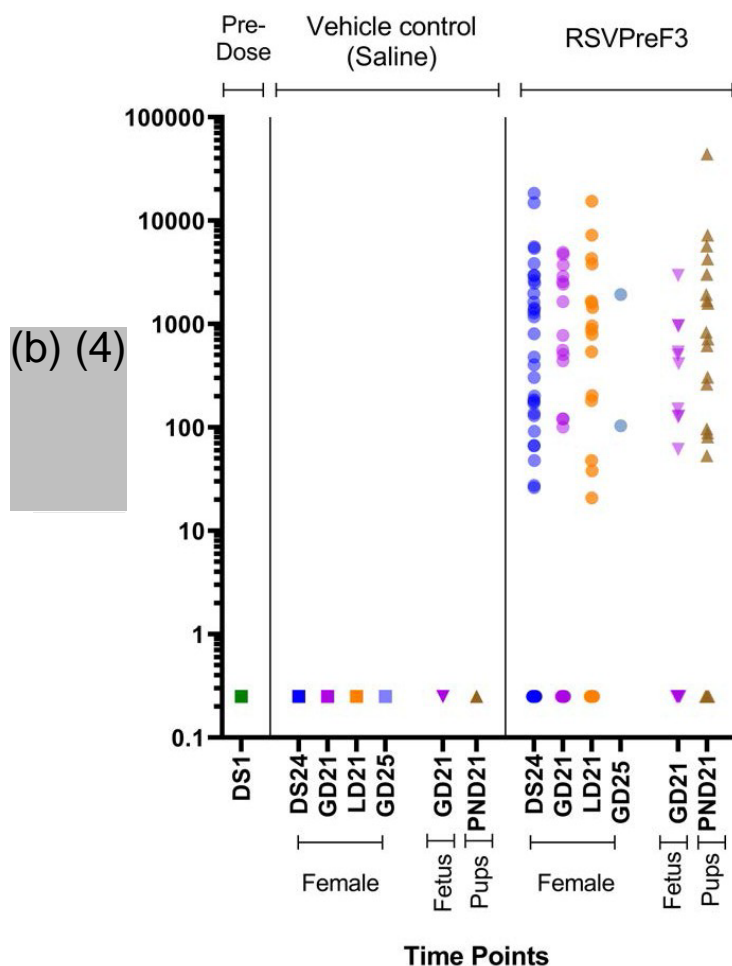


Figure 13: IgG antibodies directed against the preF3 antigen in rat serum (repro tox study # 4); sponsor provided

### Conclusion

No RSVPreF3-related effects on clinical signs, dermal observations, body weights, food consumption, ovarian weights, necropsy observations, mating and fertility, ovarian and uterine parameters, embryo-fetal development, or natural delivery, lactation, and litter parameters were reported. No RSVPreF3-related effects on growth, physical or neurobehavioral development of the F1 pups were reported. In female rats and prior to mating until termination at the end of pregnancy or lactation, anti-RSVPreF3 IgG antibody titers were detected. Passive immunity was reported with transfer of anti-RSVPreF3 IgG antibody from maternal animals to offspring. In conclusion, the RSVPreF3 vaccine (48 µg/0.2 mL injection) was well tolerated and did not adversely affect female fertility, embryo-fetal or pre- and post-natal survival, growth, or development of the offspring up to day 75 of age.

**GLP study deviations or amendments:** No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

**Investigators Brochure:** Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes ( ) or no (X).

**Over all conclusions:**

Based on nonclinical toxicity assessments, there are no significant safety issues related to female fertility, embryo-fetal or pre- and post-natal survival, growth, or development of the offspring were reported.

**Study Number 5: MPL: Subcutaneous Study of Embryo-Fetal Development in the Rat. Study number: (b) (4) 1729/7.**

Reviewer: Dr. Marion Gruber

This study has been originally submitted to BLA 125259 and has been reviewed by Dr. Marion Gruber, here only a short summary of her review is included, her full review can be found under BLA 125259.

**Summary:**

In this study the effects on MPL on the embryonic and fetal development of the rat was evaluated. Three groups of 24 mated female rats were given MPL at dose levels of 1, 10 and 100 µg/kg/day daily, from days 6 to 17 of gestation, inclusive. A further group of 24 mated females, dosed with the vehicle (phosphate buffered saline) by the same route and over the same period, served as controls. All animals were maintained to day 20 of gestation, when they were killed and their uterine contents examined.

No mortalities were observed during the study. Clinical signs, body weights and food intakes were unaffected by treatment and there were no treatment-related effects noted at necropsy. There were no adverse effects of treatment on the pregnancy rate or on the uterine/implantation or fetal data. External and visceral malformation and variations observed in this study were unaffected by treatment. They were in the range of what is observed in the historical control database and occurred in the untreated (control) as well as in the treated groups. There were no skeletal malformations. The number of fetuses showing skeletal variations was in the range of that observed in the historical control database.

In conclusion, administration of MPL to pregnant rats at dose levels of up to 100 µg/kg/day from day 6 to 17 of gestation showed no indication of maternal or embryo toxicity or of teratogenicity.

**Study 6: MPL: Subcutaneous Study of Embryo-Fetal Development in the Rabbit. Study number: (b) (4) 1729/8.**

Reviewer: Dr. Marion Gruber

This study has been originally submitted to BLA 125259 and has been reviewed by Dr. Marion Gruber, here only a short summary of her review is included, her full review can be found under BLA 125259.

**Summary:**

In this study the effect of MPL on the embryonic and fetal development of the rabbit when administered subcutaneously was evaluated. Three groups of 24 mated female rabbits were given

MPL at dose levels of 1, 10 and 100 µg/kg/day, from days 7 to 19 of gestation, inclusive. A further group of 24 mated females, dosed with the vehicle (phosphate buffered saline) by the same route and over the same period, served as controls. All animals were maintained to day 29 of gestation, when they were killed and their uterine contents examined.

One low dose female aborted on day 28 of gestation and one intermediate dose female aborted on day 29 of gestation. All fetuses found, dead or alive, were of apparent normal development for the stage of gestation. In addition, there was one female in the low and 5 females in the intermediate MPL dosing group with total embryo/fetal loss. Of these 5 animals in the intermediate dose group, 3 animals had only 1 implantation, one female had 2 and one female had 4 implantations. In all animals, all implantations were early uterine death, and it is not unusual for litters to be resorbed in rabbits when the implantation rate is low. Of note, the first treatment started on day 7, thus, low number of implantations on these animals is unlikely due to treatment with MPL. In addition, no total embryo/fetal loss occurred in the high dose MPL group.

In females with live fetuses at Caesarean sectioning, the mean numbers of corpora lutea were marginally lower in the MPL treated groups, compared to controls which resulted in a statistically significant dose-response, resulting in a small, dose related decrease in mean number of implantations. However, when compared to historical control data, all values were within the range of the historical control data. Since treatment started on day 7 of gestation it is unlikely that the lower number of corpora lutea observed in MPL treated groups is due to MPL. Mean sex ratio was unaffected by treatment.

The observed malformations are known to occur spontaneously in this strain of rabbit in the testing laboratories and their nature and intergroup- distribution do not indicate a treatment related effect. Overall, there were no adverse effects of treatment on the pregnancy rate or on the uterine/implantation, fetal or fetal defect data.

In conclusion, administration of MPL to pregnant rabbits at dose levels of up to 100 µg/kg/day from day 7 to day 19 of gestation, showed no indication of maternal or embryo toxicity or of teratogenicity.

Comment from the reviewer of BLA 125258 (for more details see Dr. Gruber's review of BLA 125258):

In this study 1729/8-D6154 there were 2 cases of major ventricular septal defects, one (1) in groups 3 (MPL intermediate dose group, 10 ug/kg/day) and one in group 4 (MPL high dose group, 100 ug/kg/day) with an incidence of 0.6 % by fetus and 5.8% by litter in group 3 and 0.55% by fetus and 4.7 % by litter in group 4. This observation did not occur in the low dose MPL group and/or in the saline control group. Control group values from 6 embryo/fetal studies that preceded this study showed that of 1139 fetuses (118 litters) evaluated, there was 1 case of ventricular septal defect in study 4 (0.088% by fetus and 0.8% by litter). In addition, the sponsor provided cumulative fetal defect data for (b) (4) rabbits, supplied by (b) (4) used in embryo-fetal studies at (b) (4) since February 1994. The cumulative incidence of ventricular septal defect (major) in rabbits was 0.12%.

It is not clear whether the finding of intraventricular septal defect observed in this study and in pivotal study (b) (4) 249 [This is a reproductive toxicology study evaluating CERVARIX submitted to BLA 125258. In this study two observations of small membranous intraventricular septal defect (IVSD) were described under minor visceral fetal abnormalities in rats, one pup from the group receiving the full vaccine and one pup from the group receiving the adjuvant alone showed this finding] is a treatment related finding, since it is isolated in nature, i.e., 1 fetus per litter/group and since it was also observed in the historical control data. However, concerning is that the incidence of ventricular septal defect in both studies is higher than in the historical control and did not occur in concurrent control groups. Furthermore, in this study (1729/8-D6154) in which animals were treated with MPL, this finding occurred in the higher dose groups only and in the pivotal study (b) (4) 249/033160) conducted in rats this finding occurred in groups 3 and 4, i.e. those groups that received HPV/AS04D or AS04D before and after mating.

The sponsor was asked to perform a post-hoc statistical analysis of the data from pivotal study (b) (4) 249 and study 1729/8 to further evaluate the statistical significance of this finding. In addition, the sponsor was asked to provide a reference supporting the statement that this finding represents a delay in fetal development and an explanation of their finding of the IVSD being “small” as used to describe the finding in study (b) (4) 249. The sponsor received this request on August 31, 2007, and provided a response October 3, 2007 (sequence #17 to the Cervarix BLA). In this response, the sponsor states that a demonstration of statistically significant increase in incidence of IVSD observed in (b) (4) 249 is not possible because the number of litters affected is below 5 and therefore, statistical tests are of minimal value in this analysis. Furthermore, sponsor states that the occurrence of IVSD in study 1729/8 is also not statistically significant. Sponsor concludes that the incidence of IVSD in rats and rabbits in studies (b) (4) 249 and (b) (4) 1729/8 is of spontaneous nature. Sponsor attributes the occurrence of membranous intraventricular septal defects to a delay in normal development that will close with further normal development. The reviewer concluded that GSK has satisfactorily refuted a potential association of IVSD with HPV/AS04 vaccine and noted that GSK is planning a pregnancy registry following licensure of Cervarix in the US which will capture outcomes of registered pregnancies. For more details see the review of the reproductive toxicology studies by Dr. Gruber under BLA 125258.

***Study 7: MPL: Subcutaneous Study of Pre- and Postnatal Development in the Rat-In (b) (4). Study number: (b) (4) 1729/17.***

Reviewer: Marion Gruber

This study has been originally submitted to BLA 125259 and has been reviewed by Dr. Marion Gruber, see her review below.

**Summary:**

This study assessed the effects of MPL on the pre- and postnatal development, including maternal function, in the rat when administered subcutaneously. Twenty-four (b) (4):CD((b) (4)) rats/group and were dosed with MPL by subcutaneous injection at 0, 1, 10 and 100 µg/kg/day. The parental females were dosed daily from day 6 of gestation to day 21 *postpartum*, inclusive. The females were allowed to litter and rear their offspring to weaning. Twenty animals of each sex were randomly selected from each group to form the F<sub>1</sub> generation. These animals were maintained untreated for 12

weeks post-weaning (maturation phase) before being paired for up to 15 days. Mated F<sub>1</sub> females were killed on day 13 of gestation and their uterine contents examined. The F<sub>1</sub> males were killed in week 17.

Administration of 1, 10 and 100 µg/kg/day MPL to the F<sub>0</sub> generation from day 6 of gestation to day 21 of lactation had no effect on the F<sub>1</sub> generation under the conditions of the study and parameters assessed. The experimental design of this study does not follow current recommendations outlined in CBER's guideline regarding developmental toxicity studies for vaccines; however, this study was conducted prior to the availability of the guidance document. Of note is that this study did not include visceral and skeletal examinations of the F<sub>1</sub> generation. Thus, the study is limited in terms of providing information on potential teratogenic effects due to MPL. However, other parameters assessed, such as uterine parameters, body weight, viability, and development of offspring suggest that MPL does not adversely affect pre- and postnatal development in the test species under the conditions of the study. A certificate of analysis for the test article, i.e., MPL was not provided nor was the expiry date for the test article indicated.

In conclusion, administration of 1, 10 and 100 µg/kg/day MPL from Day 6 of gestation to day 21 of lactation elicited no maternal toxicity and there were no adverse effects of maternal treatment on the F<sub>1</sub> generation.

***Study 8: DQ\* Immunostimulant: Study for Effects on Female Fertility, Embryo-Fetal and Pre- and Postnatal Development in the CD Rat by Intramuscular Administration (Including Pre-Mating Immunization Phase). Study number: (b) (4).***

**Summary:**

Female (b) (4):CD (b) (4) rats received DQ at doses of 4, 20 and 40 µg of QS-21/occasion by intramuscular administration or received saline control. Animals were treated on days -28 and -14 before pairing as well as on days 3, 8, 11 and 15 after mating and on day 7 of lactation.

There was no adverse effect on bodyweight or bodyweight gain during the pre-pairing, gestation and lactation period. A slight, but statistically significant reduction in food consumption and body weight gain was observed in animals receiving DQ at 40 µg of QS-21/occasion between days 3 and 6 of gestation (after the 3<sup>rd</sup> administration). Pre-coital interval, mating performance and fertility were unaffected by treatment with DQ. No effects on litter data and placental, fetal and litter weights were observed. Detailed fetal examination did not reveal any major or minor abnormalities or skeletal variants considered to be related to treatment. No treatment related effects on gestation and parturition were observed; litter size, offspring survival and sex ratio were unaffected by DQ up to 40 µg of QS-21/occasion. Offspring bodyweight on day 1 of age and bodyweight gain up to day 25 of age showed no adverse effects of maternal treatment with DQ, and there were no effects on the development of normal reflexes in the offspring.

