2.6.1 Introduction

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2.6.1 Introduction

The candidate vaccine, FCC H1N1sw, is a monovalent influenza virus vaccine, surface antigen, inactivated, with MF59C.1 adjuvant. It is a sterile suspension for injection in either pre-filled syringes or multi-dose vials. The active ingredient of the vaccine is purified hemagglutinin (HA) and neuraminidase (NA) from an H1N1 virus (Reassortant virus X-179A, derived from A/California/07/2009). The antigens contained in FCC H1N1sw are cell culture-derived; they are purified from virus grown in Madin Darby Canine Kidney (MDCK) cells.

Pending clinical confirmation, the anticipated 0.25 mL clinical dose of FCC H1N1sw will contain 3.75 µg of H1N1 hemagglutinin and 0.125 mL MF59™C.1 adjuvant (MF59). MF59 adjuvant is an oil-in-water emulsion, composed of squalene as the oil phase, stabilized with the surfactants polysorbate 80 and sorbitan trioleate, in citrate buffer. The composition of the FCC H1N1sw vaccine is shown in the table below.

Table 1: Composition of FCC H1N1sw vaccine

Ingredients	Quantity per dose	Function	Reference to Standards
Active Ingredient			
Influenza virus surface antigens (hemagglutinin and neuraminidase), H1N1	3.75µg, HA*	active ingredient	Ph.Eur.
Adjuvant			
Squalene	4.875 mg	oil phase	In-house spec.
polysorbate 80	0.588 mg	Surfactant	Ph.Eur.
sorbitan trioleate	0.588 mg	Surfactant	Ph.Eur.
sodium citrate dihydrate	0.330 mg	Buffer	Ph.Eur.
citric acid monohydrate	0.020 mg	Buffer	Ph.Eur.
Other Ingredients			
sodium chloride	2.00 mg	isotonic aid	Ph.Eur.
potassium chloride	0.05 mg	Buffer	Ph.Eur.
potassium dihydrogen phosphate	0.05 mg	Buffer	Ph.Eur.
disodium phosphate dihydrate	0.33 mg	Buffer	Ph.Eur.
magnesium chloride hexahydrate	0.025 mg	Stabiliser	Ph.Eur.
calcium chloride dihydrate	0.035 mg	Stabiliser	Ph.Eur.
water for injections	up to 0.25 ml	Diluent	Ph.Eur.

* HA = Hemagglutinin

Multidose vials will also contain thiomersal (0.01% w/v) as a preservative.

2.6.2 Pharmacology Written Summary

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2.6.2 Pharmacology Written Summary

2.6.2.1 Brief Summary

The primary pharmacological effect of an influenza vaccine is the induction of antibodies to hemagglutinin (immunogenicity), which can confer protection in challenge models. Nonclinical studies with the candidate vaccine, FCC H1N1sw, have not been completed, but relevant nonclinical data with an equivalent MF59-adjuvanted vaccine, FCC/MF59-H5N1 (made using the same process), and the parent vaccine (Optaflu®; non-adjuvanted trivalent seasonal influenza vaccine) are available. In addition, supportive data are provided by studies with Novartis products that are related to FCC H1N1sw as shown below.

Table 1: Related Novartis Influenza Vaccines

Vaccine	Description*	Status
Cell culture influenza vaccines		
Optaflu®	Cell culture-derived trivalent, interpandemic non-adjuvanted	'Parent' vaccine registered for seasonal use in EU (EU/1/07/394/001-009).
FCC H1N1sw	Cell culture-derived monovalent, A/H1N1 MF59C.1 adjuvant	Investigative H1N1 (swine origin) vaccine. Manufacturing based on Optaflu® process.
FCC/MF59 -H5N1	Cell culture-derived monovalent, A/H5N1 MF59C.1 adjuvant	Manufacturing based on Optaflu® process. Formulation, fill and packaging based on Fluad® process.
Related influenza vaccines (provide additional supportive nonclinical data)		
Aflunov®	Egg-derived monovalent, A/H5N1 MF59C.1 adjuvant	Under evaluation for use prior to an H5N1 outbreak by EMEA (CP No. EMEA/H/C/804) and the Swiss regulatory authority. Produced using Fluad® manufacturing processes.
Fluad®	Egg-derived trivalent, interpandemic MF59C.1 adjuvant	Registered for seasonal use in EU (MRP No. IT/H/0104/001) and Germany (PEI.H.01444.01.1), also registered outside EU.

*All of these vaccines are surface antigen, inactivated

The definitive GLP toxicology study supporting the FCC H1N1sw program was performed with the comparable MF59-adjuvanted vaccine containing HA from an H5N1 strain (FCC/MF59-H5N1 vaccine). This study is directly relevant to the FCC H1N1sw vaccine because the manufacturing process for the antigen, and the adjuvant contained in FCC/MF59-H5N1 vaccine, are the same as those in the FCC H1N1sw vaccine. In this 6-week rabbit toxicology study, local (intramuscular) and systemic toxicities were evaluated following repeated intramuscular dosing (3 doses of 15 µg antigen + 0.25 mL MF59

separated by 2 weeks). Under the conditions of the study, FCC/MF59-H5N1 vaccine was well tolerated and immunogenic.

A study in mice evaluated the immunogenicity of seasonal cell culture-derived vaccine (Optaflu) with and without MF59. In this study, mice received two intramuscular doses of trivalent cell culture-derived antigens adjuvanted with various amounts of MF59. Non-adjuvanted antigen was also administered. Immunogenicity was evaluated using both ELISA and HI assays. As expected, the addition of MF59 increased antibody titers. Reducing the amount of MF59 up to 10 fold did not affect antibody titers.

Support for FCC H1N1sw is also provided by the nonclinical package of studies performed to support the registration of Optaflu, the 'parent' vaccine. Optaflu is a cell culture-derived, trivalent, seasonal influenza vaccine containing a total of 45 µg of HA. The immunogenicity (mouse, rabbit, ferret), and efficacy (ferret) of Optaflu have been demonstrated.

Comparability of egg-derived and cell culture-derived antigens has been demonstrated in mice, rabbits, and ferrets. Egg- and cell culture-derived antigens have also been shown to be comparable in the clinic. Nonclinical studies performed with egg-derived MF59-adjuvanted influenza vaccines relevant to FCC H1N1sw (Aflunov and Fluad) include immunogenicity testing in mice, rabbits, and ferrets and efficacy/challenge experiments in mice and ferrets. The vaccines were immunogenic in all species and protective in mice and ferrets.

There is also a comprehensive package of nonclinical studies with MF59 that demonstrate its effectiveness as an adjuvant with influenza and non-influenza antigens. The nonclinical data demonstrating the adjuvant activity of MF59 have been confirmed in clinical studies with many antigens, including influenza antigens.

2.6.2.2 Primary Pharmacodynamics

2.6.2.2.1 Immunogenicity of MF59-adjuvanted cell culture influenza vaccines

Dedicated immunogenicity or challenge studies have not been completed with FCC H1N1sw vaccine. Relevant data are provided by studies with FCC/MF59-H5N1 and Optaflu (with and without MF59).

FCC/MF59-H5N1

The immunogenicity of an equivalent vaccine was assessed in the GLP toxicology study in rabbits (section 2.6.6.3, Study No. 466122). Rabbits received three intramuscular doses of either saline, MF59, or FCC/MF59-H5N1 vaccine (15 µg antigen; 0.25mL of MF59; total volume of 0.5 mL per dose) on days 1, 15 and 29. Blood was collected and sera prepared pre-study, prior to dosing on days 15 and 29, and prior to necropsy on days 31 and 43. Immunogenicity of FCC/MF59-H5N1 was demonstrated using the

hemagglutination inhibition (HI) assay using a heterologous (Vietnam; Clade 1) virus strain; analysis with the homologous (Indonesia; Clade 2) strain was not possible due to restrictions imposed after the study began on the use of the A/Indonesia strain by the Indonesian government.

HI assay data demonstrated that there were no antibodies in either the saline control group or the MF59-alone group at any time point (data not shown). Rabbits that received FCC/MF59-H5N1 all had titers >40 post-second dose, as shown below.

Table 2: HI titers (heterologous) in FCC/MF59-H5N1-treated rabbits

Animal Number	Pre-study	Day 15 (post 1st dose)	Day 29 (post 2nd dose)	Day 31 (post 3rd dose)	Day 43 (post 3rd dose - recovery)
17	<10	<10	160	320	—
18	<10	<10	320	320	—
19	<10	<10	160	160	—
20	<10	<10	160	320	—
21	<10	<10	320	—	320
22	<10	<10	320	—	640
23	<10	<10	160	—	320
24	<10	<10	160	—	320
41	<10	<10	160	160	—
42	<10	<10	160	160	—
43	<10	20	160	160	—
44	<10	20	320	320	—
45	<10	<10	160	—	160
46	<10	<10	320	—	320
47	<10	40	640	—	640
48	<10	20	320	—	320

Titers persisted throughout the treatment and recovery periods. Based on these results, FCC/MF59-H5N1 was immunogenic in rabbits and two doses were sufficient to elicit titers ≥ 160.

MF59-adjuvanted cell culture vaccine (Optaflu + MF59)

In this study, 8 week old female BALB/c mice received two intramuscular doses of trivalent cell culture-derived antigens (0.2 µg each A/New Caledonia, A/Wyoming and B/Jiangsu) adjuvanted with various amounts of MF59. The ratio of antigen formulation to MF59 (v/v) was 1:1, 1:0.75, 1:0.5, 1:0.25 or 1:0.1. Non-adjuvanted antigen was also

administered. Doses were administered on days 0 and 28; sera were collected on days 27 and 42. Dose volume was 100 µL. Immunogenicity was evaluated using both ELISA and HI assays.