In conclusion, DQ, at doses of 4, 20 and 40 µg of QS-21/occasion did not adversely affect female fertility, embryo-fetal or pre- and post-natal survival, growth or development of the offspring up to day 25 of age.

(b) (4)

***Study 9: DQ –Developmental Toxicity Study (Including Teratogenicity and Postnatal Investigations) by the Intramuscular Route in the Rabbit. Study number: (b) (4) AB14898.***

**Summary:**

Three groups of female (b) (4) rabbits were administered intramuscular injections of DQ adjuvant containing 20, 100 or 200 µg/mL of QS2, 28 and 14 days before the start of mating and on days 3, 8, 11, 15 and 24 of gestation, then on day 7 of lactation (littering sub-groups only). A control group of female (b) (4) rabbits was administered sterile physiological (b) (4). Animals were either assigned to a Caesarean sub-group (all females were necropsied on day 29 *post-coitum*) or littering sub-group.

There were no treatment-related clinical signs before mating or during the gestation and lactation periods. At 200 µg/mL of QS-21, a slight reduction in mean body weight gain (not statistically significant) between days 0 and 14 of the pre-mating period (after the first dosing) was observed. A statistically significant mean body weight loss occurred between days 24 and 29 of gestation, leading to a statistically significant reduction in mean body weight gain between days 0 and 29 of gestation. Conversely, a statistically significant increase in mean body weight gain was observed between days 4 and 35 of lactation, mainly due to a higher mean body weight gain between days 7 and 14 of lactation. There were no treatment-related effects on body weight or body weight gain in the 20 or 100 µg/mL QS-21 dose groups during the pre-mating, gestation or lactation periods. At 200 µg/mL of QS-21, a slight but statistically significant decrease in mean food consumption was observed between days 0 and 7 of pre-mating period. A slight decrease in mean food consumption was also noted from day 11 of gestation onwards (Caesarean sub-group), that was statistically significant between days 24 and 29 of gestation (littering sub-group). No effects on mean food consumption were observed in the 20 or 100 µg/mL QS-21 dose groups during the pre-mating or gestation periods. No treatment-related effects on food consumption were seen during the lactation period.

The adjuvant produced no effects on mating performance or fertility of the females in either the Caesarean or littering sub-groups. No treatment-related effects on gravid uterine weight were observed in the caesarean sub-group, nor were treatment-related effects on ovary weight seen in the Caesarean or littering sub-groups. A dose-related lower net (minus uterine weight) mean body weight change was observed in the treated groups, compared with control, attaining the statistical significance at the 200 µg/mL QS-21 dose level. Necropsy examination of the females did not reveal any treatment-related lesions.

In the Caesarean sub-group, 23, 22, 23 and 19 females were pregnant in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively, and all pregnant females had viable fetuses, with the exception of two control females. No effects of the DQ adjuvant on the pre- and post-implantation parameters were observed. Slightly statistically significant lower mean fetal weight was observed in the 200 µg/mL QS-21 group, however, no effects of treatment on mean fetal weight were seen in the 20 or 100 µg/mL QS-21 dose groups. No effects of treatment on the fetal sex ratio were observed in any DQ dose group.

Examination of the live fetuses revealed 7 malformed fetuses from 6 different litters in the 200 µg/mL QS-21 group, 0 malformed fetuses in the 100 µg/mL QS-21 group, 2 malformed fetuses

from separate litters in the 20 µg/mL QS-21 group, compared with 1 malformed fetus in the control group. In the 200 µg/mL QS-21 group, three fetuses from separate litters had defects of the aortic arch (retro or high arched) suggestive of a possible association with treatment at the highest dose. In the 20 or 100 µg/mL QS-21 dose groups, neither the incidence nor type of the malformations suggested any association with treatment due to their diverse nature or since they are part of the background of morphological changes in the (b) (4) rabbit strain used in this study.

**Study no.:** (b) (4) 14898

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 20 December 2012

**GLP compliance:** yes

**QA reports:** yes

**Drug, lot #, and % purity:**

DQ (QS-21-10 µg/500 µL) at 10 µg: (b) (4)

DQ (QS-21-50 µg/500 µL) at 50 µg: (b) (4)

DQ (QS-21-100 µg/500 µL) at 100 µg: (b) (4)

**Doses:** DQ adjuvant containing QS-21, DQ is the detoxified formulation of QS-21, animals received 20, 100 or 200 µg given in 2 injections of 500µL (concentration: 20, 100 or 200 µg/mL, respectively), ratio between QS-21: cholesterol: DOPC is (b) (4)

DQ at 10 µg: 20 µg/mL QS-21, 400 µg/mL DOPC and 100 µg/mL cholesterol.

DQ at 50 µg: 100 µg/mL QS-21, 2000 µg/mL DOPC and 500 µg/mL cholesterol.

DQ at 100 µg: 200 µg/mL QS-21, 4000 µg/mL DOPC and 1000 µg/mL cholesterol

Human DQ: 50 µg/mL QS-21, 1000 µg/mL DOPC and 250 µg/mL cholesterol

**Frequency:** Once, 28 days (i.e. day 0 of study) and 14 days (i.e. day 14 of study) before mating, on days 3, 8, 11, 15 and 24 of gestation (i.e. G 3, G 8, G 11, G 15 and G 4) then on day 7 of lactation (i.e. L 7; for littering sub-groups only).

**Species/strain:** (b) (4)

**Number/sex/group:** 55 females per dosage group (25 Caesarean and 30 littering)

**Route, and volume:** intramuscular, 500 µL; Bolus injection in the dorsal lumbar muscles (right and left sites for each occasion) using sterile syringe and needle after disinfection with an antiseptic solution.

**Study design:**



Group number/ Treatment	Dose level per injection (µg of QS21/0.5mL)	Dose level per occasion (µg of QS21/mL)	Dose volume per injection (mL/site)	Dose volume per occasion (mL/occasion)
1. Control	0	0	0.5	1
2. Low dose	10	20	0.5	1
3. Intermediate dose	50	100	0.5	1
4. High dose	100	200	0.5	1

Females received 2 injections of 0.5 mL for a total of 1 mL injected/occasion.

Table 93: Study design (study AB14898) (repro tox study # 9); sponsor provided

Test article was given 28 days and 14 days before mating and on days 3, 8, 11, 15 and 24 of gestation, and on day 7 of lactation (for littering sub-groups)

#### Parameters and endpoints evaluated:

**Morbidity/mortality:** All adults were observed twice daily at the beginning and at the end of each working day. Offspring were examined daily from postnatal day 1 (PND 1) with minimal interference to nursing between postnatal days 1 and 7.

**Clinical observations:** All females were observed daily for clinical signs. At the end of gestation, the females in the littering phase were inspected at least twice daily for signs of parturition. A physical examination was performed weekly (including pretest). Offspring were observed daily from postnatal day 1 (PND 1). The clinical observation was performed from outside the nesting box during the early phase of lactation (between PND 1 and 7)

**Injection site observations:** Any local reactions at the injection sites were assessed daily until disappearance. However, since reduced food consumption was noted in some females from all groups at the end of the gestation period, it was decided to not perform the injection site observations from day 27 of gestation to day 6 of lactation in the littering sub-groups, to avoid any interference with mating, gestation, littering and other reproductive parameters. During the lactation phase, the local reactions were observed on day 7 of lactation to minimize any interference with the nursing behavior, then they were observed daily until disappearance of the signs.

#### Body weight:

Individual body weights for females were recorded:

- once pretest on day -8 or -7 (at randomization)
- on day 0 (28 days before mating) and day 14 (14 days before mating) of the study
- on days 0 (day of mating), 6, 9, 11, 16, 20, 24 and 29 of gestation
- on days 4, 7, 11, 14, 17, 21, 28 and 35 of lactation (littering sub-group only).

**Food consumption:**

Food consumption of each female was recorded daily from the day of arrival to day 29 of gestation for the caesarean sub-groups and to day 35 of lactation for the littering sub-groups and reported as follows:

- daily pretest (from arrival)
- the mean (g/animal/day) was calculated for the periods (days)
- 0 to 7, 7 to 14, 14 to 21 and 21 to 28 before mating
- 0 to 6, 6 to 9, 9 to 11, 11 to 16, 16 to 20, 20 to 24 and 24 to 29 of gestation
- 0 to 4, 4 to 7, 7 to 11, 11 to 14, 14 to 17, 17 to 21, 21 to 28 and 28 to 35 of lactation (Littering sub-group only).

**Mating:**

Cohabitation started 28 days after the first administration. Each day, a number of females was paired with males of the same strain for up to 10 minutes or until copulation occurred. Following observed copulation, the males and females were left together for at least 1 hour. Unmated females were paired with a different male on the same day or on subsequent days until copulation occurred (up to 10 days).

**Parturition observations and gestation length:****Pregnancy and parturition – Littering subgroup only:**

From day 30 of gestation, each female in the littering sub-groups was observed at least 4 times a day for the onset and duration of parturition.

The following data were recorded:

- date of mating
- date of parturition (day 0 of lactation or L 0)
- duration of gestation
- abnormalities of delivery, nesting, or nursing behavior
- number of implantation sites (at necropsy).

**Litter data – Littering subgroup only**

For each litter, the following data were recorded:

- number of pups born (live and dead)
- external abnormalities of the pups
- number and weight of live pups on PND 4, 7, 11, 14, 21, 28 and 35
- physical development of the offspring, as assessed by the intra-litter onset and duration of incisor eruption, fur growth and eye opening on PND 4 then from PND 7 (any pup failing the eye-opening test was examined the following days until attainment)
- behavioural and functional tests in all pups as follows:
- pupillary reflex and auditory reflex on PND 35
- external and necropsy findings of dead pups.

**Necropsy schedule:**

Surviving females were necropsied according to the following schedule:

Caesarean sub-groups:

- on day 29 of gestation

- two females (group 1 female no. 7484 and group 4 female no. 7550) that failed to mate (mating not detected) were killed on study day 39 (end of the mating period).

**Littering sub-groups:**

- on L 35 (with litter)
- three females (group 1 female no. 7379, group 2 female no. 7385 and group 3 female no. 7435) that failed to mate (mating not detected) were killed on study day 39 (end of the mating period)
- mated females that failed to produce a viable litter were necropsied on day 35 or 37 *post-coitum*
- one female with undetected mating (group 4 female no. 7458): after completion of the mating period on study day 39
- females with total litter death were necropsied on L 0 (group 2 female no. 7411), PND 2 (group 2 female no. 7386 and group 4 female no. 7451), or PND 7 (group 4 female no. 7452)
- one female with total litter resorption (group 4 female no. 7454) was necropsied on day 35 of gestation.

**Necropsy of gestating and lactating females:**

All females, at the scheduled sacrifice (G 29 for the Caesarean sub-groups and L 35 for the littering sub-groups) were weighed and submitted to a macroscopic examination, including the thoracic, abdominal and pelvic viscera, and injection sites. Abnormal organs or tissues were sampled and preserved in 10 % neutral formalin but were not examined further.

The number and distribution of uterine implantation scars were recorded for all littering sub-group females that gave birth.

**Organ weights**

The ovaries from all surviving females from both sub-groups were weighed paired.

**Caesarean examinations - Caesarean sub-groups**

The ovaries and uterus of each female were removed and examined. The placentae were also examined. The following data were recorded:

- pregnancy status
- number of corpora lutea
- number of implantations
- number and distribution of live fetuses
- number and distribution of embryonic/fetal deaths, classified as follows:
  - early: only placenta visible at termination
  - late: both placenta and embryonic tissue visible at termination
- dead fetus
- gravid uterus weight
- individual fetal weights
- fetal sex.

**Fetal examinations - Caesarean sub-groups:**

All live fetuses were examined for external defects and killed by oral intubation of (b) (4). Dead fetuses were examined externally, preserved in (b) (4) but not examined further. All live fetuses were examined viscally and sexed at the time of Caesarean section. Following this, the heads of approximately half of the fetuses in each litter were removed and fixed for subsequent examination by serial sectioning. The eviscerated fetal carcasses were fixed and processed for skeletal examination. The ossified skeleton was (b) (4).

**Necropsy of pups - Littering sub-groups**

All pups were given a macroscopic examination of the thoracic, abdominal and pelvic viscera for structural or pathological changes following an intracardiac injection of (b) (4). Abnormal organs or tissues were sampled and preserved in (b) (4) but were not examined further. The pups (including decedents) were sexed, where possible, by internal inspection.

**Reproductive assessment:**

Fetal abnormalities are categorized as follows:

- Malformations - structural defects which are rare in the control population and are thought to be life threatening or of major physiological consequence.
- Anomalies - minor abnormalities or defects which are relatively rare in the control population and/or are considered not to be of major physiological consequence.
- Variations - minor abnormalities, defects or alternative forms which are either common in the control population or are of no known physiological consequence.

For the Caesarean sub-groups, the following parameters were calculated:

$$\text{Pre-implantation loss (in \%): } \frac{\text{Number of corpora lutea} - \text{Number of implantations}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Post-implantation loss (in \%): } \frac{\text{Number of implantations} - \text{Number of viable fetuses}}{\text{Number of implantations}} \times 100$$

The following reproductive indices were calculated for the littering sub-groups:

$$\text{Gestation index (in \%):} \quad \frac{\text{number of females with live pups}}{\text{number of pregnant females}} \times 100$$

$$\text{Lactation index (in \%):} \quad \frac{\text{number of pups alive on PND 35}}{\text{number of pups alive on PND 4}} \times 100$$

$$\text{Live birth index (in \%):} \quad \frac{\text{number of pups born alive}}{\text{number of pups born}} \times 100$$

The following indices were calculated for both Caesarean and littering sub-groups:

$$\text{Pre-coital interval (in days):} \quad \frac{\text{sum of days until successful insemination}}{\text{number of inseminated females}}$$

$$\text{Copulation (mating) index (in \%):} \quad \frac{\text{number of inseminated females}}{\text{number of paired females}} \times 100$$

$$\text{Fertility index (in \%):} \quad \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

$$\text{Sex ratio (proportion of male) (in \%):} \quad \frac{\text{number of males}}{\text{number of pups}} \times 100$$

## Statistical methods

### Gestation, lactation and fetus/pup-related data analysis:

The data were checked for homogeneity of variance across groups using Bartlett's test.