Immunogenicity data from sera collected on day 42 (14 days post-second dose) are presented in Figure 1 and Figure 2. The addition of MF59 increased antibody titers. Reducing the amount of MF59 up to 10 fold did not affect antibody titers.

Figure 1: Antibody titers 14 days post second dose (ELISA)

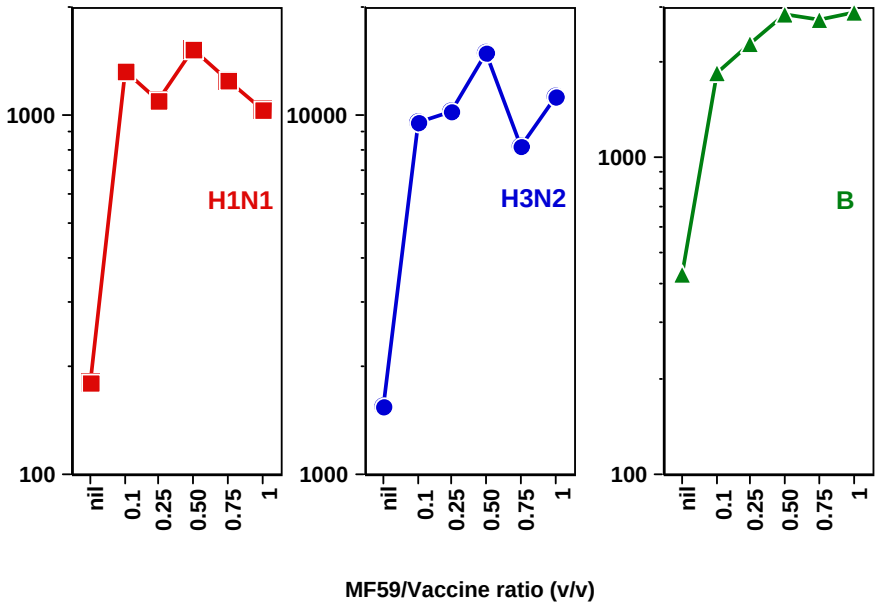
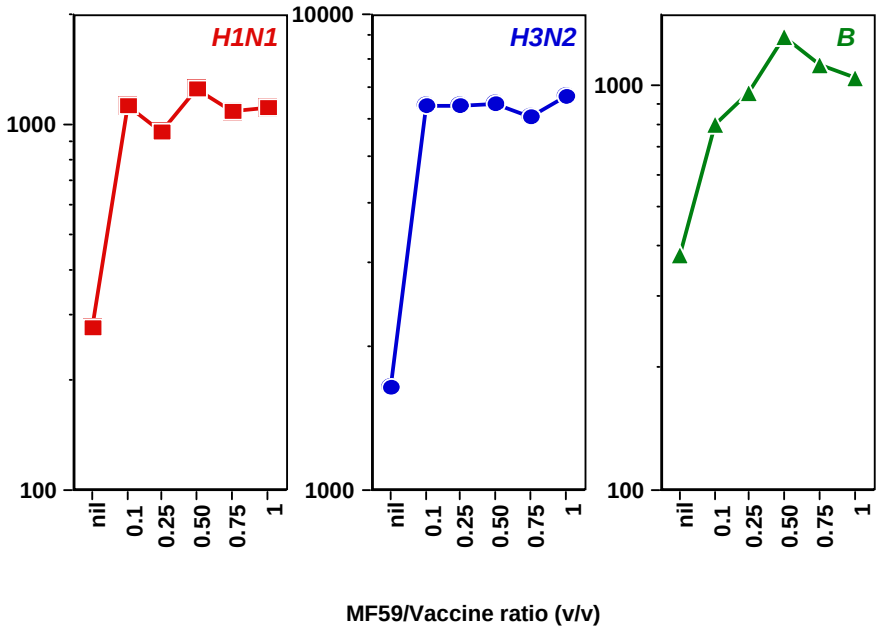


Figure 2: Antibody titers 14 days post second dose (HI)



2.6.2.2.2 Immunogenicity of Optaflu (non-adjuvanted parent vaccine)

Studies with Optaflu were conducted in mice, rabbits and ferrets. Mouse studies demonstrated a dose-response and immunological equivalence between Optaflu and an egg-derived comparator vaccine (Agrippal). Immunogenicity in rabbits was demonstrated in the GLP toxicology study (Study No. 191-44) and the GLP reproductive and developmental toxicity study (Study No. UBA00037). The latter study also demonstrated the ability of maternal vaccination to lead to hemagglutinin-inhibiting antibodies in fetuses; these antibodies persisted in offspring for 4 weeks following birth. Challenge studies in ferrets evaluated both immunogenicity and efficacy. In ferrets, Optaflu was immunogenic, and although vaccinated animals exhibited some flu-like symptoms, bodyweight loss, body temperature increase, and leukocyte counts were all reduced when compared to the negative control group. Optaflu and the positive control vaccine were comparable in all species tested. These nonclinical data have been confirmed in clinical immunogenicity/comparability studies.

Study No. KOE 090697: Immunogenicity testing of influenza antigens in mice

In this preliminary study, the immunogenicity of in-process egg-derived and MDCK-derived vaccine antigens was compared. Twenty mice per group received three intraperitoneal doses (0.5 mL each, on days 0, 7 and 14) of influenza antigens as shown below in Table 3. Antigen preparations were adjusted to 1000 HA-Units. No adjuvant was used. Control animals received phosphate buffered saline. Serum was collected on day 28 and immunogenicity testing was performed for A/Nanchang/933/95 (H3N2) and

B/Panama/45/90 antigens. Serum pools and individual samples were tested for hemagglutination-inhibiting (HI) antibodies against homologous virus.

As shown in the table below, all mice seroconverted and developed high and consistent HI antibody titers against the HA of the immunizing strain. These high titers were due to the use of 3 immunizations. For subsequent experiments, only 2 immunizations were used in order not to mask any minor differences between groups. The monovalent bulk egg-derived and MDCK-derived antigens appeared to be equivalent, as there were no statistically significant differences between the means and ranges. Conventionally, a serological titer difference of less than a factor of 4 (or two serum dilution steps) is not considered relevant, as this is within the variation of the methods.

Table 3: Mean HI antibody titers induced by egg-derived and MDCK-derived monovalent bulk antigens

Virus grown in:	**Reciprocal HI antibody titers** [a]		
	Mean titer	**Minimum titer**	**Maximum titer**
Antigen: A/Nanchang/ 933/95			
MDCK (gradient purified)	3520	1280	5120
MDCK (gradient purified; stored 4 months, HA activity reduced)	1536	640	5120
Negative Control	40 (serum pool titer)	Not tested	Not tested
Antigen: B/Panama/45/90			
MDCK (unpurified cell culture harvest)	1952	640	5120
EGG (gradient purified)	1416	640	2560
Negative Control	40 (serum pool titer)	Not tested	Not tested

a Mean titers: serum pools where number of animals = 20. Minimum and maximums: single sera samples

Both antigen production systems were comparable and showed only small variations between individual sera. No non-responders were observed.

Study No. KOE 050601: Immunogenicity testing of influenza antigens in mice

Thirteen mice per group received two 0.5 mL intraperitoneal doses (day 0 and 7) of either conventional egg-derived subunit vaccine process material (monovalent bulk; Agrippal) or MDCK cell-derived, chromatography purified, bulk material (monovalent and trivalent). Antigen preparations were adjusted to 15µg HA per 0.5 mL dose for monovalent formulations (total 45µg for trivalent). No adjuvant was used. Control animals received phosphate buffered saline. Serum was collected on day 21 and immunogenicity testing was performed on single serum samples (trivalent formulation) or on two separate serum pools for the monovalent formulations (mice 1–6 and mice 7–13 pooled) for hemagglutination-inhibiting (HI) antibodies against homologous and heterologous HA. As shown below, mice developed the same HI antibody titers against the HA of the immunizing strain from the two different sources. The pairs of antigen compared were equivalent in terms of homologous reactivity as well as for heterologous reactivity, as there was no statistically significant difference.

Table 4: Mean HI antibody titers against homologous and heterologous virus following administration of egg-derived and MDCK-derived monovalent bulk antigens

Vaccination Antigen	Virus grown in	Reciprocal HI Antibody Titers [a]		
		A/New Caledonia	A/Panama	B/Guangdong
A/New Caledonia/20/99; IVR 116 (H1N1)	MDCK	640	320	160
	EGG	640	320	80
A/Panama/2007/99; Resvir 17 (H3N2)	MDCK	160	2560	160
	EGG	160	2560	80
B/Guangdong/120/2000	MDCK	160	320	1280
	EGG	160	320	1280
Control	---	80	320	40

a Each value represents data from 2 separate pools of sera, total number of animals = 13 per group.

Table 5: Mean HI antibody titers and ranges against homologous virus following administration of MDCK-derived trivalent antigens

MDCK-derived vaccine antigens	Reciprocal HI Antibody Titers [a]		
	A/New Caledonia	A/Panama	B/Guangdong
A/New Caledonia/20/99; IVR 116 (H1N1) A/Panama/2007/99; Resvir 17 (H3N2) B/Guangdong/120/2000	788 (320-1280) Median: 640	2658 (1280-5120) Median: 2560	1083 (640-1280) Median: 1280
Control	80	320	40

a Values shown are mean, (range), and median of single sera samples. Number of animals = 13

Homologous HI titers for both egg- and MDCK-derived antigens were comparable and HI titer ranges for trivalent MDCK antigens were within one serum dilution step around the median. Antigens produced using the two production systems were comparable.

Study No. KOE 090702: Immunogenicity testing of influenza antigens in mice

Thirteen mice per group received two 0.5 mL intraperitoneal doses (day 0 and 7) of MDCK cell-derived, chromatography purified, trivalent bulk material. The strains tested included A/New Caledonia, A/Panama and B/Guangdong. Mice received either 15, 1.5 or 0.15 µg HA from each strain per dose. Antigen preparations did not contain adjuvant. Control animals received phosphate buffered saline. Serum was collected on day 21 and immunogenicity testing was performed on single serum samples (13/group) or on two separate serum pools (mice 1–6 and mice 7–13 pooled) for hemagglutination-inhibiting (HI) antibodies against homologous and heterologous HA. The results are shown below.

Table 6: HI titers in mice following administration of three concentrations of trivalent MDCK cell-derived influenza vaccine

MDCK cell-produced influenza antigens	µg antigen per strain per 0.5 mL dose	Reciprocal HI antibody titers [a]		
		A/New Caledonia	A/Panama	B/Guangdong
A/New Caledonia/20/99; IVR 116 (H1N1) A/Panama/2007/99; Resvir 17 (H3N2) B/Guangdong/120/00	15	738 (640-1280)	2757 (2560-5120)	699 (320-1280)
	1.5	689 (640-1280)	2166 (1280-2560)	541 (320-1280)
	0.15	320 (160-640)	985 (320-2560)	190 (80-320)
Control	0	105 (80-160)	394 (320-640)	86 (40-160)

a Values shown are mean and (range) of single sera samples. Number of animals per group = 13.

Antibody titers in mice receiving 15 or 1.5 µg antigens per strain were similar and corresponded with titers seen in Study KOE 050601 for MDCK and egg-derived antigens. Reducing the amount of antigen administered to 1/100th of the clinical dose (0.15 µg antigens per strain) resulted in a reduction in HI titers. In all groups all mice responded to all three antigens; there were no non-responders.

Immunogenicity in rabbits

Study No. 191-44: Two dose intramuscular toxicity study of Influenza vaccine formulation in New Zealand White rabbits

This GLP toxicity study is discussed in detail in section 2.6.6.3.1. Briefly, New Zealand White rabbits received 2 intramuscular injections 1 week apart. Groups of 6 animals per sex received placebo (phosphate buffered saline), the test article (Optaflu) or a comparator vaccine (Agrippal). Agrippal and Optaflu contained 15 µg of antigen from each of 3 strains for a total of 45 µg. The 3 strains used included B/Guangdong/120/00, A/New Caledonia/20/99 and A/Panama/2007/99. Half the animals were necropsied on day 10 (males) or 11 (females), the remainder on day 22 (males) or 23 (females). Serum was collected for antibody analysis from all animals on study days 1 and 8. Additional samples were collected from half the animals prior to necropsy (days 10 or 11, terminal necropsy). For the remaining animals, samples were collected on day 15 and prior to recovery necropsy on day 22 or 23.

Immunogenicity results are shown in the table below.

Table 7: Rabbit HI antibody titers ± standard deviation

Group [a]	B/Guangdong/ 120/00 (B)		A/New Caledonia/ 20/99 (H1N1)		A/Panama/ 2007/99 (H3N2)	
Sex	M	F	M	F	M	F
1 (Saline)						
Day 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	67 ± 20	60 ± 22
Day 8	0 ± 0	7 ± 10	0 ± 0	18 ± 20	93 ± 55	80 ± 44
Day 10/11 or 15	0 ± 0	0 ± 0	10 ± 17	0 ± 0	67 ± 48	87 ± 39
Day 22/23	0 ± 0	0 ± 0	0 ± 0	0 ± 0	33 ± 12	40 ± 0
2 (Optaflu)						
Day 1	3 ± 8	3 ± 8	0 ± 0	3 ± 8	67 ± 16	57 ± 27
Day 8	5 ± 12	17 ± 15	13 ± 16	27 ± 33	60 ± 22	93 ± 55
Day 10/11 or 15	123 ± 138	318 ± 266	47 ± 39	127 ± 109	73 ± 16	140 ± 33
Day 22/23	320 ± 0	427 ± 185	267 ± 92	360 ± 262	133 ± 46	213 ± 93
3 (Agrippal vaccine)						
Day 1	3 ± 8	3 ± 8	0 ± 0	3 ± 8	53 ± 16	47 ± 16
Day 8	33 ± 10	37 ± 8	47 ± 30	50 ± 40	126 ± 53	120 ± 36
Day 10/11 or 15	123 ± 115	160 ± 104	37 ± 27	70 ± 47	107 ± 41	107 ± 41
Day 22/23	227 ± 101	640 ± 320	120 ± 40	426 ± 185	160 ± 0	160 ± 0

a Number of animals evaluated was 6/sex per timepoint except at day 22/23 (3/sex)

HI results for serum antibodies to two of the strains (A/New Caledonia and B/Guangdong) showed no titer pre-treatment and an increase in titer after the second vaccine dose of Optaflu or Agrippal (reference vaccine). There was no titer against these strains in control animals. In contrast, there were background titers against strain A/Panama in control animals and pre-treatment in the Optaflu and Agrippal-treated animals, which were attributed to nonspecific binding. However, a trend towards increasing amounts of A/Panama antibody titer could be seen after the second vaccine dose.

Study No. UBA00037: Intramuscular reproductive and developmental toxicity study of FCC Vaccine in rabbits, including a postnatal evaluation

The GLP reproductive and developmental toxicity study is discussed in detail in section 2.6.6.6.1. In this study, female New Zealand White rabbits were assigned to one of two study cohorts, Caesarean-sectioning on day 29 of gestation or natural delivery on day 29 of lactation (48 animals per cohort). In each of these cohorts, one group of 24 animals received intramuscular injections of saline as the control article while the other group of 24 animals received intramuscular injections of Optaflu at the clinical dose of 45 µg in

0.5 mL. Each dose of Optaflu contained 15 µg of each of three influenza strains: A/New Caledonia/20/99, A/New York 55/2004x-157 and B/Jiangsu/10/2003 for a total antigen dose of 45 µg. Injections were given prior to mating (on days 1, 15 and 29 of study) and during gestation on days 7 and 20. Blood samples were collected prior to study initiation and prior to injection of saline or Optaflu on days 15 and 29 of study and days 7 and 20 of gestation, and at the time of euthanasia on day 29 of gestation (Caesarean-sectioning cohort) or day 29 of lactation (natural delivery cohort).

A subset of serum samples was analyzed using the hemagglutination inhibition assay (against the A/New Caledonia strain). Immunogenicity results are shown below.

Table 8: Rabbit A/New Caledonia HI antibody titers – geometric mean and (range)

Sample [a]	Caesarean-sectioned		Natural Delivery	
	Group I Control	Group II Optaflu	Group III Control	Group IV Optaflu
Pre-Study	–	<10 (<10 – <10)	–	–
Study day 15	–	<10 (<10 – 40)	–	–
Study day 29	–	320.0 (160 – 1280)	–	–
Gestation day 7	–	269.1 (80 – 1280)	–	–
Gestation day 20	–	519.2 (160 – 1280)	<10 (<10 – <10)	367.1 (160 – 1280)
Gestation day 29	<10 (<10 – <10)	226.3 (40 – 640)	–	–
Pooled fetal samples (Gestation day 29)		293.4 (80 – 1280) [b]	–	–
Lactation day 29	–	–	<10 (<10 – <10)	56.6 (40 – 160)
Pup samples (Lactation day 29)	–	–	–	17.6 (<10 – 80) [c]

[a] Mean of titers from n = 8 does, and where applicable, their fetuses or pups.
[b] Mean of titers from n = 8 pools, each pool made up of sera from fetuses from a single C-sectioned doe
[c] Mean of n = 59 individual pups
– Samples collected but not tested.
To calculate geometric means a value of 5 was assigned to samples where titer was <10.

As expected, the vaccine was immunogenic in female rabbits. Neutralizing anti-HA antibodies were measured on day 29 of the study following two injections of Optaflu. Maternal transfer of elicited antibodies was demonstrated by the presence of comparable

titers in the pooled fetal serum samples of all Optaflu-treated does. At the end of the lactation period (4 weeks after birth), does and pups still had measurable titers although they had decreased slightly compared to the levels in does and fetuses which were Caesarean-sectioned just prior to delivery on day 29 of gestation.

Influenza challenge in ferrets

Ferrets have been established as a good animal model for the study of influenza virus infection. In this model, animals can be naive or primed with heterologous virus. Animals are then immunized one or more times with a candidate and/or control vaccine. After immunization, the animals are challenged with live virus. The amount of virus used to infect the animals is established by evaluating the effects of several dilutions in naive animals. Infectivity is evaluated based on clinical signs, body weight and body temperature, presence of serum antibodies, and inflammatory cells and virus in nasal washes.