- Homogeneous data were then analyzed by parametric methods, i.e. one-way analysis of variance (ANOVA) followed by Dunnett's test if the ANOVA was significant.
- Non-homogeneous data were analyzed by non-parametric methods, i.e. Kruskal-Wallis test followed by Dunn's test if the Kruskal-Wallis was significant.

The numbers of resorptions and all litter-based percentages were analyzed using the above non-parametric methods. Selected incidence data were analyzed using a chi2 test for all groups

followed by two-tailed Fisher's exact test with Bonferroni correction for each treated group versus the control if the chi2 was significant.

#### **Pretest body weight, pretest food consumption, terminal body weight and organ weight data analysis**

For pretest body weights, pretest food consumption, terminal body weights, absolute and relative organ weights, statistical analyses were performed by the Provantis data acquisition system as follows:

The best transformation for the data (none, log or rank) was determined depending upon the kurtosis of the data, the probability of the Bartlett's test for homogeneity of the variances and the similarity of the group sizes.

- Non- or log-transformed data were analyzed by parametric methods. Rank transformed data were analyzed using non-parametric methods.
- The homogeneity of means was assessed by one-way analysis of variance (ANOVA).

Data were then analyzed to test for a dose-related trend to detect the lowest dose at which there was a significant effect, based on the Williams test for parametric data or Shirley's test for non-parametric data. If no trend was found and the means were not homogeneous, the data were analyzed by a stepwise parametric or non-parametric Dunnett's test to look for significant differences from the control group.

#### **Pre-coital interval data analysis**

The pre-coital interval were analyzed using the following methods:

- Levene's test was used to test the equality of variance across groups and Shapiro-Wilk's test was used to assess the normality of the data distribution in each group.
- Data with homogeneous variances and a normal distribution in all groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test if the ANOVA was significant.
- Data showing non-homogeneous variances or a non-normal distribution in at least one group were analyzed using Kruskal-Wallis test followed by the Wilcoxon's rank sum test if the Kruskal-Wallis test was significant.

### **Results:**

#### **F0 Generation**

##### Mortality/Clinical signs:

One female given 100 µg/mL of QS-21 was prematurely sacrificed on day 18 of gestation following severe clinical signs, mainly characterized by diarrhea and subdued behavior, marked body weight loss (-451 g between days 9 and 16 of gestation) associated with no food intake between days 11 and 18 of gestation. At necropsy, no treatment-related macroscopic findings were observed. Since this premature death was not observed in the high dose group, this isolated case was considered to be not treatment related.

One female given 200 µg/mL of QS-21 (Caesarean sub-group no. 7570) was sacrificed after aborting on day 28 of gestation. No specific clinical signs were observed during the gestation period. A body weight loss of -103 g was noted between days 11 and 20 associated with reduced

food consumption between days 11 and 28 of gestation. No macroscopic findings were noted at necropsy. This female had 13 implantation sites but no live fetus. The sponsor considered this case to be isolated and not treatment-related, since abortion can be noted in the historical control data (2/25 pregnant females aborted and 1/21 pregnant females aborted in two different studies in 2010). However, since this animal also showed significant weight loss it could also be a consequence of the observed maternal toxicity in the high dose group.

Red fluid in the cage, red vaginal discharge, few, soft or no feces, hair loss and/or scratches on the back (outside the treatment area) were observed on some occasions during the study and were considered to be incidental since they were also observed in controls or during the pretest period and can occur spontaneously in the rabbit.

Some local reactions, including hematoma (grade 1 to 4), very slight to slight edema, small induration and/or very slight to well defined erythema were noted after each injection in all groups, including the control group. The incidence and the duration of these reactions were similar between the control and treated groups and were considered to be related to the administration procedure.

However, in three female animals, severe edema was observed:

- 2 days after the fourth injection (day 8 of gestation) in one female treated at 100 µg/mL of QS-21
- 1 day after the fifth injection (day 11 of gestation) in one female treated at 100 µg/mL of QS-21
- 2 days after the third injection (day 3 of gestation) in one female treated at 20 µg/mL of QS-21

This local reaction disappeared three to five days after the injection for the first two females and 19 days after the injection for the third.

Observation at the injection site (summary of all events)	Injection	F0 generation			
		Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Bleeding		56	59	50	61
Induration (grade:1/2/3/4)	1 <sup>st</sup>	(4/0/0/0)	(0/0/0/0)	(1/0/0/0)	(2/0/0/0)
	2 <sup>nd</sup>	(0/0/0/0)	(4/0/0/0)	(0/0/0/0)	(1/0/0/0)
	3 <sup>rd</sup>	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	4 <sup>th</sup>	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	5 <sup>th</sup>	(0/0/0/0)	(0/0/0/0)	(2/0/0/0)	(0/0/0/0)
	6 <sup>th</sup>	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	7 <sup>th</sup>	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
	8 <sup>th</sup>	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)	(0/0/0/0)
Edema/Erythema (grade:1/2/3/4)	1 <sup>st</sup>	(9/2/0/0)	(6/1/0/0)	(2/0/0/0)	(2/1/0/0)
	2 <sup>nd</sup>	(/0/0/0)	(0/0/0/0)	(5/0/0/0)	(3/0/0/0)
	3 <sup>rd</sup>	(0/0/0/0)	(1/7/6/4)**	(1/0/0/0)	(4/0/0/0)
	4 <sup>th</sup>	(0/0/0/0)	(13/2/0/0)	(3/1/0/2)	(7/6/0/0)
	5 <sup>th</sup>	(0/0/0/0)	(4/2/1/0)	(2/0/0/2)	(4/0/0/0)
	6 <sup>th</sup>	(3/0/0/0)	(1/3/0/0)	(2/0/0/0)	(0/0/0/0)
	7 <sup>th</sup>	(2/0/0/0)	(2/0/0/0)	(5/0/0/0)	(12/0/0/0)
	8 <sup>th</sup>	(0/0/0/0)	(1/0/0/0)	(1/0/0/0)	(5/0/0/0)
Hematoma	1 <sup>st</sup>	(4/8/1/1)	(16/0/1/0)	(15/1/0/0)	(6/1/2/0)

Observation at the injection site (summary of all events)	Injection on	F0 generation			
		Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Bleeding		56	59	50	61
(grade:1/2/3/4)	2 <sup>nd</sup>	(6/0/0/0)	(11/2/0/0)	(6/4/0/0)	(12/3/2/0)
	3 <sup>rd</sup>	(5/0/0/0)	(7/0/0/0)	(4/0/0/0)	(13/0/0/0)
	4 <sup>th</sup>	(11/0/0/0)	(1/0/0/0)	(3/0/0/0)	(8/0/0/0)
	5 <sup>th</sup>	(5/0/0/0)	(19/7/0/0)	(17/2/0/0)	(13/5/0/0)
	6 <sup>th</sup>	(16/6/0/0)	(10/3/0/0)	(15/3/0/0)	(10/8/0/0)
	7 <sup>th</sup>	(12/3/0/0)	(26/0/0/0)	(13/0/0/0)	(20/0/0/0)
	8 <sup>th</sup>	(3/4/0/0)	(10/3/0/0)	(7/0/0/0)	(2/0/0/0)

\* Each animal received 2 administrations on each dosing day. \*\* data for grade 2, 3 and 4 edemas are seen in one animal over several days

Table 94: Injection site observations (study AB14898) in the littering and caesarian sub-group combined (repro tox study # 9); sponsor provided

### ***Erythema and eschar formation***

No erythema	0
Very slight erythema (barely visible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beetroot red) or eschar formation (deep lesion) making observation of intensity of erythema impossible	4

### ***Oedema formation***

No oedema 0	
Very slight oedema (barely visible)	1
Slight oedema (edges of area well-defined)	2
Moderate oedema (edges raised approximately 1 mm)	3
Severe oedema (edges raised more than 1 mm and extended)	4

### ***Induration***

No induration	0
Induration of an area < 1 cm <sup>2</sup>	1
Induration of an area > 1 cm <sup>2</sup> and < 2 cm <sup>2</sup>	2
Induration of an area > 2 cm <sup>2</sup> and < 3 cm <sup>2</sup>	3
Induration of an area > 3 cm <sup>2</sup>	4

### ***Hematoma***

No hematoma	0
Hematoma of an area < 1 cm <sup>2</sup>	1
Hematoma of an area > 1 cm <sup>2</sup> and < 2 cm <sup>2</sup>	2
Hematoma of an area > 2 cm <sup>2</sup> and < 3 cm <sup>2</sup>	3
Hematoma of an area > 3 cm <sup>2</sup>	4

### **Body weight:**



There was a slight and not statistically significant reduction in mean body weight gain between days 0 and 14 of the pre-mating period in the 200 µg/mL QS-21 group from both sub-groups (mean body weight gain of 225 g and 257 g in females from Cesarean and littering sub-groups, respectively, compared to a gain of 253 or 322 g in the controls). There was a statistically significant mean body weight loss between days 24 and 29 of gestation (after the last dosing on day 24 of gestation) in the 200 µg/mL QS-21 group sub-group; a mean loss in body weight of 88g (-88g) was observed between day 24 and 29 in animals receiving 200 µg/mL QS-21 while a mean weight gain of 72 g (+72 g) was seen in the control group. This effect resulted in a statistically significantly lower mean body weight gain between days 0 and 29 of gestation (mean overall body weight gain of 296 g and 299 g in females from the Cesarean and littering sub-groups, respectively, compared to a gain of 515 or 449 g in the controls). There was a statistically significantly higher mean body weight gain between days 4 and 35 of lactation in the 200 µg/mL QS-21 group, mainly due to a higher mean body weight gain between days 7 and 11 of lactation and between days 11 and 14 of lactation.

There were no relevant effects of treatment on body weight or body weight gain in the 20 or 100 µg/mL QS-21 dose groups during the pre-mating, gestation or lactation periods.

#### **Food consumption:**

There was a slight but statistically significant decrease in mean food consumption in the 200 µg/mL QS-21 group between days 0 and 7 of pre-mating period (-9 % and -15 % in the Cesarean and littering sub-group, respectively, compared with controls). This reduced mean food consumption was consistent with the body weight effect.

In the Cesarean sub-group, there was a slight decrease in mean food consumption from day 11 of gestation onwards in the 200 µg/mL QS-21 group, compared with the control group and/or the historical control data, leading to a statistically significantly lower mean food consumption between days 24 and 29 of gestation (-37 %, when compared with controls). Consequently, average mean food consumption was slightly reduced during the gestation period (days 0 and 29) in this dose group, compared with controls (-12 %).

The same tendency was noted in the littering high dose group but between days 24 and 29 of gestation only (-20 %, compared with controls). However, the mean food consumption was comparable with the control group during the gestation period (days 0 and 29 of gestation). This reduced mean food consumption was consistent with the body weight effect.

There was no effect on mean food consumption in the lower dose groups during the pre-mating and gestation periods. There were no effects of treatment on food consumption during the lactation period.

#### **Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):**

**Reproductive parameters examined (in F<sub>0</sub> animals), natural birth group:**

GROUP TREATMENT	1 0 µg/adm	2 10 µg/adm	3 50 µg/adm	4 100 µg/adm
<u>LITTERING AND CAESAREAN SUB-GROUPS:</u>				
NUMBER OF FEMALES:				
Paired	55	55	55	55
Failed to mate	2	1	1	1
Inseminated	53	54	54	54
Pregnant	51	51	50	48
Not pregnant	2	3	4	6
Aborted	0	0	0	1
With viable fetuses at caesarean section	21	22	23	19
No viable fetuses at caesarean section	2	0	0	0
Pregnant females allowed to litter	28	29	27	28
Total litter death <i>post-partum</i>	0	2	0	2
Total litter resorption	0	0	0	1
Elective sacrifice	0	0	1	0
Mistimed pregnancy	0	0	0	1
Reared pups to weaning	28	27	26	24
PRE - COITAL INTERVAL - DAYS				
MEAN	1.62	1.52	1.46	1.74
S.D.	1.30	1.48	1.46	1.37
N	53	54	54	53
COPULATION INDEX (%)	96	98	98	98
FERTILITY INDEX (%)	96	94	93	89
LACTATION INDEX (%)	100	93	96	86

Table 95: Summary of cohabitation data and maternal performance (study AB14898) in the littering and caesarean sub-group (repro tox study # 9); sponsor provided

All paired females mated with the exception of 2, 1, 1 and 1 in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively. The majority of animals mated on the first day of pairing and the mean pre-coital interval was consequently comparable in all groups. Most mated females became pregnant with the exception of 2, 3, 4 and 6 in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively. The slightly higher incidence of non-pregnant females in the group given 200 µg/mL QS-21 was due to 2 of the mating males (nos. 28 and 30) that between them failed to induce pregnancy in 5 females. The fertility index was consequently incidentally slightly lower (89 %) in the high dose group compared with the control (96 %), without statistical significance.

One female given 100 µg/mL QS-21 was prematurely sacrificed for ethical reasons on day 18 of gestation but was pregnant. One female given 200 µg/mL QS-21 for which mating was not detected (mistimed date of mating) was prematurely sacrificed, as soon as possible after the end of the mating period, while pregnant. For both females, fetuses were not submitted to examination due to their small size. One female given 200 µg/mL QS-21 was prematurely sacrificed following abortion.

#### **Maternal organ weights and terminal body weights:**

There were no treatment-related effects on ovary weights from females in the Caesarean or littering sub-groups. The ovary weights in the adjuvant groups were comparable with the concurrent control.

There were no treatment-related effects on gravid uterine weight in the Caesarean sub-groups. One female given 200 µg/mL QS-21 had a lower gravid uterus weight (173.1 g, compared with a mean control value of 489.2 g), however, this female only had two fetuses. A dose-related lower net (minus uterine weight) mean body weight change was observed in the treated groups, compared with control, attaining statistical significance at the 200 µg/mL QS-21 dose level. This is a reflection of the reduced maternal body weight gain and observed maternal toxicity in the high dose group.

	Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Net mean body weight change (g)	515	511	458	296**
Mean gravid uterine weight (g)	489	526	491	440
Net weight change minus uterine weight (g)	26	-15	-33	-144**

\* Each animal received 2 administrations on each dosing day; \*\*p<0.01; Net body weight change + terminal body weight minus day 0 body weight; net weight change = net body weight change minus uterine weight change.

Table 96: Summary of gravid uterus weight and body weight change (g) (study AB14898) (repro tox study # 9); sponsor provided

Parameter	F0 generation			
	Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Mating index (natural birth group)	96.7%	96.7%	96.7%	100.0%
Female Fertility Index (natural birth group)	96.6%	100.0%	93.1%	93.3%
Gestation Index (natural birth group)	100.0%	100.0%	96.3%	92.9%
Gestation Length (natural birth group)	31.5 days	31.4 days	31.6 days	31.3 days
Females completing delivery % (N)	93.3% (28)	96.7% (29)	86.7% (26)	86.7% (26)

ND: Not determined; \* each animal received 2 administrations on each dosing day

Table 97: Reproductive parameters (study AB14898), natural birth group, (repro tox study # 9); sponsor provided

#### Results F1: Caesarean data:

Parameter	F0 generation			
	Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Pregnant dams with no viable fetuses	2	0	0	0
Pregnant dams with viable fetuses	21	22	23	19
Corpora lutea per animal	9.9	10.3	10.2	9.8
Implantation sites per animal	8.4	9.0	8.6	8.9
Preimplantation loss per animal (% per animal)	1.5 (17.2%)	1.4 (12.5%)	1.7 (14.4%)	0.9 (9.0%)
Live fetuses per animal (% males/% female)	7.7 (47.0/53.0)	8.5 (43.4/56.7)	8.4 (48.2/51.8)	8.1 (51.5/48.5)
Postimplantation loss	15	11	3	16
Dead fetuses	0	0	0	0

Parameter	F0 generation			
	Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Resorption: early	10	2	1	11
Resorption: late	5	9	2	5
Fetal body weight	39.8	41.8	40.1	36.2**

\* Each animal received 2 administrations on each dosing day; \*\*p<0.05.

Table 98: Summary of Caesarean section data (study AB14898) (repro tox study # 9); sponsor provided

There were 23, 22, 23 and 19 pregnant females in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively, at the terminal Caesarean examinations. All pregnant females had viable fetuses, with the exception of two control females. One other pregnant female in the 200 µg/mL QS-21 group aborted earlier in gestation. This female had 13 implantation sites but no live fetus. This finding could be a background finding since abortion can be noted in the historical control data (2/25 pregnant females aborted and 1/21 pregnant females aborted in two different studies in 2010) or a consequence of maternal toxicity.

The pre-implantation data (mean numbers of corpora lutea, implantation sites and the percentage of pre-implantation loss) were comparable with the concurrent control and/or historical control range in all treated groups. The percentage pre-implantation loss was incidentally slightly higher in the control group (17.2 %) but remained within the historical control range (7.6 to 18.2 %).

The mean number of early resorptions was slightly higher in the 200 µg/mL QS-21 group (0.6 per female) in comparison with the mean historical control value (0.2) and the concurrent control value (0.4). However, the difference was essentially due to one atypical female within the group (no. 7565) with 8 early resorptions. It should be noted that 7 early resorptions were observed in one control female in one study performed in 2011 in the same laboratory. Consequently, this isolated finding was considered to be incidental. Mean live litter size was marginally higher in all treated groups compared with the control due to the incidentally high pre-implantation loss in the control group.

There was a slightly statistically significant lower mean fetal weight (36.2 g) in the 200 µg/mL QS-21 group (-9 %, compared with control 39.8 g; P<0.05) and historical control range (38.6 to 44.0 g).

### Results F1 generation (natural birth group)

Reproductive parameters:

GENERATION		F <sub>1</sub> LITTER			
		Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
FEMALES ON STUDY	N	30	30	30	30
MATING INDEX	(%)	96.7	96.7	96.7	100.0
GESTATION INDEX	N	100.0	100.0	96.3	92.9
FEMALES COMPLETING DELIVERY	N (%)	28 (100)	29 (96.7)	26 (86.7)	26 (86.7)
FEMALES WITH STILLBORN PUPS	N (%)	8 (28.6)	7 (24.1)	5 (19.2)	4 (15.4)

GENERATION		F <sub>1</sub> LITTER			
		Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
LITTERS WITH LIVEBORN BUT NO PUPS ALIVE DAY4		0	2	0	1
DAY 35		0	2	0	2
MEAN DURATION OF GESTATION	days	31.5	31.4	31.6	31.3
LITTER SIZE					
Litters with liveborn pups	N MEAN	28	29	26	26
Liveborn	N	226	216	212	218
Live Birth Index	%	93.8	91.9	97.7	97.8
Stillborn	N	15	19	5	5
	%	(6.2)	(8.1)	(2.3)	(2.2)
Pups dying. Missing an/or cannibalized Day 0	N	0	3	1	0
	%	0.0	1.4	0.5	0.0
Day 1-7	N	19	21	13	38*
	%	8.4	9.7	6.1	17.4
Day 8-35	N	13	14	16	10
	%	5.8	6.5	7.5	4.6
Day 0-4	N	5	17*	10	25**
	%	2.2	7.9	4.7	11.5
Day 0-35	N	32	38	30	48
	%	14.2	17.6	14.2	22.0
Pups surviving 4 days	N	221	199**	202	193***
Viability Index	%	97.8	92.1	95.3	88.5
Pups surviving 35 days	N	194	178	182	170
Lactation Index	%	87.8	89.4	90.1	88.1
LITTER WEIGHT IN G (♂/♀)					
Day 4	GRAM	83.0	83.3	83.5	77.1
Day 7	GRAM	111.7	111.7	113.4	108.8
Day 11	GRAM	115.4	155.4	155.7	152.1
Day 14	GRAM	185.2	183.8	189.8	182.9
Day 21	GRAM	256.2	267.4	276.4	266.9
Day 28	GRAM	454.1	178.0	479.6	472.1
Day 35	GRAM	752.9	744.3	771.1	753.9
SEX RATIO (M%)	Day 1	53.5	50%	49.1%	53.1%

\* Each animal received 2 administrations on each dosing day; \* each animal received 2 administrations on each dosing day; \*\* p<0.05; \*\*\* p<0.01

Table 99: Reproductive parameter (study AB14898) (repro tox study # 9); sponsor provided

There was no treatment-related effect on parturition and gestation length in any group. There were 28, 29, 26 and 26 females that completed delivery in the control, 20, 100 or 200 µg/mL QS-21 groups, respectively. The mean duration of gestation was comparable (approximately 31 days) in the treated and control groups. The mean numbers of implantation sites and delivered pups were comparable in all groups. As a consequence, there was no influence of treatment in any group on pre-birth loss.

The total number of live pups (226, 216, 212 and 218 in the control, 20, 100 or 200 µg/mL QS-21 groups, respectively) was comparable in all groups and the corresponding live birth index (93.8, 91.9, 97.7 and 97.8 %, respectively) were slightly superior in the 100 and 200 µg/mL QS-

21 groups due to fewer stillborn pups when compared with the control group (15, 19, 5 and 5 stillborn pups in the control, 20, 100 or 200 µg/mL QS-21 groups, respectively).

During the first week post-partum, there was a statistically significantly greater number of pups dying or missing in the 200 µg/mL QS-21 group, compared with the concurrent control (38 pups compared with 19 in the control group). This finding was due to two females (nos. 7451 and 7452) with total litter loss accounting for 16 of the affected pups. In the absence of an increase in pup death amongst the other females in the high dose group and since the pup viability index on PND 4 and the lactation index on PND 35 remained within the historical control range (84.7 % to 99.3 % for the viability index and 83.5 % to 93.7 % for the lactation index), no clear association with treatment was considered to have occurred.

There was also a statistically significantly higher incidence of pups dying or missing in the 20 µg/mL QS-21 groups over the first four days post-partum, which was due to two females with total litter loss (i.e. female nos. 7386 and 7411). The number of live pups per litter after PND 7 and through to PND 35 remained comparable between the treated groups and controls. Litter sizes in the treated groups were comparable with controls throughout lactation.

There was a slightly statistically significant lower mean fetal weight (36.2 g) in the 200 µg/mL QS-21 group (-9 %, compared with control 39.8 g;  $P < 0.05$ ) and historical control range (38.6 to 44.0 g). There was no effect of treatment on mean fetal weight in the lower dose groups. There was no effect of treatment on fetal sex ratio in any dose group.

#### Fetal alterations: F<sub>1</sub> generation: Caesarian data

			Control	10 µg/admin *	50 µg/admin*	100 µg/admin*
Litters evaluated		N	21	22	23	19
Fetuses evaluated		N	178	186	194	153
<b>Fetal external observations</b>						
Anasacra (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Cranial: acephalia (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Limbs: malrotated (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
<b>Fetal visceral observations</b>						
Thymus large (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Ventricular Septum defect (M)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Carotid: Narrowed (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Great vessels: malformed (M)	Fetal incidence	N	0	1	0	3
	Litter incidence	N	0	1	0	3
Common Carotid trunk: absent (V)	Fetal incidence	N	96	106	117	85
	Litter incidence	N	20	20	23	19
Innominate: absent	Fetal incidence	N	0	0	0	2
	Litter incidence	N	0	0	0	2

			Control	10 µg/admin *	50 µg/admin*	100 µg/admin*
Litters evaluated		N	21	22	23	19
Fetuses evaluated		N	178	186	194	153
Lung: azygos love absent (V)	Fetal incidence	N	3	1	7	3
	Litter incidence	N	3	1	4	3
Lung: small	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Diaphragmatic hernia (M)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Liver: dark raised area (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Liver: discolored (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Liver: additional fissure (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Liver: supernumerary lobe (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Liver: pale area (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Kidney: malposition	Fetal incidence	N	0	1	0	1
	Litter incidence	N	0	1	0	1
Kidney: malformed	Fetal incidence	N	0	0	0	2
	Litter incidence	N	0	0	0	0
Kidney: dilated renal pelvis, slight (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Kidney: pale (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Ovary: cyst (A)	Fetal incidence	N	4	0	1	1
	Litter incidence	N	3	0	1	1
Eyes: discolored (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
<b>Fetal skeletal observations</b>						
Phalanx: unossified 1 <sup>st</sup> or 5 <sup>th</sup> digit, forepaw (V)	Fetal incidence	N	9	19	16	24
	Litter incidence	N	8	6	8	11
Phalanx: incomplete ossification, forepaw (V)	Fetal incidence	N	0	0	0	3
	Litter incidence	N	0	0	0	3
Metacarpal: unossified 1 <sup>st</sup> digit, (A)	Fetal incidence	N	8	4	6	9
	Litter incidence	N	5	2	4	6
Phalanx: incomplete ossification, hindpaw (V)	Fetal incidence	N	1	1	1	3
	Litter incidence	N	1	1	1	2
Phalanx: unossified hindpaw (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Tarsal bone: incomplete ossification (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Sternebra: incomplete ossification (V)	Fetal incidence	N	19	25	12	14
	Litter incidence	N	9	12	9	9
Sternebra: 5 <sup>th</sup> unossified (V)	Fetal incidence	N	18	34	33	15
	Litter incidence	N	9	15	16	9
Sternebra: bibartite ossification (V)	Fetal incidence	N	3	2	1	3
	Litter incidence	N	3	2	1	2
	Fetal incidence	N	16	3	1	11

			Control	10 µg/admin *	50 µg/admin*	100 µg/admin*
Litters evaluated		N	21	22	23	19
Fetuses evaluated		N	178	186	194	153
Sternebra: incomplete ossification of 2 <sup>nd</sup> /4 <sup>th</sup> (V)	Litter incidence	N	4	3	1	6
Sternebra: minor fusion (V)	Fetal incidence	N	2	0	0	0
	Litter incidence	N	2	0	0	0
Sternebra: asymmetric (V)	Fetal incidence	N	3	1	1	0
	Litter incidence	N	1	1	1	0
Sternebra: 6 <sup>th</sup> unossified (V)	Fetal incidence	N	5	2	2	3
	Litter incidence	N	3	2	2	2
Sternebra: 2 <sup>nd</sup> /4 <sup>th</sup> unossified (V)	Fetal incidence	N	1	0	0	1
	Litter incidence	N	1	0	0	1
Sternebra: extra ossification site (V)	Fetal incidence	N	1	0	1	0
	Litter incidence	N	1	0	1	0
Sternebra: incomplete ossification of 1 <sup>st</sup> and 3 <sup>rd</sup> (V)	Fetal incidence	N	4	0	0	0
	Litter incidence	N	2	0	0	0
Number of full ribs 12/13 (V)	Fetal incidence	N	37	25	40	11
	Litter incidence	N	17	15	18	7
Rib: short (A)	Fetal incidence	N	48	55	59	33
	Litter incidence	N	18	19	20	14
Rib: detached (A)	Fetal incidence	N	6	8	5	6
	Litter incidence	N	5	5	2	6
Number of full ribs 12/12 (V)	Fetal incidence	N	62	77	49	45
	Litter incidence	N	16	22	18	11
Rib: incomplete ossification (A)	Fetal incidence	N	8	6	5	6
	Litter incidence	N	5	6	5	5
Rib: thickened (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Rib: absent (A)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Rib: unossified area (A)	Fetal incidence	N	0	1	1	1
	Litter incidence	N	0	1	1	1
Rib: cervical (A)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Cervical vertebra supernumerary (A)	Fetal incidence	N	3	0	1	0
	Litter incidence	N	3	0	1	0
Cervical vertebra: incomplete ossification (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Thoracic vertebra: incomplete ossification (A)	Fetal incidence	N	0	1	0	1
	Litter incidence	N	0	1	0	1
Thoracic vertebra: malformed (M)	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Lumbar vertebra: misshaped arch (A)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Lumbar vertebra: number 7 (A)	Fetal incidence	N	2	0	1	4
	Litter incidence	N	2	0	1	3
Lumbar vertebra: number 5 (A)	Fetal incidence	N	1	2	1	0
	Litter incidence	N	1	1	1	0
Lumbar vertebra: bipartite ossification (A)	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Caudal vertebra fused (A)	Fetal incidence	N	0	0	1	0