Depending upon the type of influenza vaccine being studied, ferrets may be immunologically naive or may be primed with a heterologous virus prior to the vaccination and challenge phases of the study. With subunit vaccines in general, it has been shown that immunological responses of ferrets are enhanced when animals are primed with heterologous virus prior to vaccination and challenge (Govorkova 2006, Hampson 2006, Potter 1975). Novartis has performed challenge studies with Optaflu in ferrets using both approaches. After experimentation with the model (Study Nos. CB001 through CB006; data not shown), we concluded that a study design utilizing heterologous priming optimizes the ability to evaluate Optaflu. Study No. CBI-PCS-007 was performed with the heterologous priming approach. This study is summarized in detail below. The reports for the earlier studies with naive/unprimed ferrets are available upon request.

Study No. CBI-PCS-007: Determining the efficacy of an influenza vaccine in the ferret experimental challenge model

In this study, the efficacy of Optaflu was evaluated using the ferret challenge model. Prior to study initiation, ferrets were implanted with transponders for identification and collection of body temperature data. On day 0, three groups of 8 males per group were infected intranasally (primed) with A/Panama/2007/99 virus (titer 5.12 HAU). On days 28 and 49, animals were injected intramuscularly with 0.5 mL of Optaflu, positive control vaccine (egg-derived Agrippal) or the negative control (water for injection). The test and control vaccines contained 15µg each of A/New Caledonia/20/99 (H1N1, IVR-116 reassortant), A/New York/55/2004 (H3N2, X-157 reassortant) and B/Jiangsu/10/2003 antigens for a total of 45µg per dose. On day 56, animals were challenged intranasally with A/New Caledonia/20/99 virus (titer 5.12 HAU). Animals were euthanized on day 61, five days post-challenge.

Body weights, body temperature and clinical symptoms including nasal discharge, sneezing, reduced activity or inactivity were evaluated. Serum antibodies were evaluated

pre-study, and on days 28, 49, 56 and 61. Nasal washes were collected on days 57 through 61 for virus shedding and leukocyte count evaluations. After euthanasia on day 61, nasal turbinates and lungs were extracted and tested for the presence of virus.

The focus of this section is the candidate vaccine (Optaflu). However, the positive control vaccine (Agrippal) is discussed where results differed versus Optaflu. In general, the two vaccines were similar in this challenge study.

One negative control animal was euthanized on study day 5 due to weight loss following the priming infection. All other animals survived to the scheduled necropsy on day 61.

During the challenge phase (study days 56 through 61), Optaflu-treated animals lost less body weight than negative control animals ($p = 0.022$). The sum of weight loss was also reduced in Optaflu-treated animals compared to negative controls ($p = 0.034$).

Body temperatures were measured twice daily (morning and afternoon). The mean maximum temperature change in Optaflu-treated animals was less than in negative control animals in the morning ($p = 0.04$) but was not significantly different in the afternoon.

Clinical symptom scores during the challenge phase were not statistically different between groups. The amount of shed virus recovered from nasal washes from vaccinated animals was lower compared to the negative control group. This reduction was not statistically significant for the Optaflu-treated group, however the positive control group did reach significance ($p = 0.028$). The proportion of animals shedding virus during the challenge phase reached or approached significance for the positive control group on 4 out of 5 days, but differences between the Optaflu-treated groups and the control groups were not significant.

Maximum leukocyte counts from nasal washes recorded during the challenge phase were reduced for the Optaflu-treated group compared to the negative control group and approached significance ($p = 0.089$).

Antibody titers were measured throughout the study. Most animals receiving either Optaflu (6/8) or the positive control vaccine (5/8) had a $\geq$ 4-fold increase in titer after the first vaccination. All animals seroconverted and titers increased further after the second vaccination. Antibodies persisted throughout the study. Titers for negative control animals remained at baseline levels for all timepoints. Antibody titers are shown in the table below.

Table 9: A/New Caledonia/20/99 HI titers in ferrets

Group	Animal ID	HI titer (HAU) [a]				
		Day of study [b]				
		-7	28	49	56	61
		Days post-challenge				
		-63	-28	-7	0	5
Test article (Optaflu)	1336	5	5	80	320	80
	1337	5	5	40	160	80
	1338	5	5	80	320	160
	1339	5	5	40	160	80
	1340	5	5	80	320	160
	1341	5	5	5	80	40
	1342	5	5	10	80	40
	1343	5	5	20	80	20
Positive control (Agrippal)	1344	5	5	160	1280	320
	1345	5	5	5	40	40
	1346	5	5	20	320	160
	1347	5	5	40	320	160
	1348	5	5	20	320	80
	1349	5	5	5	80	40
	1350	5	5	5	80	40
	1351	5	5	40	320	160
Negative control (water)	1352	5	5	5	5	5
	1353	5	5	5	5	5
	1355	5	5	5	5	5
	1356	5	5	5	5	5
	1357	5	5	5	5	5
	1358	5	5	5	5	5
	1359	5	5	5	5	5

a All values of 5 HAU were without detectable antibody

b Animals were primed with A/Panama/2007/99 on study day 0. Test and control articles were administered on study days 28 and 49. Animals were challenged with A/New Caledonia/20/99 on study day 56.

Optaflu was considered comparable to the positive control vaccine because differences between the two groups were not statistically significant. Both vaccines induced protective levels of antibody, and although vaccinated animals exhibited some flu-like

symptoms following challenge, bodyweight loss, body temperature increase, and leukocyte counts were all reduced when compared to the negative control group.

2.6.2.2.3 Supportive studies with MF59-adjuvanted egg-derived influenza vaccines

There is a substantial nonclinical data package supporting the use of MF59-adjuvanted influenza antigens. The studies listed below were performed using antigen manufactured using an egg-based process, and demonstrate that in mice, rabbits, and ferrets MF59-adjuvanted influenza antigens are immunogenic, and where tested, protective. These studies are summarized briefly, and reports are provided in Module 4.

Supportive immunogenicity and efficacy studies with egg-derived vaccines

Study number	Study type	Species	Vaccine (strain)
[redacted] Mouse Study	Challenge with homologous and heterologous wild-type virus	Mouse	Aflunov (Vietnam)
UBA00021	Reproductive and developmental toxicity	Rabbit	Aflunov (Vietnam)
780-N007104 (report in preparation)	Challenge ~4 months post-vaccination with wild-type virus homologous and heterologous to the vaccine strain	Ferret	Aflunov (Vietnam) Aflunov (Turkey)
765-N106857	Challenge with wild-type virus homologous and heterologous to the vaccine strain	Ferret	Aflunov (Vietnam) and Aflunov (Turkey)
673-N106850	Challenge with homologous wild-type virus	Ferret	Aflunov (Vietnam)
CBI-PCS-008	Challenge with homologous reverse genetics virus	Ferret	Aflunov (Vietnam)
488182	Repeat dose toxicity study with Fluad and Fluad formulations	Rabbit	Fluad
94-0184, 93-847	Antibody response and lymphoproliferative response in young and old mice	Mouse	Fluad equivalent
94-0307, 94-0214, 94-0215	Antigen dose versus antibody response, Post-challenge lung viral load, Protective efficacy against challenge	Mouse	Fluad equivalent
MF-1/MF-2 2003/04	Potency study of MF59 for influenza trivalent subunit vaccine in Balb/c mice of 8 weeks and 18 months of age	Mouse	Fluad

Aflunov

Efficacy in mice and ferrets has been demonstrated with vaccine formulations equivalent to Focetria. The vaccine was immunogenic in mice ([redacted] Mouse Study) and prevented infection and viral replication in brain, lung and spleen in mice challenged with homologous (Vietnam) or heterologous (Indonesia) virus.

Immunogenicity data in rabbits was generated during a reproductive and developmental toxicity study (Study No. UBA00021). The vaccine was administered by intramuscular injection (15 µg per dose) three times before mating and twice during gestation. The vaccine was well tolerated, did not cause maternal or embryofetal toxicity, was not teratogenic, and had no effects on post-natal development. Additionally, the vaccine was immunogenic in maternal rabbits, developing foetuses had comparable titres, and antibodies persisted through the first 4 weeks of life in F1 offspring.

Immunogenicity and protection were also evaluated in the ferret challenge model. Three studies were done using highly pathogenic avian influenza virus (HPAI) to challenge animals. A fourth study, the earliest chronologically, was conducted using challenge with a non-lethal reverse-genetics virus strain.

In Study No. 765-N106857, the protective and cross-protective efficacy of Focetria (Aflunov Turkey and Aflunov Vietnam in the study report) vaccines was evaluated in ferrets. Animals received one or two doses of vaccine containing either 3.75 or 7.5 µg antigen per dose before challenge with highly pathogenic avian influenza (A/Vietnam/1203/04). Based on mortality, body weights, body temperatures, and clinical signs, administration of vaccine homologous or heterologous to the challenge strain protected ferrets from challenge.

In Study No. 780-N007104, ferrets were vaccinated twice three weeks apart with Focetria (Aflunov Vietnam or Aflunov Turkey in the study report) containing 3.75 or 7.5 µg antigen per dose. Serum samples were analysed monthly. Approximately 4 months later, when antibody titres had waned, animals were challenged with HPAI (A/Vietnam/1203/04). Based on mortality, body weights, body temperatures, and clinical signs, administration of vaccine homologous or heterologous to the challenge strain protected ferrets from challenge.

In Study No. 673-N106850, animals were vaccinated, and then challenged with highly pathogenic wild-type virus (A/Vietnam/1203/04). Four of eight control animals died. There was no mortality in animals that received vaccine. Body weight, body temperature, nasal viral titres, and homologous immunogenicity data show that one or two doses of vaccine was immunogenic, prevented body weight loss and fever, and decreased nasal viral titres.