			Control	10 µg/admin *	50 µg/admin*	100 µg/admin*
Litters evaluated		N	21	22	23	19
Fetuses evaluated		N	178	186	194	153
	Litter incidence	N	0	0	1	0
Caudal vertebra misshapes (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Pelvis: incomplete ossification of pubis (A)	Fetal incidence	N	2	0	2	10
	Litter incidence	N	2	0	2	5
Pelvis: unossified pubis (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Arcania (M)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Cranium: parietal: unossified area (A)	Fetal incidence	N	2	1	0	0
	Litter incidence	N	2	1	0	0
Cranium: frontal: fused (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Cranium: frontal: structural bone (A)	Fetal incidence	N	0	1	1	0
	Litter incidence	N	0	1	1	0
Nasal: structural bone (A)	Fetal incidence	N	0	1	0	2
	Litter incidence	N	0	1	0	2
Mandibular: hyoid: incomplete ossification (V)	Fetal incidence	N	5	6	0	5
	Litter incidence	N	4	6	0	4
Maxilla: incomplete ossification (A)	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1

M-Malformation, V-Variation, A-Anomaly

Table 100: Fetal alterations (study AB14898) (repro tox study # 9); sponsor provided

Dose level of QS21 (µg/mL)	Female number	Male number	Fetus number	Malformation(s) <sup>#</sup>
0	7477	5	6	Diaphragmatic hernia
20	7501	13	1	Malformed thoracic vertebrae
	7504	15	5	Ventricular septum defect, malformed great vessels
	7515	10	7	Malpositioned kidney (left)
100	/		/	/
200	7551	25	6	Acephaly/acrania, narrowed carotid arteries
	7553	27	10	Malformed great vessels
	7557	31	2	Malpositioned kidneys (both), malformed kidney (right)
			8	Malpositioned kidney (right)
	7558	29	8	Malformed great vessels
	7566	25	2	Anasarca
	7574	31	1	Malformed great vessels Fused, malformed and malpositioned kidneys (both)

Individual descriptions; # including external, visceral and skeletal examinations, /: No malformation observed

Table 101: Summary of malformations (study AB14898) (repro tox study # 9); sponsor provided

### External observations:

There were two malformed fetuses from separate litters in the 200 µg/mL QS-21 group. One fetus had acephaly and the other had anasarca. There were no external malformations in the lower dose groups. These malformations were considered to be not treatment-related in view of the isolated nature of these findings (0.7 %, 1/153 fetuses) and since they are part of the background of changes noted in the strain of rabbit used (1 fetus had acrania and 1 fetus had local edema between 2007 and 2012).

Further, one fetus from the 200 µg/mL QS-21 group had malrotated hindlimbs. This anomaly was considered to be not treatment-related in view of the isolated nature of this finding restricted to a single litter and since it is part of the background of changes noted in the strain of rabbit used (5 fetuses between 2007 and 2012).

### Visceral observations:

There were 1 (1), 2 (2), 0 (0) and 6 (5) fetuses (litters) with visceral malformations in the control, 20, 100 and 200 µg/mL QS-21 groups, respectively.

Common findings included three fetuses from separate litters with malformed great blood vessels with defects of the aortic arch (retro or high arched) in the 200 µg/mL QS-21 group (female nos.

7553, 7558 and 7574) in comparison with none in the control and intermediate dose groups. One fetus from female no. 7504 in the 20 µg/mL QS-21 group also had malformed great blood vessels associated with persistent truncus arteriosus with pulmonary arteries arising from the descending aorta, and a ventricular septal defect. Two fetuses in the 200 µg/mL QS-21 group had malformed kidneys (one fetus from female no. 7557 had small right kidney and one fetus from female no. 7574 had fused kidneys on midline).

Other findings amongst the treated groups included malpositioned kidneys for three fetuses in the 200 µg/mL QS-21 group (two fetuses from female no. 7557 and one fetus from female no. 7574) and one fetus (female no. 7515) in the 20 µg/mL QS-21 group and narrowed carotid arteries in another fetus in the 200 µg/mL QS-21 group (female no. 7551). One control fetus had a diaphragmatic hernia (female no. 7477). These findings are isolated, part of the background of changes noted in the strain of rabbit used and were considered to be spontaneous in origin (historical control data: mal positioned kidneys and diaphragmatic hernia were observed in 2/1699 fetuses (0.12 %) between 2010 and 2012, 1/3915 fetuses (0.03 %) between 2007 and 2009, respectively).

There were no fetuses with visceral malformations in the 100 µg/mL QS-21 group.

The incidences of other less severe soft tissue anomalies/variations, including absent common carotid trunk, absent innominate artery, absent azygos lobe of the lungs, small lungs, dark or pale areas/additional fissure/supernumerary lobe in the liver, dilated renal pelvis, pale kidneys, ovarian cystic areas and/or discolored eyes did not suggest any association with treatment.

#### **Skeletal observations:**

There was no treatment-related effect on fetal skeletal development.

Skeletal malformations were observed in one fetus in each of the 20 and 200 µg/mL QS-21 groups. The fetus in the 20 µg/mL QS-21 group (female no. 7501) had malformed thoracic vertebrae with associated scoliosis and the fetus in the 200 µg/mL QS-21 (female no. 7551) had acrania. These findings are part of the background of changes noted in the strain of rabbit used (6 fetuses between 2010 and 2012 with a malformed thoracic vertebrae, 2 fetuses between 2010 and 2012 with scoliosis and 1 fetus between 2007 and 2012 with acrania) and were considered to be incidental.

The incidence of fetuses with incomplete ossification of the pubis was slightly higher in the 200 µg/mL QS-21 group (6.5 %) than in the control group (1.1 %) and the historical control data (2.4 %). Since this anomaly is part of the background of changes noted in the strain of rabbit used and was considered to be spontaneous in origin, this delay in ossification was considered to be of no toxicological significance.

The incidence of other skeletal anomalies and variations in the treated groups were comparable with the concurrent control and/or historical control data and therefore did not suggest any association with treatment.

Pre-weaning examinations F1					
		Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Incisor eruption	Day 4 (% positive of pups)	100%	100%	100%	100%
Fur growth	Day 4 (% positive of pups)	100%	100%	100%	100%
Pupil reflex	Day 35 (% positive of pups)	100%	100%	100%	100%
Auditory reflex	Day 35 (% positive of pups)	100%	100%	100%	100%
Eye opening	Day 8 (% positive of pups)	0%	0%	0%	0%
	Day 9 (% positive of pups)	0%	7%	5%	3%
	Day 10 (% positive of pups)	14%	33%	34%	27%
	Day 11 (% positive of pups)	50%	73%	76%	51%
	Day 12 (% positive of pups)	77%	92%	95%	82%
	Day 13 (% positive of pups)	96%	96%	99%	92%
	Day 14 (% positive of pups)	100%	100%	100%	95%
	Day 15 (% positive of pups)	100%	100%	100%	97%*

\*Less than 100% due to death of pups.

Table 102: Summary of reflex and physical development (study AB14898) (repro tox study # 9); sponsor provided

Pup physical development, as assessed by the day of occurrence of incisor eruption, fur growth and eye opening, was similar in the control and treated groups. The evaluation of pup reflexes (surface righting, pupil and auditory responses) did not reveal any evidence of functional defects in the adjuvant groups.

#### Pub necropsy observations:

			Control	10 µg/admin*	50 µg/admin*	100 µg/admin*
Litters evaluated		N	28	27	26	24
Fetuses Live evaluated		N	226	207	211	200
Fetuses Stillborn evaluated		N	15	10	5	5
Gross Exam: Omphalocele	Fetal incidence	N	0	1	0	0
	Litter incidence	N	0	1	0	0
Lung: dark area	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Kidney: malpositioned	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Kidney: small	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Kidney: abnormal shape	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Dilated renal pelvis	Fetal incidence	N	0	0	0	1
	Litter incidence	N	0	0	0	1
Abnormal cavity: autolysis	Fetal incidence	N	5	2	6	6
	Litter incidence	N	3	2	4	4
Abnormal cavity: clear fluid	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
Urinary bladder dilatation	Fetal incidence	N	1	1	0	0
	Litter incidence	N	1	1	0	0
Abnormal cavity: cannibalized	Fetal incidence	N	0	2	0	4
	Litter incidence	N	0	2	0	3
Limb/paw: cannibalized	Fetal incidence	N	0	3	1	0

			Control	10 µg/admin *	50 µg/admin*	100 µg/admin*
Litters evaluated		N	28	27	26	24
Fetuses Live evaluated		N	226	207	211	200
Fetuses Stillborn evaluated		N	15	10	5	5
	Litter incidence	N	0	3	1	0
Limb/paw: hyperextension	Fetal incidence	N	1	0	0	0
	Litter incidence	N	1	0	0	0
	Fetal incidence	N	0	1	0	0
Uterus: enlarged	Litter incidence	N	0	1	0	0
	Fetal incidence	N	4	2	5	7
Thoracic cavity: autolysis	Litter incidence	N	3	2	3	4
	Fetal incidence	N	0	0	0	2
Thoracic cavity: cannibalized	Litter incidence	N	0	0	0	1
	Fetal incidence	N	1	0	0	1
Tale: cannibalized	Litter incidence	N	1	0	0	1
	Fetal incidence	N	1	0	0	1

Table 103: Pub necropsy observations (repro tox study # 9); sponsor provided

Neither the incidence nor type of pup observations noted suggested any association with treatment in any group. One pup from female no. 7409 given 20 µg/mL QS-21 was prematurely sacrificed on PND 0 due to an omphalocele with protusion of the intestines. Since this malformation was observed in only one pup at the low dose level and was not seen for any fetus in the Caesarean sub-groups, this finding was considered to be incidental. One pup from female 7448 given 200 µg/mL QS-21 was found dead on PND 4. At necropsy, a malpositioned, small and abnormally shaped kidney was observed. One pup from female 7457 given 200 µg/mL QS-21 was prematurely sacrificed on PND 6 and dilated renal pelvis with reduced papillae was observed at necropsy. These isolated findings were considered to be incidental.

## Conclusions

Intramuscular administrations of DQ adjuvant containing 200 µg/mL of QS-21 to (b) (4) rabbits starting 28 and 14 days before the start of mating and on gestation days 3, 8, 11, 15 and 24 and on day 7 of lactation induced a significant maternal mean body weight loss associated with reduced mean food consumption at the end of the gestation period. In addition, lower mean fetal weight was noted at this dose level. Defects of the aortic arch (retro or high arched) were observed in three fetuses from separate litters suggestive of a possible association with treatment at this dose. Doses of DQ adjuvant containing 100 or 20 µg/mL of QS-21 did not induce any adverse effects on maternal condition or embryo-fetal and post-natal development. Under the defined experimental conditions of the study, the dose level of DQ adjuvant containing 100 µg/mL of QS-21 (corresponding to 30 µg of QS-21/kg body weight considering a mean body weight of 3.33 kg for female rabbits) was selected as the NOAEL (No Observed Adverse Effect Level) for maternal, pre- and post-natal toxicity.

## Genotoxicology Studies Reviews:

### Genotoxicology studies: *in vivo*

Reviewer: Nabil Al-Humadi

**Study # 1: Genetic Toxicology (Micronucleus Test). Study number.:** (b) (4)

**Study title:** Comparison of different test formulations in the rat micronucleus test.

**Study no.:** (b) (4) 317/032657

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** March 04, 2003.

**Date of study completion:** June 05, 2003.

**GLP compliance:** Yes

**Drug, lot #, and % purity:**

IV1, (b) (4). Stable for 15 days.

IM1, (b) (4) end of filling (pool).

IM2, (b) (4). Stable for 15 days.

IM3, (b) (4) end of filling (pool).

**Strains/species/cell line:** (b) (4) (CD) Rat.

**Breeder/supplier:** (b) (4).

**Number of animal per group and sex:** 5/sex/group

**Age:** Not provided.

**Body weight range:** 120-150 g males, 120-140 g females

**Route, site, and frequency of administration:**

Animals in the vehicle and IV1 groups were dosed by intravenous injection at a volume dosage of 0.5 mL.

Animals in IM1, IM2, and IM3 groups were dosed by intramuscular injection at a volume dosage of 0.1 mL in each hind limb. Dosing was administered on two occasions, the second dose administered approximately 24 hours after the first dose.

No positive control was used.

**Volume of injection:** For vehicle and IV1 groups 0.5 mL/day, and for IM1, IM2, and IM3 groups 0.2 mL/day.

## Methods

### Study design

Toxicity test 1: Used to determine the suitable dose level for use in the micronucleus test.

Group Number	Description	Dose Route	Dosage Volume* (mL/day)	Dose Concentration (µg/animal)	Animal Numbers	
					Male	Female
1	Vehicle (Saline)	Intravenous	0.5	0	5	5
2	IV1	Intravenous	0.5	1.05	5	5
3	IM1	Intramuscular	0.2 <sup>a</sup>	0.66	5	5
4	IM2	Intramuscular	0.2 <sup>a</sup>	8.92	5	5
5	IM3	Intramuscular	0.2 <sup>a</sup>	0.50	5	5

\* Constant volume irrespective of individual bodyweight.

<sup>a</sup> 0.1 mL in each hind limb

Table 104: Experimental design ((b) (4) study # 1).

**Doses used:** (b) (4) CD rats in the vehicle control group (sterile 0.9% w/v physiological saline) and IV1 treatment group were dosed by intravenous injection at a volume dosage of 0.5 ml. The aim of the study was to evaluate the possible risk of a contamination with (b) (4). Concentration levels of this compound were measured in

the batches used in the micronucleus test and the compound was found to be stable for 15 days in the concentration range between 1.5 and 42.4 µg/ml. Animals in the IM1, IM2 and IM3 groups were dosed by intramuscular injection at a volume dosage of 0.1 ml in each hind limb. Dosing was administered on two occasions, the second dose administered approximately 24 hours after the first dose. All animals were sacrificed approximately 24 hours after the second treatment.

Positive controls: No positive control was used.

Sample preparations: The femurs were cleared of tissue and the proximal epiphysis removed from each bone. The bone marrow of both femurs from each animal was flushed out. Six bone marrow smears from each animal were prepared and examined microscopically. The (b) (4) smears were examined by (b) (4) to determine the incidence of micronucleated cells per 2000 polychromatic erythrocytes per animal. One smear per animal was examined and the remaining smears were held temporarily in reserve in case of technical problems with the first smear.

## **Results**

Study validity: The study met all criteria for validity. Five animals/sex/group was used for bone marrow analyses. Up to 2000 immature erythrocytes were scored for the presence of micronuclei for each animal. The proportion of immature erythrocytes was assessed by examination of a total of at least 1000 erythrocytes per animal and the number of micronucleated mature erythrocytes was recorded.

A positive response is normally indicated by a statistically significant increase in the incidence of micronucleated immature erythrocytes for the treatment group compared with the vehicle control group ( $P < 0.01$ ); individual and/or group mean values should exceed the laboratory historical control range.

A negative result is indicated where individual and group mean incidences of micronucleated immature erythrocytes for the group treated with the test substance are not significantly greater than incidences for the vehicle control group ( $P > 0.01$ ) and where these values fall within the historical control range.

An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response. Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant decrease in the proportion of immature erythrocytes ( $P < 0.01$ ).

Study outcome: Following administration of the second treatment, animals treated with IM3 showed clinical signs including underactivity, raised gait and reluctant, slow use of hind limbs. No other adverse clinical signs were reported in the treated groups over the duration of the test. All animals had recovered by the time of scheduled termination.

During the slide preparation process, cell pellets from animals treated with IM3 formed gelatinous clumps after the addition of (b) (4) and did not form a homogenous cell suspension for smearing on to the microscope slides. Various methods were tried to break up the clumps (e.g. agitation, stirring) but without success. The clumping effect observed in the IM3

treated group was not observed in cells from any animal in the vehicle control or IV1, IM1 and IM2 treated groups, indicating the possibility of a treatment related effect. Slides were prepared from all animals and on examination, prior to coding, the smears prepared from animals treated with IM3 did not appear to show any morphological differences compared to the slides for the vehicle control group.

IV1, IM1 and IM2 did not show any evidence of causing chromosome damage or bone marrow cell toxicity in this in vivo test procedure. IM3 showed no evidence of causing micronuclei but did show evidence of causing bone marrow cell toxicity in this in vivo test procedure.

Sampling time after 2nd dose	Treatment	Dose volume (ml/day)	% ie/(ie+me) †	incidence mie (mean)	ncidence mme (group mean) <sup>b</sup>
24 Hours	Vehicle	0.5	40	1.2	0.3
	V	0.5	37	1.7	0.3
	M	0.2a	38	0.4	0.3
	M2	0.2a	41	1.0	0.3
	M3	0.2a	31***	0.3	0.3

Vehicle:

(b) (4)

a 0.2 ml in each hind limb

% ie/(ie+me) Proportion of immature erythrocytes

ie Immature erythrocytes

mie Number of micronucleated cells observed per 2000 immature erythrocytes examined

me Mature erythrocytes

mme Number of micronucleated cells calculated per 2000 mature erythrocytes

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

\*\*\* P < 0.001 (highly significant)

Otherwise, P > 0.01 (not significant)

† Occasional apparent errors of  $\pm$  % may occur due to rounding of values for presentation in the table

<sup>b</sup> Formula for calculation of incidence mme (group mean): Sum of group incidence mme scored x 2000/Sum of group me scored

Table 105: Micronucleus test results (genotox study # 1); sponsor provided

#### Test article related effects and assessments:

IV1, IM1 and IM2 did not show any evidence of causing chromosome damage or bone marrow cell toxicity in this in vivo test procedure. IM3 showed no evidence of causing micronuclei but did show evidence of causing bone marrow cell toxicity in this in vivo test procedure.

Study # 2: Genetic Toxicology (Bone Marrow and Blood Cells Assessment. **Study number:**

(b) (4).

**Study title:** Assessment of effect on blood cells and bone marrow following intramuscular administration to (b) (4) rats.

(b) (4)



**Key findings:** The ability of the bone marrow to produce red blood cells in male rats was not affected by the treatment, for 1 or 2 days, with the adjuvant AS01B. A decrease in the proportion of immature erythrocytes in the bone marrow and some cell clumping during preparation of the smear was identified in the previous study. No decrease in the proportion of immature erythrocytes was apparent in this study; however, cell clumping was seen on day 3 of study for the majority of animals treated with AS01B.

**Study no.:** (b) (4) 681/043748

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** October 27, 2004

**Date of study completion:** March 23, 2005.

**GLP compliance:** Yes

**QA reports:** Yes (x); No ( )

**Drug, lot #, and % purity:** AS01B, Batch No. (b) (4), no purity reported.

**Strains/species:** (b) (4): CD® (b) (4) rats

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

**Number of animal per group and sex:** 10/group

**Age:** Not provided.

**Body weight range:** 132-146 g males

**Route, site, and frequency of administration:**

Each animal received 0.2 mL/rat/day; this was administered as one injection of 0.1 mL into each anterior thigh muscle.

No positive control was used.

**Volume of injection:** For vehicle and AS01B adjuvant 0.2 mL/rat/day.

## Methods

### Study design

Group Number	Description	Days of Dosing	Animal Numbers Male	Dosage Concentration (µg/rat/day)	Dosage Volume (mL/rat/day)	Days of kill
1	Vehicle (Saline)	1, 2	10	0	0.2 <sup>a</sup>	3
2	Vehicle (Saline)	1	10	0	0.2 <sup>a</sup>	13
3	AS01B adjuvant	1, 2	10	20	0.2 <sup>a</sup>	3
4	AS01B adjuvant	1, 2	10	20	0.2 <sup>a</sup>	13
5	AS01B adjuvant	1	10	20	0.2 <sup>a</sup>	3
6	AS01B adjuvant	1	10	20	0.2 <sup>a</sup>	13

<sup>a</sup> 0.1 mL in each anterior thigh muscle

Table 106: Experimental design (b) (4) study # 2)

Doses used in definitive study: 0.2 mL/rat/day.

Composition per vial (0.5 mL):

KH<sub>2</sub>PO<sub>4</sub>: 41 mM

Na<sub>2</sub>HPO<sub>4</sub>: 9mM

NaCl: 100mM

Dose used and sample collections: Treatment: Ten male rats per group were treated with 0.2 mL/rat/day on either day 1 or on days 1 and 2. The rats were observed for three or thirteen days for treatment-related deaths, clinical signs (twice daily), and body weights (week -1 and days 1, 3, 6, 9, 12 and before necropsy). Blood samples were collected from the sublingual vein on days 3, 6, 9, and 12 of study for hematology assessment. The following were measured: hematocrit (Hct), hemoglobin conc. (Hb), mean corp. Hb (MCH), mean corp. Hb conc. (MCHC), mean corp. volume (MCV), total erythrocyte count (RBC), total white cell count (WBC), differential WBC count (neutrophils, lymphocytes, eosinophils, basophils, monocytes and large unstained cells), and platelet count (Plt). Bone marrow samples were obtained, on either day 3 or day 13, from the tibia of all animals and smears were prepared from these samples. All animals were subject to a detailed necropsy.

Negative controls: No negative control was used

Positive controls: No positive control was used

## Results

There were no treatment-related clinical signs recorded. There was significant decrease in body weight of animals treated with AS01B on day 1 only or on days 1 and 2 (56 and 68% of group 2 for groups 5 and 6, respectively, and 19 and 31% of group 1 for groups 3 and 4). Thus, effect was substantially greater in animals dosed twice in comparison with those dosed once.

Low hematocrit, hemoglobin concentration, mean cell volume and mean cell hemoglobin and abnormal red cells forms apparent but no effect on red cell numbers were reported in the treated animals (day 3/6). Differences were minimal but there was evidence that the degree of these differences were dose related (2 doses > 1 dose). As the post-dose period progressed, these effects lessened and some evidence of a response to low oxygen carrying capacity was apparent as marginally higher reticulocyte counts and macrocytosis. This may reflect early release of immature red cells into the circulation. Pro-erythrocyte counts were slightly high and no treatment-related differences in the normocyte counts were reported in the bone marrow. This suggests the early stages of up-regulation of red cell production and indicates that AS01B does not impair the ability of the body to produce red cells. A direct effect on AS01B on circulating red cells could be occurring and interaction of AS01B with red cell membranes may be the cause of the cell clumping observed during preparation of blood smears on day 3. The recovery of hemoglobin concentration by day 12 and the absence of cell clumping in the smears produced on day 13 indicate this is a short-term effect. Total white cell counts were increased (day 6 onwards) due to increases in neutrophil and lymphocyte counts.

**Test article related effects and assessments:**

AS01B does not affect the ability of the bone marrow to produce red blood cells in male rats after 1- or 2-days treatment.

**Study # 3: MPL (b) (4) Rat Micronucleus Test (Reviewed by Steve Kunder in 2009)**

**Test article:** 3-O-desacyl-4'-monophosphoryl lipid A (MPL). Study number BVR 730/052198.

**Test for induction of chromosome damage, aneuploidy**

**Treatment schedule:** 2 doses/24 hr

**Study No.** (b) (4) 730/052198

**Species/Strains:** CD Rats.

**Sampling time:** 24h post dose 2.

**Location:** (b) (4)

**Animal weight:** 162-180 g.

**Method of administration:** intramuscular.

**Cells evaluated:** Bone marrow immature erythrocytes.

**Vehicle formulation:** Saline solution

**GLP Compliance:** Yes.

**Results**

No of cells analyzed/animal: 2000 Date of dosing: Dec 2004 Test article	Dose	Proportion immature erythrocytes (%)	Incidence of No of micronucleated cells / 2000 immature erythrocytes (mean)
Saline	0.2 ml	44 %	2.8
MPL (1.05 mg/ml)	0.2 ml (approx 0.4mg/kg)	41%	2.8
Cyclophosphamide (2 mg/ml)	20 mg/kg	37%	35.1***

\*\*\* P < 0.001. Note: the MPL dose used in this study compares to the dose used in toxicology studies of about 0.1 mg/kg in rats

Table 107: Erythrocytes results (b) (4) study # 3); sponsor provided

MPL (b) (4) did not cause any statistically significant increases in the number of micronucleated immature erythrocytes when compared to vehicle control values. MPL (b) (4) did not cause any substantial increases in the incidence of micronucleated mature erythrocytes. MPL was not positive in the rat micronucleus assay.

**Study # 4: An Assessment of the Effects of AS01B on Red Blood Cells in Peripheral Blood and Bone Marrow (Study number (b) (4) . Study number: (b) (4)**

**Purpose of the study:** To investigate the effect of adjuvant AS01B on red blood cells in peripheral blood and bone marrow.

**Key findings:** In rats, AS01B did not cause any, reproducible, effect on the proportion immature erythrocytes in bone marrow and no effect on erythrocyte count in peripheral blood was reported. Rabbits in six repeated dose studies produced marginal reductions in hemoglobin, hematocrit, and red cell count which were inconsistent with respect to time after dose administration but did occur with some reproducibility between the studies. No evidence of AS01B effect on cell counts in the bone marrow in rabbits was reported.

**Study no.:** (b) (4) 0026/070209

**Conducting laboratory and location:** (b) (4)

**Date of first study:** June 06, 2003

**Date of study completion:** January 25, 2008

**GLP compliance:** Yes

**QA reports:** Yes (x), No ( )

**Drug, lot #, and % purity:** AS01B (contains 100 µg/mL monophosphoryl lipid A (MPL), a non-toxic derivative of lipopolysaccharide (LPS), 100µg/mL QS- 21, a purified form of saponin and 500 µg/mL cholesterol in a liposomal formulation)

**Introduction:** A rat micronucleus study (study 1 below) on AS01B resulted in a statistically significant reduction in the percentage of immature erythrocytes in the bone marrow. To further investigate this, a more extensive rat study (study 2 below) was performed which assessed red cells in bone marrow and peripheral blood. Six repeated dose rabbit studies have been performed using AS01B and various novel vaccine antigens, during which red cell indices were assessed. The objective of this report is to summarize the effects of AS01B on red blood cells in peripheral blood and bone marrow in eight studies, reported between 2003 and 2007, and to assess the toxicological significance of those effects.

Study summaries:

1- Comparison of Different Test Formulations in the Rat Micronucleus Test. (b) (4)

Report Number (b) (4) 317/032657, 6 June 2003.

A group of 5 male and 5 female (b) (4) rats was treated with AS01B by intramuscular injection into both hind limbs on two occasions, one day apart. They were given a dose volume of 200 µL (100µL/limb) of the formulation and thus received 20 µg MPL, 20 µg QS-21 and 100 µg cholesterol. Control group was treated with 0.9% saline intravenously at 500 µL/rat (as a control specifically for one of the other adjuvants administered).