In Study No. CBI-PCS-008, ferrets were vaccinated prior to challenge with the non-lethal reverse-genetics virus (Vietnam). Serological testing of samples taken from this study

showed cross-reaction with a heterologous (Turkey) reverse genetics virus by hemagglutination inhibition (HI) and microneutralization assays (Study No. VIV-PCS-001).

These studies demonstrated that the vaccine is immunogenic (mice, rabbits, and ferrets) and protects against challenge with virus homologous and heterologous to the vaccine strain.

Fluad

A series of studies (Study Nos. 94-0184, 93-847, 94-0307, 94-0214, and 94-0215) was conducted to investigate the immune response in young (2-3 months) and old (18 months) mice after subcutaneous or intramuscular administration of influenza vaccines. In these studies, the non-adjuvanted influenza vaccine Agrippal was administered alone or in combination with MF59 adjuvant (equivalent to Fluad).

These studies demonstrated that in all cases, dose-related antigen-specific antibody response were elicited to all three HA antigens present in the vaccine. An antigen-specific antibody response was also elicited in seropositive mice, which had previously been infected with influenza virus. Other beneficial effects associated with immunisation included proliferation of spleen-derived lymphocytes, reduction in lung viral load following challenge with influenza virus, and protection against challenge with a lethal dose of influenza virus. In all cases, the presence of the MF59 adjuvant significantly improved the immune response, particularly in old mice.

A mouse immunogenicity study (Study No. MF-1/MF-2 2003/04) was conducted to explore the antigen dose-response and to confirm the role of adjuvant (MF59) in the enhancement of immunogenicity. A fixed ratio of antigen to MF59 (1:1) was utilized. This study confirmed that MF59 enhanced the humoral immune responses in young and old mice and that MF59 results in antigen-sparing.

In a repeat-dose toxicity study (Study No. 488182) with Fluad and Fluad formulations, groups of eight rabbits/sex/group were dosed three times, two weeks apart, with saline (control group), Fluad, or one of two Fluad-like formulations. Fluad was immunogenic in all males and females on Days 15 and 29 (14 days after the first and second dose, respectively) and after a recovery period (Day 43).

2.6.2.2.4 MF59 Adjuvant

MF59 adjuvant (MF59C.1) is an oil-in-water emulsion with a squalene internal oil phase and a citrate buffer external aqueous phase. Two nonionic surfactants, sorbitan trioleate and polysorbate 80, serve to stabilize the emulsion. The components of MF59 adjuvant are provided below.

Table 10: MF59C.1 Adjuvant composition

Component	Quantity per mL
Squalene	39 mg
Polysorbate 80	4.7 mg
Sorbitan trioleate	4.7 mg
Sodium citrate, dihydrate	2.65 mg
Citric acid, monohydrate	0.17 mg
Water for injection	q.s.

In general, a single clinical dose of MF59-adjuvanted vaccine contains 0.25 mL MF59 combined with 0.25 mL antigen.

During the development of MF59, various formulations were tested. A water-based formulation of MF59 (referred to as MF59 water, MF59-0, or MF59W.1) was later optimized by the addition of citrate buffer to provide increased stability, and is designated MF59C.1. Information pertaining to both formulations is relevant because citrate is a common, well-tolerated excipient, and immunogenicity and toxicology studies have identified no notable differences between the two formulations.

MF59 administered alone and in combination with a variety of antigens has been extensively tested in a number of animal models. Antigens adjuvanted with MF59 include recombinant proteins or glycoproteins from herpes simplex virus (HSV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), cytomegalovirus (CMV), hepatitis B virus (HBV), human papilloma virus (HPV), and malaria, as well as natural glycoproteins from influenza virus. In all cases, the antigen+MF59 combinations generated high antigen-specific antibody titers and, where tested, high virus neutralizing titers.

2.6.2.3 Secondary Pharmacodynamics

No specific secondary pharmacodynamics studies have been done with FCC H1N1sw, FCC/MF59-H5N1, or Optaflu, which is justifiable based on the nature of these products.

The potential for undesirable pharmacological activities of MF59 (cardiovascular and neurological safety pharmacology) was assessed as a component of repeated-dose toxicology studies in dogs (section 2.6.2.4).

2.6.2.4 Safety Pharmacology

Dedicated safety pharmacology (or secondary pharmacodynamics) studies were not performed with FCC H1N1sw, FCC/MF59-H5N1, or Optaflu. Nonclinical and clinical studies have not identified overt effects of MF59-adjuvanted vaccines on physiological

functions (e.g. central nervous system, respiratory, cardiovascular and renal functions). Inactivated purified influenza antigens do not fall into categories 'of concern' for possessing unexpected innate pharmacological activities.

However, there is pertinent information on MF59 adjuvant that provides supportive secondary or safety pharmacological data. During the early development of MF59 adjuvant, two repeat-dose dog studies were conducted to evaluate the toxicity of vaccine formulations with antigens that are unrelated to this dossier. Both studies had an MF59 group and a saline/buffer control group, and included the evaluation of cardiovascular and neurological parameters. An overview of the study designs and results is provided below.

Table 11: Cardiovascular and neurological evaluations during repeat-dose studies with MF59 in dogs

Study number	Test materials and intramuscular dosing schedule	Number of animals (M/F)	Cardiovascular and neurological evaluations*
89-6193	0.5 ml saline (control) or 1:1 saline:MF59, 3 injections on days 1, 16 and 29	2/2	Cardiovascular: no relevant changes noted Neurology: all dogs showed normal reactions
90-6231	0.5 ml buffer (control) or 1:1 buffer:MF59 3 injections on days 1, 15 and 29	2/2	Cardiovascular: no treatment-related abnormalities Neurology: no abnormalities detected

* Evaluated pretest, and prior to necropsy. Animals were necropsied 1week post-last dose.

Based on the cardiovascular and neurological evaluations performed in dogs that received intramuscular injections of MF59 adjuvant, and the known safety of MF59-adjuvanted influenza antigens in animals and humans, the risk of unanticipated secondary or safety pharmacological effects in vaccinees receiving FCC H1N1sw is considered extremely unlikely.

2.6.2.5 Pharmacodynamic Drug Interactions

No formal pharmacodynamic studies have been conducted with FCC H1N1sw in accordance with its status as a vaccine.

2.6.2.6 Discussion and Conclusions

The candidate vaccine, FCC H1N1sw, has not been tested in nonclinical studies, but relevant nonclinical data are provided by studies with a comparable MF59-adjuvanted vaccine, FCC/MF59-H5N1 (made using the same process), and the parent vaccine (Optaflu; non-adjuvanted trivalent seasonal influenza vaccine). Studies in mice with Optaflu formulations showed that cell-derived antigen was comparable to egg-derived, and when cell-derived antigens were adjuvanted with MF59, immunogenicity increased.

Efficacy studies in the ferret challenge model were performed with both egg- and cell-derived antigens; the vaccines were immunogenic and protective. Supportive nonclinical data is provided by studies with the egg-derived MF59-adjuvanted vaccines Aflunov (H5N1) and Fluad (seasonal).

2.6.2.7 Tables and Figures

All tables are included in the text.

2.6.2.8 References

Govorkova EA, Webby RJ, Humberd J, Seiler JP, Webster RG. Immunization with reverse-genetics-produced H5N1 influenza vaccine protects ferrets against homologous and heterologous challenge. J Infect Dis. 2006 Jul 15;194(2):159-67.

Hampson AW. Ferrets and the challenges of H5N1 vaccine formulation. J Infect Dis. 2006 Jul 15;194(2):143-5.

Potter CW, McLaren C, Jennings R. Assessment of resistance to influenza virus infection in animal models. Dev Biol Stand. 1975;28:307-18.

2.6.3 Pharmacology Tabulated Summary

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2.6.3 Pharmacology Tabulated Summary

2.6.3.1 Pharmacology Overview

Study Title	Test System	Method of Administration	Testing Facility	Study Number	Location
Primary Pharmacodynamics - studies with MF59-adjuvanted cell culture-derived vaccines					
Immunogenicity of MF59-adjuvanted trivalent influenza vaccine in mice	Mouse	Intramuscular	Novartis	None assigned	Not applicable
6-Week vaccine toxicity study with H5N1 FCC + MF59 + Thiomersal vaccine by 3 intramuscular injections in NZW rabbits	Rabbit	Intramuscular	[redacted] NL	466122	4.2.3.2-1
Primary Pharmacodynamics - studies with non-adjuvanted cell culture-derived vaccines					
Immunogenicity of antigen produced in egg versus cell culture	Mouse	Intraperitoneal	Chiron	KOE 090697	4.2.1.1-1
Immunogenicity of antigen produced in egg versus cell culture	Mouse	Intraperitoneal	Chiron	KOE 050601	4.2.1.1-2
Immunogenicity after administration of 0.15, 1.5 or 15 µg of antigen	Mouse	Intraperitoneal	Chiron	KOE 090702	4.2.1.1-3
Immunogenicity in rabbit (repeat dose toxicity)	Rabbit	Intramuscular	[redacted]	191-44	4.2.3.2-2
Immunogenicity in rabbit (reproductive and developmental toxicity)	Rabbit	Intramuscular	[redacted]	UBA00037	4.2.3.5-1
Post-vaccination challenge with A/New Caledonia/20/99	Ferret	Intramuscular	[redacted]	CBI-PCS-007	4.2.1.1-8
Post-vaccination challenge with A/New Caledonia/20/99	Ferret	Intramuscular	[redacted]	CB002	Available on request