Bone marrow smears were prepared and examined for the presence of micronuclei.

During the slide preparation process, cell pellets from animals treated with AS01B formed gelatinous clumps after the addition of fetal calf serum and did not form a homogenous cell suspension for preparation of smears for microscopic evaluation. Various methods were tried to break up the clumps (e.g. agitation, stirring) but without success. This clumping effect was not reported in cells from any animal in either the vehicle control or other adjuvants assessed.

No evidence of micronuclei formation was reported. However, reduction in the proportion of immature erythrocytes in rats treated with AS01B was reported. The mean percentage of immature erythrocytes decreased from 40% in the controls to 31% in the AS01B treated group ( $p < 0.001$ ). This shows that the values for AS01B treated rats are the lowest in comparison with the controls and the other three adjuvant groups assessed (IV1, IM1 and IM2). This is an indication of bone marrow cell toxicity.

2- AS01B Assessment of Effect on Blood Cells and Bone Marrow Following Intramuscular

Administration to CD Rats. (b) (4) Report Number (b) (4) 681/043748.  
23 March 2005.

Groups of 10 male (b) (4) rats were treated with AS01B by intramuscular injection into both hind limbs on one or two occasions and euthanized 1 or 12 days after the last dose. They were treated with a dose volume of 200  $\mu$ L of the formulation and thus received 20  $\mu$ g MPL, 20  $\mu$ g QS-21 and 100  $\mu$ g cholesterol per dose.

Hematological investigations were performed on days 3, 6, 9 and 12, that is 2, 5, 8 and 11 days after one dose or 1, 4, 7 and 10 days after two daily doses.

Hematocrit and hemoglobin concentration were reduced from day 3 to between 0.94 and 0.97X control with recovery on days 9 or 12. Red blood cell count was not affected by treatment. Consequently, mean cell hemoglobin and mean cell volume were low. Abnormalities (hypochromasia from day 3 and macrocytosis and anisocytosis from day 6) were reported. These abnormalities were not recovering by day 12. Minimal evidence of increase in reticulocytes was reported on days 9 and 12.

Bone myelograms were assessed on days 3 or 13 (two or twelve days after one dose or one or eleven days after the second dose) for proportion (percentage) of each cell type.

No treatment-related effects were reported on erythroid series cell lines in rats euthanized on day 3. Statistically significantly higher percentage pro-erythrocytes were reported in rats euthanized on day 13 (dosed once or twice). Lower percentage late normoblasts (dosed twice [ $P < 0.05$  or  $P < 0.01$ ]) was also reported.

In marrow smears, no decreases in proportion of immature erythrocytes were reported. However, cell clumping during the preparation of the slides from rats euthanized on day 3 was reported for all rats that had received one or two doses of AS01B. This effect was not reported in slides prepared from rats euthanized on day 13.

3- RTS,S/AS01B Versus RTS,S/AS02V Malaria Candidate Vaccines Toxicity Study by  
Repeated (4 times) Intramuscular Administration to Rabbits. (b) (4) Report  
Number  
(b) (4) 033/022086, 15 April 2005.

Two groups of 10 male and 10 female (b) (4) rabbits were treated with the malaria vaccine RTS,S/AS01B by intramuscular injection into the gastrocnemius muscles of each hind limb in turn on four occasions 14 days apart. The formulation administered contained 100  $\mu$ g/ml RTS,S antigen, in AS01B. The low dose group was given a dose volume of 125  $\mu$ L and the high dose group 500  $\mu$ L of the formulation. The low dose rabbits thus received 12.5  $\mu$ g MPL, 12.5  $\mu$ g QS-21 and 62.5  $\mu$ g cholesterol per dose. The high dose rabbits received 50  $\mu$ g MPL, 50  $\mu$ g QS-21 and 250  $\mu$ g cholesterol per dose. Five rabbits per sex per group were euthanized 3 or 28 days after the last dose.

Only data for rabbits given 500  $\mu$ L has been assessed for comparability with other studies below. No test article-related effect on red blood cell count, hemoglobin, hematocrit or reticulocytes, assessed by routine hematology, was reported. No test article-related effect on bone marrow, assessed histopathologically, was reported. There were no statistically significant differences

from control in these parameters at any time point. Mean values were very similar to control (generally no lower than 0.96X control). However, there were some occasions when the degree of difference from control (0.94-0.95X) was similar to that described in rabbit studies below as being test article related when statistically significant. These were hematocrit for males 1 day after the first dose; hemoglobin for females 1 day after the first dose and 28 days after the fourth dose; and red cell count for females 1 day after the first dose.

4- AS01B Versus AS02V Toxicity Study by Repeated (5 times) Intramuscular Administration to Rabbits. (b) (4) Report Number (b) (4) 045/022412, 15 December 2006

Group of 10 male and 10 female (b) (4) rabbits was treated with AS01B by intramuscular injection into the gastrocnemius muscles of each hind limb over five occasions 14 days apart. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL, 50 µg QS-21 and 250 µg cholesterol. Five rabbits per sex were euthanized 3 or 28 days after the last dose. Control group treated with 0.9% saline was also included and were euthanized at the same times.

No test article-related effect on red blood cell count, haemoglobin, haematocrit, or reticulocytes was reported. No test article-related effect on bone marrow, assessed histopathologically, was reported. There were no statistically significant differences from control in these parameters at any time point. Mean values were very similar to control (generally no lower than 0.96X control). However, there were some occasions when the degree of difference from control (0.91-0.95X) was similar to that described in rabbit studies below as being test article related when statistically significant.

5- (b) (4) /AS01B Versus (b) (4) /AS02V Toxicity Study by Repeated (5 times) Intramuscular Administration to Rabbits. (b) (4) Report Number (b) (4) 049/022268, 4 January 2007.

Group of 10 male and 10 female (b) (4) rabbits were treated with HIV vaccine (b) (4) in combination with AS01B by intramuscular injection into the gastrocnemius muscles of each hind limb on five occasions 14 days apart. The formulation administered contained 200 µg/ml (b) (4) antigen and 40 µg/ml (b) (4) antigen, in AS01B. The rabbits were given a dose volume of 500 µL of formulation and thus received 50 µg MPL, 50 µg QS- 21 and 250 µg cholesterol per dose. Five rabbits per sex per group were euthanized 3 or 28 days after the last dose. A similarly sized control group was given 0.9% saline at the same dose volume and euthanized at the same times.

No test article-related effect on red blood cell count, hemoglobin, hematocrit, or reticulocytes was reported. No test article-related effect on bone marrow, assessed histopathologically, was reported. The only statistically significant differences from control in these parameters were  $P < 0.05$  for hemoglobin in males 1 and 3 days after the first dose and 3 and 28 days after the last dose.

6- Repeated-dose Toxicity Study with (b) (4) ASCI Candidates (b) (4)/AS01B and (b) (4)/AS15) Administered Intramuscularly (Seven times) to Male and Female Rabbits. (b) (4) Report Number V7464, 7 November 2007

Group of 10 male and 10 female (b) (4) rabbits were treated with AS01B by intramuscular injection into the muscles of each hind limb on seven occasions 14 days apart. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL and 50 µg QS-21 in a liposomal formulation per dose. Five rabbits per sex per group (including control group) were euthanized 3 or 28 days after the last dose.

Red cell count, hemoglobin, and hematocrit levels were reduced in male rabbits only. This reduction was one and three days after administration of the first dose (typically 0.95X control) with statistical significance attained ( $p < 0.05$  or  $p < 0.01$ ). There were no effects of similar magnitude at other time points in males or at any time point in females. There were no effects on reticulocyte proportions. There were no effects on bone marrow assessed histopathologically.

7- Repeat Dose Toxicity Study with HIV Candidate Vaccines (HIV-(b) (4) versus HIV-(b) (4)/AS01B) Administered Intramuscularly (Four times) to Male and Female Rabbits. (b) (4) report number V6794, 3 April 2007.

Group of 10 male and 10 female (b) (4) rabbits were treated with AS01B by intramuscular injection into the muscles of each hind limb on four occasions 14 days apart. This group was acting as a control in the assessment of a cancer vaccine containing AS01B. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL and 50 µg QS-21 in a liposomal formulation per dose. Five rabbits per sex per group (including control group) were euthanized 3 or 28 days after the last dose.

Red cell count, hemoglobin, and hematocrit levels were reduced in female rabbits only. Red cell count was reduced 28 days after the last dose only. Hemoglobin and hematocrit were reduced 1 day after the first dose and 1 and 28 days after the last dose (but not 3 days after the last dose). These differences were considered as test article related because statistical significance was attained ( $p < 0.05$  or  $p < 0.01$ ). No similar differences were reported in males. There were no effects on reticulocyte proportions.

8- Repeat Dose Toxicity Study with a VZV Candidate Vaccine (gE 100 µg/AS01B) Administered Intramuscularly (Three times) to Male and Female Rabbits. (b) (4) report number V6721, 3 April 2007.

Group of 10 male and 10 female (b) (4) rabbits were treated with AS01B by intramuscular injection into the muscles of each hind limb on three occasions 14 days apart. They were given a dose volume of 500 µL of the formulation and thus received 50 µg MPL and 50 µg QS-21 in a liposomal formulation per dose. Five rabbits per sex per group (including control group) were euthanized 3 or 28 days after the last dose.

Red cell count, hemoglobin, and hematocrit levels were reduced intermittently in both sexes, typically 0.95X control. In males these parameters were reduced 1 and 3 days after the first dose. In females they were reduced 1 day after the first dose and 3 days after the last dose. Reticulocyte proportion was increased in males only three days after the first dose only. These

differences were considered as test article related because statistical significance was attained ( $p < 0.05$  or  $p < 0.01$ ). The data for other time points shows no effect.

### **Assessment of results**

#### Rats

AS01B produced a reduction in the proportion of immature erythrocytes in bone marrow smears generated during a micronucleus study (study 1). As a result, another study was carried out to assess peripheral blood hematology parameters and bone marrow in rats (study 2). This study failed to repeat the effect on proportion of immature erythrocytes in blood smears reported in study 1. In study 1, gelatinous clumping was reported when preparing blood smears from rats killed one day after the last dose. This effect was also reported in study 2 for rats killed one or two days after the last dose but not for rats killed 13 days after the last dose. It is possible that this clumping was caused by the presence of AS01B in the blood. It affected the quality of the smears and consequently the assessment of red cell proportion in study 1. In Study 2, hematocrit and hemoglobin levels were reduced to between 0.94X and 0.97X control between 1 and 7 days after one or two doses, with recovery from 10 days after dosing. Red cell count was not affected by treatment (no statistically significant differences, means never less than 0.97X control).

#### Rabbits

In the six rabbit studies reviewed, hematocrit, hemoglobin concentration and red blood cell count were assessed 1 and 3 days after the first dose, 10 days after an intermediate dose and 1, 3 and 28 days after the last dose when the number of doses varied from 3 to 7 between studies, all at 14-day intervals. The effects on hematological parameters other than red cells, that is white cells and fibrinogen, were similar in the studies in which AS01B was given intramuscularly to rabbits alone (studies 4, 6, 7, 8) or in combination with (b) (4) (study 3) or (b) (4) (study 5). This indicates that the findings were principally attributable to AS01B, that the presence of the vaccine antigen did not generate any findings that might have masked an effect of AS01B on red cells and consequently, studies 3 and 5 are also useful in the assessment of the effect of AS01B on red cells.

Bone marrow was examined during routine histopathology in four of the rabbit studies and no effects of treatment were reported.

Hematocrit, hemoglobin, and red cell count showed marginally lower values (typically 0.91X-0.95X control) intermittently and inconsistently in five of the six studies. Many of the differences were statistically significant.

It was concluded that the incidence of marginally lower mean hematocrit, hemoglobin, and red cell count levels were affected by treatment with AS01B in rabbit.

### **Conclusion:**

Treatment with AS01B did not reproducibly affect the proportion immature erythrocytes in the bone marrow of the rats. In addition, AS01B did not affect the erythrocyte count in peripheral blood. Rabbits in six repeated dose studies produced marginal reductions in hemoglobin, hematocrit, and red cell count which were inconsistent with respect to time after dose



administration but did occur with some reproducibility between the studies. No evidence of AS01B effect on cell counts in the bone marrow in rabbits was reported.

**Study # 5: Bone Marrow Micronucleus Test with DQ in Rats (Study no.: V20204/04). Study number: V20204/04**

**Purpose of the study:** To investigate the potential of DQ to cause damage to the chromosomes and/or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow of rats.

**Key findings:** In male rats treated by IV administration of the maximum (tolerable) dose of DQ containing 160 µg QS-21/kg-bw, no test article-related chromosomal damage and/or damage to the mitotic spindle apparatus of the bone marrow target cells was reported.

**Study no.:** V20204/04

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** November 13, 2012

**Date of study completion:** March 03, 2013.

**GLP compliance:** Yes

**QA reports:** Yes (x), No ( )

**Drug, lot #, and % purity:**

DQ (QS-21 = 100 µg/dose), Batch No.; (b) (4), expiration date; (b) (4), No purity reported.

Water for injection, Batch No.; (b) (4), expiration date; (b) (4).

Negative control: physiological saline, expiration date; (b) (4).