Study Title	Test System	Method of Administration	Testing Facility	Study Number	Location
Post-vaccination challenge with A/Panama/2007/99	Ferret	Intramuscular	[redacted]	CB003	Available on request
Post-vaccination challenge with A/New Caledonia/20/99	Ferret	Intramuscular	[redacted]	CB004	Available on request
Post-vaccination challenge with B/Guangdong/120/00	Ferret	Intramuscular	[redacted]	CB006	Available on request
Supportive Primary Pharmacodynamics – studies with egg-derived MF59-adjuvanted vaccines					
Aflunov					
Immunogenicity, challenge and viral titers in mice	Mouse	Intramuscular	[redacted] USA	None assigned	4.2.1.1-7
Intramuscular reproductive and developmental toxicity study of Fluad H5N1 vaccine in rabbits, including a postnatal evaluation	Rabbit	Intramuscular	[redacted] US	UBA00021	4.2.3.5-2
Evaluation of the protective and cross-protective efficacy of Aflunov Turkey and Aflunov Vietnam vaccines in ferrets challenged with highly pathogenic avian influenza	Ferret	Intramuscular	[redacted] US	765-N106857	4.2.1.1-9
Prolonged Homologous/Heterologous Vaccine Efficacy in Ferrets Challenge with HPAI	Ferret	Intramuscular	[redacted] US	780-N007104	Report in preparation
Evaluation of the Protective Efficacy of Fluad H5N1 Vaccine in Ferrets Challenged with Highly Pathogenic Avian Influenza	Ferret	Intramuscular	[redacted] US	673-N106850	4.2.1.1-10

Study Title	Test System	Method of Administration	Testing Facility	Study Number	Location
Efficacy of an H5N1 vaccine adjuvanted with MF59C.1	Ferret	Intramuscular	[redacted] UK	CBI-PCS-008 & VIV-PCS-001	4.2.1.1-11 4.2.1.1-12 4.2.1.1-13
Fluad formulations					
Antibody Response with Agrippal S1 vaccine in young and old mice	Mouse	Subcutaneous	Not specified	94-0184 / 93-847 (Experiment 1)	4.2.1.1-4
Lymphoproliferative Response with Agrippal S1 vaccine in young and old mice	Mouse	Subcutaneous	Not specified	94-0184 / 93-847 (Experiment 2)	4.2.1.1-4
Antibody Response with Agrippal S1 vaccine in seropositive young and old mice	Mouse	Subcutaneous	Not specified	94-0184 / 93-847 (Experiment 3)	4.2.1.1-4
Antibody response with Biocine Flu Vaccine alone or mixed with MF59-0 water	Mouse	Intramuscular	Not specified	94-0307 / 94-0214 94-0215 (Experiment 1)	4.2.1.1-4
Post Challenge Relative Viral Load in the Lungs of Immunized Mice	Mouse	Intramuscular	Not specified	94-0307 / 94-0214 94-0215 (Experiment 2)	4.2.1.1-5
Protective Efficacy of Biocine Flu Vaccine with and without MF59	Mouse	Intramuscular	Not specified	94-0307 / 94-0214 94-0215 (Experiment 3)	4.2.1.1-5
Potency study of the MF59 adjuvant for influenza trivalent subunit vaccine in Balb/c mice of 8 weeks and 18 months of age	Mouse	Subcutaneous	[redacted]	MF-1/MF-2 2003/04	4.2.1.1-6
4-Week Toxicity Study with Fluad®, Fluad High B, and Fluad High H3+IC31® Influenza Vaccine Formulations by 3 Intramuscular Injections in NZW Rabbits Followed by a 2-week Recovery Period	Rabbit	Intramuscular	[redacted] NL	488182	4.2.3.7-1

Study Title	Test System	Method of Administration	Testing Facility	Study Number	Location
Secondary Pharmacodynamics					
Not applicable	—	—	—	—	—
Safety Pharmacology (applicable to MF59 adjuvant only)					
Intramuscular Tolerability Study in Dogs	Dog	Intramuscular	Ciba-Geigy	89-6193	4.2.1.3-1
Comparative Intramuscular Tolerability Study in Dogs	Dog	Intramuscular	Ciba-Geigy	90-6231	4.2.1.3-2
Pharmacodynamic Drug Interactions					
Not applicable	—	—	—	—	—

2.6.4 Pharmacokinetics Written Summary

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2.6.4 Pharmacokinetics Written Summary

2.6.4.1 Brief Summary

Pharmacokinetic studies with were not performed and are not considered relevant for vaccines.

Classic absorption, distribution, metabolism and excretion (ADME) studies were not performed because they are not considered relevant for vaccines. However, the squalene component of MF59C.1 is involved in normal metabolic pathways and is discussed below (2.6.4.8).

2.6.4.2 Methods of Analysis

Hemagglutination Inhibition Assay

A standard HI assay using horse blood cells and virus was used to quantify the antibody response to vaccination. The assay was qualified for use in rabbit sera and a report prepared (Study No. QD-126).

Microneutralization Assay

A standard MN assay using Madin-Darby Canine Kidney (MDCK) cells and virus was used to quantify the antibody response to vaccination.

2.6.4.3 Absorption

Not applicable.

2.6.4.4 Distribution

Not applicable.

2.6.4.5 Metabolism

Not applicable.

2.6.4.6 Excretion

Not applicable.

2.6.4.7 Pharmacokinetic Drug Interactions

Not applicable.

2.6.4.8 Other Pharmacokinetic Studies

Distribution and clearance of squalene, a component of MF59C.1

Squalene is an intermediate in the biosynthesis of cholesterol and is a natural constituent in dietary products, which include vegetable and fish oils (Lehninger 1975, Price 1996). The small volume of squalene administered with each vaccination is unlikely to have measurable metabolic implications.

The distribution of MF59 after intramuscular injection in mice was evaluated (Dupuis 1999). The goal of the study was to determine the distribution of MF59 injected with soluble antigen gD2 from type 2 herpes simplex virus (HSV) and to compare the distribution of gD2 injected with or without MF59. At 4 h, 36% of the injected dose of labeled MF59 was in the quadriceps muscle and about 50% was in the inguinal fat surrounding the muscle. Half of the initial amount of labelled MF59 in muscle was detected 42 h after injection. The amount of labeled MF59 in the draining lymph nodes was maximal 2 d after injection, which represented 0.1±0.3% of the injected dose. At 4 h, 12% of the injected dose of labeled gD2 was found in the muscle. The presence of MF59 did not significantly modify the distribution of gD2. The results indicate that MF59 and gD2 distribute and are cleared independently after intramuscular injection.

Clearance studies in rabbits injected intramuscularly with ^{125}I-squalene labeled MF59 demonstrated that the adjuvant is rapidly cleared (Ott 1995). Only 10% of the administered squalene remained at the injection site at 6 hours post-injection and decreased to 5% at 120 hours after injection.

2.6.4.9 Discussion and Conclusion

In accordance with current guidelines, pharmacokinetic or classic absorption, distribution, metabolism and excretion studies with FCC H1N1sw vaccine (or MF59) were not conducted because such studies are not considered relevant for a vaccine.

2.6.4.10 Tables and Figures

Not applicable.

2.6.4.11 References

Dupuis M, McDonald DM, Ott G. Distribution of adjuvant MF59 and antigen gD2 after intramuscular injection in mice. Vaccine. 1999 Oct 14;18(5-6):434-9.

Lehninger AL. The Biosynthesis of Lipids. In: Worth, editor. Biochemistry. Second ed. New York: The Johns Hopkins University School of Medicine; 1975. p. 679 - 86.

Ott G, Barchfeld G, Chernoff D, Radhakrishnan R, van Hoogevest P, Van Nest G. Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines. In: Powell M,

Newman M, editors. Vaccine Design: The Subunit and Adjuvant Approach. New York: Plenum Press; 1995.

Price RJ. What is Squalene and Squalane? University of California, Davis, CA., 95616-8598: Seafood Network Information Center; 1996.

2.6.5 Pharmacokinetics Tabulated Summary

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2.6.5 Pharmacokinetics Tabulated Summary

No tabulated summary is presented because pharmacokinetic studies with FCC H1N1sw vaccine have not been performed.