Positive control: (b) (4) Batch No.; (b) (4), expiration date; (b) (4)

**Strains/species:** (b) (4) outbred male rats

**Breeder/supplier:** (b) (4).

**Number of animals per group and sex:** 43 rats

**Age:** 6 weeks

**Body weight range:** Not provided

**Route, site, and frequency of administration:** Intravenous.

**Volume of injection:** See study design below.

## Methods

### Study design

The experimental design of the bone marrow micronucleus test was as follows:

Group	Treatment	Color	Dosing volume <sup>1</sup>	Dose level	n
1	Negative control <sup>2</sup>	White	10	-	5
2	DQ <sup>3</sup>	Blue	10	40 µg QS-21/kg-bw	5
3	DQ <sup>3</sup>	Green	10	80 µg QS-21/kg-bw	5

Group	Treatment	Color	Dosing volume <sup>1</sup>	Dose level	n
4	DQ <sup>3</sup>	Red	10	160 µg QS-21/kg-bw	7
5	Positive control <sup>4</sup>	Yellow	10	1.5 mg/kg-bw	5

<sup>1</sup> Dosing volume in ml/kg-bw/day

<sup>2</sup> Rats of this group were dosed twice intravenously with physiological saline on two consecutive days with an interval of approximately 24 h

<sup>3</sup> Rats of these groups were dosed twice intravenously with DQ on two consecutive days with an interval of approximately 24 h

<sup>4</sup> Rats of this group were dosed once intraperitoneally with 1.5 ml/kg-bw (b) (4) freshly formulated in physiological saline

Table 108: Study design (b) (4) study # 5)

The following criteria were used for the scoring of cells:

- 1- A polychromatic erythrocyte (PE) is an immature erythrocyte that still contains ribosomes and can be distinguished from mature, normochromatic erythrocytes by a (b) (4).
- 2- A normochromatic erythrocyte (NE) is a mature erythrocyte that lacks ribosomes and can be distinguished from immature, polychromatic erythrocytes by a (b) (4).
- 3- A micronucleus is a small, normally round, nucleus with a diameter of circa 1/20 to 1/5 of an erythrocyte, distinguished from the cytoplasm by a (b) (4).

The numbers of PE and NE were recorded in a total of at least 200 erythrocytes (E) per animal. If micronuclei were observed, these were recorded as micronucleated polychromatic erythrocytes (MPE) or micronucleated normochromatic erythrocytes (MNE). Once a total of 200 E (PE + NE) was scored, an additional number of PE was scored for the presence of micronuclei until a total of 2000 PE was scored.

## Results

### Preliminary dose range finding study

First, animals were treated with a single dose of DQ containing 500 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration. Approximately 4 h after dosing, lethargy, hunched posture, blepharospasm, and piloerection were reported. Approximately 7 h after dosing, dyspnoea and nasal swelling were also reported. Based on these clinical signs the animal was killed for ethical reasons.

Second, another rat received a dose of DQ containing 125 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration twice on two consecutive days with an interval of approximately 24 h between doses. No abnormalities were observed up to approximately 72 h after administration of the second dose.

Third, one rat received a single dose of DQ containing 250 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration. Approximately 7 h after dosing, piloerection was reported and approximately 24 h after dosing hunched posture and

blepharospasm were also reported. In addition, a >11% body weight reduction compared to the body weight prior to dosing was reported.

Fourth, another rat received a dose of DQ containing 160 µg QS-21/kg-bw (diluted in water for injection, dosing volume 10 ml/kg-bw) by intravenous administration twice on two consecutive days with an interval of approximately 24h between doses. Approximately 24h after the first dose and 1h after the second dose piloerection was reported. Approximately 4h after the second dose, nasal encrustations were also reported. No clinical signs were reported approximately 24h after the second dose. Prior to the second dose, a body weight reduction of approximately 7% compared to the body weight prior to the first dose was reported. No further weight loss was observed after the second dose.

Based on these observations, the maximum tolerable intravenous dose for the bone marrow micronucleus test was established as a DQ dose containing 160 µg QS-21/kg-bw per day for two consecutive days with an interval of approximately 24h between doses.

#### Bone marrow micronucleus test

##### *Clinical signs*

Approximately 7 h after the final treatment, one animal treated with DQ containing 160 µg QS-21/kg-bw showed nasal and eye encrustations. This finding disappeared approximately 24 h after the final treatment.

##### *Body weights*

One day after the first treatment, body weight reduction (mean body weight reduction of 7.2%) was reported in all animals treated with DQ containing 160 µg QS-21/kg-bw. No other changes were reported.

##### *Bone marrow micronucleus test*

The groups mean numbers of MPE/2000E and PE/200E are presented in Table 109.

The number of micronucleated polychromatic erythrocytes (MPE) per 2000 polychromatic erythrocytes (PE) and number of PE per 200 erythrocytes (E) reported in the micronucleus test (group mean  $\pm$  SD) are listed in the following table:

Group	Treatment	Dose level	MPE/2000PE	PE/200E
1	Negative control <sup>1</sup>	-	2.0 $\pm$ 1.6	114 $\pm$ 18
2	DQ <sup>2</sup>	40 µg QS-21/kg-bw	3.0 $\pm$ 0.7	112 $\pm$ 17
3	DQ <sup>2</sup>	80 µg QS-21/kg-bw	1.6 $\pm$ 0.5	101 $\pm$ 8
4	DQ <sup>2</sup>	160 µg QS-21/kg-bw	1.8 $\pm$ 1.3	95 $\pm$ 9
5	Positive control <sup>3</sup>	1.5 mg/kg-bw	41.4 $\pm$ 13.3*	86 $\pm$ 12*

<sup>1</sup> Rats of this group were dosed twice intravenously with physiological saline on two consecutive days with an interval of approximately 24 h

<sup>2</sup> Rats of these groups were dosed twice intravenously with DQ on two consecutive days with an interval of approximately 24 h

<sup>3</sup> Rats of this group were dosed once intraperitoneally with 1.5 ml/kg-bw (b) (4) freshly formulated in physiological saline

\* Statistically significant difference from negative control group

Table 109: MPE and PE results ((b) (4) study # 5); sponsor provided

#### *Validity of the study*

Statistically significant increase ( $p=0.0060$ ) in the mean number of MPE/2000PE were reported in the positive control animals (group 5) compared to the negative control animals (group 1). The mean number of MPE/2000E reported in the positive control mitomycin C was within the range of means of the historical data.

Statistically significant decrease ( $p=0.0208$ ) in the mean number of PE/200E was reported in the positive control animals (group 5) compared to the negative control animals (group 1). This confirms that the positive control substance (b) (4) reached the bone marrow.

The positive control (b) (4) demonstrated the expected response, and the negative control was within the range of historical data. Therefore, the study was considered valid.

#### *Treatment groups DQ (40, 80 and 160 $\mu\text{g}$ QS-21/kg-bw)*

No statistically significant increase in the mean number of MPE/2000PE was reported in groups 2, 3, or 4 when compared to group 1.

No statistically significant decrease in the mean number of PE/200E was reported in groups 2, 3, or 4 when compared to group 1.

#### **Conclusion**

No test article-related chromosomal damage and/or damage to the mitotic spindle apparatus of the bone marrow target cells was reported in male rats treated by IV administration of the maximum (tolerable) dose of DQ containing 160  $\mu\text{g}$  QS-21/kg-bw.

#### **Genotoxicology studies: *in vitro***

Reviewer: Claudia Wrzesinski

(b) (4)

5 Pages have been determined to be not releasable

**Local tolerance studies:*****Study 1: Single Dose Toxicity and Local Tolerance Study with Zoster Candidate Vaccine (gE/AS01B) Administered Subcutaneously to Male and Female Rabbits (V 9912/05)***

The Zoster candidate vaccine (gE/AS01B) was evaluated for acute toxicity and local reactogenicity after a single subcutaneous injection in a group of three male and three female (b) (4) rabbits, sacrificed 3 days after the injection. The animals received 0.5 ml per injection, equivalent to the intended full human dose. The reactions of the Zoster candidate vaccine were compared to an adjuvant alone group (AS01B) and to a saline control group. Local reactions at the injection site were recorded approximately 3, 24, 48 and 72 hours after injection. General and local clinical signs, body weights, macroscopic changes at necropsy and histopathology of the injection site collected 3 days after injection (necropsy) were evaluated in this study.

No treatment-related changes were observed in general and local clinical signs and body weights of the animals treated with the Zoster candidate vaccine. Macroscopically, a hemorrhage at the injection site in treated animals as well as control animals. This was most likely the result of a punctured blood vessel during injection. Microscopically, a slight to severe diffuse mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes) was reported at the injection site of all animals receiving gE/AS01B as well as a slight to moderate diffuse mixed inflammatory cell infiltration (small and medium sized macrophages, plasma cells and granulocytes) in 2/3 male and female animals receiving the adjuvant alone. A slight acanthosis was observed in 1/3 male animal receiving gE/AS01B or the adjuvant alone. The severity of the histopathological changes was slightly lower in females than in males and slightly lower in animals treated with the adjuvant alone (AS01B) than in those treated with gE/AS01B.

***Study 2: Single Dose Toxicity and Local Tolerance Study with a VZV Candidate Vaccine (gE 100 gE/AS01B) Administered Intramuscularly to Male and Female Rabbits (v 6812/02)***

The VZV candidate vaccine (gE 100 µg/AS01B) was examined for its local reactogenicity after a single subcutaneous injection in a group of three male and three female (b) (4) rabbits, sacrificed 3 days after the injection. The animals received 0.5 ml per injection (right calf muscle), equivalent to the intended full human dose. The reactions of the Zoster candidate vaccine were compared to an adjuvant alone group (AS01B) and to a saline control group. Local reactions at the injection site were recorded approximately 3, 24, 48 and 72 hours after injection. General and local clinical signs, body weights, macroscopic changes at necropsy and histopathology of the injection site collected 3 days after injection (necropsy) were evaluated in this study.

No treatment-related changes were observed in general and local clinical signs and body weights of the animals treated with the Zoster candidate vaccine. Microscopically, a treatment related very slight to slight widespread, predominantly extramuscular, mononuclear cell infiltrate was observed in all adjuvant and vaccine formulation treated animals, while mononuclear cell infiltrates were observed in the control animals.

***Study 3: Single Dose Toxicity and Local Tolerance Study with DQ Administered Intramuscularly to Male and Female Rabbits (V 20212/02)***

Three male and 3 female (b) (4) rabbits received a single intramuscular dose of 20, 100 and 200 µg/mL DQ (0.5 mL at the right and left thigh) and were compared to a saline control group. General and local clinical signs, body weights, as well histopathology of the injection sites collected 3 days after injection were evaluated in this study. No treatment related changes were observed regarding clinical signs or body weights. During the necropsy, three days after the administration discoloration (white area) at the injection site was observed in one male and one female animal receiving 100 µg/mL and a hemorrhage at the injection site in one male animal receiving 200 µg/mL of DQ.

Microscopically, animals in the treatment and control group showed minimal to mild localized mononuclear (lymphocytes and small macrophages) inflammatory response. In 1/3 saline control females, 2/3 DQ 100 µg/mL females and 1/3 DQ 200 µg/mL females, a minimal multifocal mononuclear inflammatory cell infiltrate was observed, characterized by scattered small foci of inflammatory cells. A mild widespread (extended along the epimysium and diffusely between the muscle fibers) mononuclear cell infiltration was reported in animal receiving 100 or 200 µg/mL DQ (1/3 DQ 100 µg/mL males, 2/3 DQ 200 µg/mL males and 1/3 DQ 200 µg/mL females at the right anterior thigh muscle, and 1/3 DQ 200 µg/mL males and 2/3 DQ 200 µg/mL females at the left anterior thigh muscle) which also involved a mild mixed cell infiltration (besides lymphocytes and macrophages, polymorphonuclear inflammatory cells were present) in animals receiving 200 µg/mL DQ (1/3 DQ 200 µg/mL males and 2/3 DQ 200 µg/mL females at the right anterior thigh muscle). Minimal to mild hemorrhage were observed at the injection site of male animals receiving 200 µg/mL DQ. Minimal mineralization and muscle fiber degeneration were observed as part of the local response in few treated animals but also in a few saline control animals.

It was considered that 20 µg DQ/rabbit is the NOEL. The NOAEL of DQ was considered to be above 200 µg DQ/rabbit, since the local effects observed were all considered to be non-adverse effects related to the purpose and intended use of DQ.

***Study 4: Single Dose Toxicity and Local Tolerance Study with DQ Administered Intramuscularly to Male and Female Rats (V 20212/01)***

DQ was evaluated for acute toxicity and local tolerance in 5 male and 5 female (b) (4) rats after a single intramuscular dose 4, 20 or 40 µg/rat DQ compared to a sham-treated saline control group. Animals received 0.2 mL of the various formulations, given as two injections of 0.1 mL in the right and left anterior thigh muscles and were sacrificed 3 days after the injection. Clinical signs, body weight, hematology, clinical chemistry, gross changes at necropsy and histopathological examination of the injection sites collected 3 days after injection were evaluated.

No treatment related changes were observed in general and local clinical signs, body weight and clinical chemistry. Hematological, a dose-dependent increase in fibrinogen was observed in animals receiving DQ on day 1 and on day 3 after injection. In the DQ 100 µg/mL (20 µg/rat) and the DQ 200 µg/mL (40 µg/rat) males a dose-dependent WBC response, consisting of an

increase in neutrophils and a decrease in lymphocytes, were observed on day 1 and on day 3 after injection. In the DQ 100 µg/mL and the DQ 200 µg/mL females, an increase in neutrophils and a decrease in lymphocytes were observed on day 1 after. The changes were considered to be part of the inflammatory process following injection of an immunostimulant.

Three days post injection, the main treatment related microscopic findings at the injection sites were either a localized or a widespread mononuclear inflammatory response. Generally, the response increased in severity and extension with increasing DQ dose, i.e. minimal to mild localized in DQ 20 µg/mL animals, mild to moderate localized in DQ 100 µg/mL animals, and mild to moderate widespread in DQ 200 µg/mL animals. In the saline control animals, a minimal localized response was observed.

The sponsor considered the NOEL to be below 4 µg DQ/rat since a minimal to mild inflammatory reaction was observed at the 20 µg/mL injection site (4 µg DQ/rat), and systemic increases in fibrinogen, neutrophils and lymphocytes. However, the observed local effects and the systemic effects were all considered non-adverse and, therefore, the NOAEL of DQ was considered to be above 40 µg DQ/rat when administered as a single injection.

#### **Overall conclusion:**

Based on nonclinical toxicity assessments, there are no significant safety issues to report.

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