2.6.6 毒性試験の概要文

目　次

表 一 覧

略号一覧

略号	省略していない表現（英）	省略していない表現（日）
MDCK	Madin-Darby Canine Kidney	イヌ腎由来 MDCK 細胞

1 緒言

FCC H1N1sw ワクチンの承認申請に際して提出する CTD2.6.6 毒性試験の概要文の構成について説明する。

本資料は，本剤のドイツにおける承認申請に際して提出した CTD（以下，ドイツ CTD）を主な申請資料として構成している。しかしながら，ウイルスの培養細胞として使用しているイヌ腎臓由来 MDCK 細胞（Madin-Darby Canine Kidney，MDCK）の腫瘍原性及びがん原性に関しては，海外では細胞培養由来ワクチンである Optaful の承認申請時に既に評価されているため，ドイツ CTD には含まれていない。したがって，当該資料として Optaful の CTD2.6.6 Toxicology Written Summary を添付した。また，本剤にアジュバントとして配合している MF59 アジュバントに関しては，海外では広く使用されているため，一部の試験報告書（重要な試験に関する資料）を除いてドイツ CTD には添付されていない。したがって，試験の記載に重複が生じるが，MF59 アジュバントに関する補足資料として，MF59 アジュバントの Drug Master File（DMF）から 2.6.6 Toxicology Written Summary を添付した。

MDCK 細胞関連資料の記載箇所をTable 1-1に，MF59 アジュバント関連資料の記載箇所をTable 1-2にそれぞれ記載した。

Table 1-1 MDCK 細胞関連資料の CTD 記載箇所

試験の種類	試験系	試験番号	CTD 記載箇所
要約	―	―	OP: 2.6.6. Toxicology Written Summary, p.16-18
腫瘍原性 (細胞)	ヌードマウス	48329	OP: 2.6.6. Toxicology Written Summary, p.19-25
がん原性 (溶解液)	ヌードマウス (幼若)	48330	OP: 2.6.6. Toxicology Written Summary, p.29-31
	ラット (幼若)	48332	OP: 2.6.6. Toxicology Written Summary, p.31-32
	ハムスター (幼若)	48331	OP: 2.6.6. Toxicology Written Summary, p.32-33
がん原性 (DNA)	ヌードマウス (幼若)	48333	OP: 2.6.6. Toxicology Written Summary, p.36-37
	ラット (幼若)	48335	OP: 2.6.6. Toxicology Written Summary, p.37-39
	ハムスター (幼若)	48334	OP: 2.6.6. Toxicology Written Summary, p39-40
腫瘍原性 (細胞/溶解液)	ラット (幼若)	B012888/02	OP: 2.6.6. Toxicology Written Summary, p.40-41
腫瘍原性 (細胞)	ヌードマウス	B96YG21.001	OP: 2.6.6. Toxicology Written Summary, p.41-43

OP: OPTAFLU®の EMEA への申請資料（2006 年 5 月作成）

Table 1-2 MF59 アジュバント関連資料の CTD 記載箇所

試験の種類	試験系	試験番号	CTD 記載箇所
Pivotal MF59 studies			
反復投与毒性			
	ウサギ	90-6081	CTD2.6.6. Toxicology Written Summary, p.26-28
遺伝毒性			
Ames test	*In vitro*	G96AQ62.502	CTD2.6.6. Toxicology Written Summary, p.29
		G96AQ61.502	CTD2.6.6. Toxicology Written Summary, p.29
小核	マウス	G96AQ62.122	CTD2.6.6. Toxicology Written Summary, p.29-30
		G96AQ61.122	CTD2.6.6. Toxicology Written Summary, p.29-30
皮膚感作性	モルモット	564278	CTD2.6.6. Toxicology Written Summary, p.30-31
生殖発生毒性			
胚・胎児発生	ラット	1303-002	CTD2.6.6. Toxicology Written Summary, p.31-34
	ウサギ	1303-001P	CTD2.6.6. Toxicology Written Summary, p.34-35
Nonpivotal MF59 studies			
単回投与毒性	ウサギ	501464	CTD2.6.6. Toxicology Written Summary, p.35-36
		00-2672	CTD2.6.6. Toxicology Written Summary, p.36-37
反復投与毒性	ウサギ	89-6280	CTD2.6.6. Toxicology Written Summary, p.37
		2777-102	CTD2.6.6. Toxicology Written Summary, p.37-38
		89-6192	CTD2.6.6. Toxicology Written Summary, p.38
		90-6230	CTD2.6.6. Toxicology Written Summary, p.38-39
		2670-101	CTD2.6.6. Toxicology Written Summary, p.39
		759-002	CTD2.6.6. Toxicology Written Summary, p.39-40
		950031	CTD2.6.6. Toxicology Written Summary, p.40
		656583	CTD2.6.6. Toxicology Written Summary, p.40-41
		501438	CTD2.6.6. Toxicology Written Summary, p.41
		00-2673	MF59: 2.6.6 Toxicology Written Summary, p.11-12 CTD2.6.6. Toxicology Written Summary, p.42

試験の種類	試験系	試験番号	CTD 記載箇所
反復投与毒性 (続き)	ウサギ	2670-100	MF59: 2.6.6 Toxicology Written Summary, p.12-14 CTD2.6.6. Toxicology Written Summary, p.42-43
		466122	CTD2.6.6. Toxicology Written Summary, p.6-10
	イヌ	89-6281	CTD2.6.6. Toxicology Written Summary, p.43
安全性薬理	イヌ	89-6193	CTD2.6.6. Toxicology Written Summary, p.43 CTD2.6.2. Pharmacology Written Summary, p.22-23
		90-6231	CTD2.6.6. Toxicology Written Summary, p.43 CTD2.6.2. Pharmacology Written Summary, p.22-23

CTD: 本ワクチンのドイツへの申請資料（CTD）
MF59: MF59 Drug Master File

2.6.6 Toxicology Written Summary

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2.6.6.1 Brief Summary

The candidate vaccine, FCC H1N1sw, is a monovalent influenza virus vaccine, surface antigen, inactivated, with MF59C.1 adjuvant. It is a sterile suspension for injection in either pre-filled syringes or multi-dose vials. The active ingredient of the vaccine is purified hemagglutinin (HA) and neuraminidase (NA) from an H1N1 virus (Reassortant virus X-179A, derived from A/California/07/2009). The antigens contained in FCC H1N1sw are cell culture-derived; they are purified from virus grown in Madin Darby Canine Kidney (MDCK) cells.

Pending clinical confirmation, the anticipated 0.25 mL clinical dose of FCC H1N1sw will contain 3.75 µg of H1N1 hemagglutinin and 0.125 mL MF59C.1 adjuvant (MF59). MF59 adjuvant is an oil-in-water emulsion, composed of squalene as the oil phase, stabilized with the surfactants polysorbate 80 and sorbitan trioleate, in citrate buffer.

The candidate vaccine, FCC H1N1sw, has not been tested in nonclinical studies, however studies with a comparable MF59-adjuvanted vaccine, FCC/MF59-H5N1, and the parent vaccine Optaflu provide relevant data. Both FCC/MF59-H5N1 and Optaflu are manufactured using the cell culture process. FCC/MF59-H5N1 and FCC H1N1sw are both MF59-adjuvanted monovalent influenza vaccines intended for pandemic use. Optaflu is a non-adjuvanted, trivalent, seasonal influenza vaccine.

A GLP rabbit toxicity study was performed to assess local and systemic effects following three intramuscular administrations of FCC/MF59-H5N1 at a dose of 15 µg of antigen and 0.25 mL adjuvant per administration. The doses of antigen and adjuvant used in this study exceed the proposed clinical dose for FCC H1N1sw. Immunogenicity was also assessed. FCC/MF59-H5N1 was immunogenic and well tolerated locally and systemically.

The local and systemic toxicity of Optaflu was evaluated in a GLP rabbit toxicology study. Two clinical (0.5 mL) doses of Optaflu were administered intramuscularly. The vaccine was well tolerated locally, based on the low order of reactogenicity seen microscopically at the intramuscular injection sites, and there were no systemic toxicological effects. The toxicological profiles of Optaflu and Agrippal (egg-derived influenza vaccine) were comparable.

In a GLP reproductive and developmental toxicity study in rabbits, five intramuscular injections of Optaflu were administered prior to mating (3 doses) and during gestation (2 doses) at the clinical dose and volume. Evaluations included potential effects of the vaccine or elicited antibodies on mating, fertility, gestation, lactation and maternal behavior in addition to potential effects on offspring of treated does. There were no effects of Optaflu on any aspect of reproductive or developmental health under the conditions of this study. Optaflu was not teratogenic.

Supportive data are provided by studies with Novartis products that are related to FCC H1N1sw as shown below.

Table 1: Related Novartis Influenza Vaccines

Vaccine	Description*	Status
Cell culture influenza vaccines		
Optaflu®	Cell culture-derived trivalent, interpandemic non-adjuvanted	'Parent' vaccine registered for seasonal use in EU (EU/1/07/394/001-009).
FCC H1N1sw	Cell culture-derived monovalent, A/H1N1 MF59C.1 adjuvant	Investigative H1N1 (swine origin) vaccine. Manufacturing based on Optaflu® process.
FCC/MF59 -H5N1	Cell culture-derived monovalent, A/H5N1 MF59C.1 adjuvant	Manufacturing based on Optaflu® process. Formulation, fill and packaging based on Fluad® process.
Related influenza vaccines (provide supportive nonclinical data)		
Aflunov®	Egg-derived monovalent, A/H5N1 MF59C.1 adjuvant	Under evaluation for use prior to an H5N1 outbreak by EMEA (CP No. EMEA/H/C/804) and the Swiss regulatory authority. Produced using Fluad® manufacturing processes.
Fluad®	Egg-derived trivalent, interpandemic MF59C.1 adjuvant	Registered for seasonal use in EU (MRP No. IT/H/0104/001) and Germany (PEI.H.01444.01.1), also registered outside EU.

*All of these vaccines are surface antigen, inactivated

These related vaccines are supportive because nonclinical studies have demonstrated the comparability of egg-derived and cell culture-derived antigens. Egg- and cell culture-derived antigens have also been shown to be comparable in the clinic. A listing of nonclinical studies with these related vaccines is provided in section 2.6.2.2.3.

There is also a comprehensive package of nonclinical studies with MF59 that demonstrate its safety and effectiveness as an adjuvant with influenza and non-influenza antigens, and the nonclinical data demonstrating the adjuvant activity of MF59 have been confirmed in clinical studies. MF59 is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen and minor inflammatory and degenerative changes at the injection site are consistent with the effects of intramuscular injections of an immunological adjuvant. These findings are readily reversible within days to 1 to 2 weeks. MF59 is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat).

2.6.6.2 Single Dose Toxicity

No single dose toxicity studies have been conducted with FCC H1N1sw.

In Study No. 466122 (FCC/MF59-H5N1 vaccine) no necropsy was performed after a single dose, however animals were evaluated for signs of local and systemic toxicity. Based on clinical signs, injection site reactions, body weights, food consumption, heart rate, respiratory rate and body temperatures, hematology and clinical biochemistry, no adverse effects were identified following a single dose of vaccine.

2.6.6.3 Repeat Dose Toxicity

No repeat dose toxicity studies have been conducted with FCC H1N1sw, however the comparable vaccine FCC/MF59-H5N1 has been evaluated; this study is described below.

In this study, the doses of antigen and adjuvant in the FCC/MF59-H5N1 vaccine tested exceeded those proposed for the FCC H1N1sw vaccine. Each of the three doses administered to rabbits (~3.5 kg) contained 15 µg of antigen and 0.25 mL adjuvant, resulting in a cumulative dose of 45 µg antigen and 0.75 mL adjuvant. In comparison, the proposed 2-dose clinical regimen of FCC H1N1sw will contain 3.75 µg of antigen and 0.125 mL adjuvant per dose, for a cumulative dose of 7.5 µg antigen and 0.25 mL adjuvant. Therefore, comparing on a body weight basis, rabbits received approximately 17× more antigen than a 10 kg child will receive, and approximately 8.5× more adjuvant.

6-Week vaccine toxicity study with H5N1 FCC + MF59 + Thiomersal vaccine by 3 intramuscular injections in NZW rabbits (Study No. 466122)

This GLP rabbit toxicity study was performed to assess immunogenicity and any local or systemic effects following three intramuscular administrations of FCC/MF59-H5N1 (called H5N1 FCC+MF59+Thiomersal in the study report) at a dose of 15 µg of antigen per administration (section 2.6.7.7, Study No. 466122).

The drug substance used to formulate the test article (FCC/MF59-H5N1) was HA from the A/Indonesia strain, however following RT-PCR analysis, the drug substance was found to contain DNA sequences from both A/Indonesia and A/turkey/Turkey strains. The decision was made to use this lot in the GLP toxicology study, because the 15 µg HA antigen formulated with MF59 adjuvant that was used was prepared by a process comparable to the production methods that will be used for GMP clinical lots. The viral antigen in the test material is representative of the material to be tested in clinical studies.

The test and control materials used in this study contained 100 µg/mL (50 µg/dose) thiomersal. Thiomersal may be used if multidose vials and/or stockpiling is required, therefore it was included in the formulations used in this study as a ‘worst case’ scenario. The study design is shown below.

Table 2: Study No. 466122 – Study Design

Group	Number of animals	Treatment 0.5 mL per intramuscular dose days 1, 15 and 29	Main necropsy	Recovery necropsy
			Day 31	Day 43
1	8/sex	Phosphate buffered saline (PBS) (saline control, 0.5 mL)	4/sex	4/sex
2	8/sex	MF59 + PBS (adjuvant control, 0.25 mL MF59)	4/sex	4/sex
3	8/sex	FCC/MF59-H5N1 (15 µg antigen, 0.25 mL MF59)	4/sex	4/sex

Note: all test and control materials contained 100 µg/mL thiomersal

New Zealand White (NZW) rabbits received three intramuscular injections in alternating hind legs, starting with the right leg. The following parameters were evaluated during the treatment and recovery periods: clinical signs (at least once daily; twice daily on dosing days), skin reactions at the intramuscular sites of injection (24 and 48 hours post-dose), body weights (weekly), food consumption (twice weekly). Ophthalmoscopy was performed pre-study for all animals, on day 30 for main necropsy animals, and on day 42 for recovery animals. Heart rate, respiratory rate and rectal body temperature were evaluated pre-study, prior to dosing, and approximately 2 hours post-dose. Hematology and clinical biochemistry were evaluated pre-study and on days 8, 17 and 31 for all animals, and on day 43 for recovery animals. Blood samples were collected for antibody analyses pre-study, prior to dosing on days 15 and 29, on day 31 (main necropsy animals), and on day 43 (recovery necropsy animals).

A comprehensive macroscopic evaluation was performed at termination. Selected organs (adrenals, brain including brainstem, heart, kidneys, liver, ovaries, spleen, testes and thymus) were weighed, and evaluation of the complete WHO tissue list was performed.

Due to a laboratory error, data from one male in recovery group 1 and one female in recovery group 3 had to be excluded from day 29 onward. Therefore, only three males in group 1 and three females in group 3 (instead of the planned 4 per sex per group) were evaluated during the recovery period. The available data was considered sufficient for toxicological evaluation.

There was no treatment-related mortality and no adverse dermal reactions at the injection sites were observed in-life. There were no toxicologically relevant changes in clinical signs, body weights, food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, clinical biochemistry, macroscopic observations, or organ weights.

Increased fibrinogen levels and shortened prothrombin times were observed in males and females treated with MF59 alone or FCC/MF59-H5N1 on days 17 and 31 compared to the PBS control group. Both parameters returned to control values following the recovery

period. The increased fibrinogen levels (and the related effects on prothrombin time) reflect a temporary immune/inflammatory response to adjuvant and vaccine; these findings are consistent with other nonclinical studies where MF59 was administered alone or with other antigens.

Slightly higher rectal body temperatures (mean increase +0.4°C) were measured only in males 2 hours after the third dose of FCC/MF59-H5N1 (day 29).

An increase in globulin levels (resulting in increased total protein levels and a reduced albumin/globulin ratio) was noted after the second and third dose with either MF59 alone or FCC/MF59-H5N1 and reflects an anticipated effect of an immunological response.

The immunogenicity of FCC/MF59-H5N1 was demonstrated using the hemagglutination inhibition (HI) assay using a heterologous (Vietnam; Clade 1) virus strain; analysis with the homologous (Indonesia; Clade 2) strain was not possible because permission to use the Indonesia strain could not be obtained. In FCC/MF59-H5N1-treated animals, low titers 2 weeks after the first dose were seen in a few animals, and two weeks after the second dose all animals had titers ≥160. Titers persisted throughout the treatment and recovery periods. There were no antibodies in either the saline control group or the MF59-alone group at any time point. The immunogenicity results are presented in section 2.6.2.2.1.

There were no adverse treatment-related macroscopic observations or effects on organ weights. There were no adverse treatment-related microscopic findings in the systemic organs that were examined. Histopathological findings at the injection sites consisted of the expected inflammatory changes associated with intramuscular injections. The findings in all groups included inflammation, infiltration and hemorrhage as shown in the tables below. The first and third injections were into the right hind limb; the second injection was into the left hind limb. The findings in the left injection site (single administration) are summarized in the table below.

Table 3: Study No. 466122 – Incidence/severity of histopathology findings at the left injection site (injection on day 15)

Group	**1 (PBS)**		**2 (MF59)**		**3 (Vaccine)**	
Finding	**M**	**F**	**M**	**F**	**M**	**F**
Injection site – Left (day 31 main necropsy) – 16 days post last injection						
Number examined / Number affected	**4 / 0**	**4 / 1**	**4 / 4**	**4 / 3**	**4 / 3**	**4 / 1**
Intermuscular connective tissue macrophages	0	0	0	0	2+	0
Dermal hemorrhage	0	0	1+	0	0	1++
Panniculus muscle fiber degeneration	0	1+	2++	3+	2++	0
Deep muscle focal degeneration	0	0	1++ 1+++	0	0	0
Deep muscle hemorrhage	0	0	1++	0	0	0
Injection site – Left (day 43 recovery necropsy) – 28 days post last injection						
Number examined / Number affected	**3 / 0**	**4 / 1**	**4 / 0**	**4 / 0**	**4 / 1**	**3 / 1**
Muscle fiber degeneration	0	1+	0	0	1++	1++

Note: findings are presented as number affected followed by severity.
Severity is scored as minimal (+), mild (++), or moderate (+++).

Comparison of the left injection sites (one injection on day 15) from animals necropsied on day 31 to the injection sites from the recovery animals necropsied on day 43 indicates reversibility. By day 43 both the incidence and severity of findings had markedly decreased.

The findings in the right injections site are summarized in the table below.

Table 4: Study No. 466122 – Incidence/severity of histopathology findings at the right injection site (injections on days 1 and 29)

Group	**1 (PBS)**		**2 (MF59)**		**3 (Vaccine)**	
Finding	**M**	**F**	**M**	**F**	**M**	**F**
Injection site – Right (day 31 main necropsy) – 2 days post last injection						
Number examined / Number affected	**4 / 0**	**4 / 3**	**4 / 4**	**4 / 2**	**4 / 1**	**4 / 3**
Intermuscular connective tissue macrophages	0	0	1++	1++	0	0
Dermal hemorrhage	0	1++	0	0	1++	0
Panniculus muscle fiber degeneration	0	1+	2+ 1+++	1++	0	0
Deep epidermal acute inflammation	0	0	1+	0	0	1+ 1++
Deep dermal diffuse macrophage infiltration	0	0	1++	2++	0	3+
Panniculus focal macrophage infiltration	0	0	1++	0	0	0
Intermuscular acute inflammation	0	0	2++	0	0	0
Deep muscle focal degeneration	0	1++	0	1+	0	0
Deep muscle hemorrhage	0	1+	0	0	0	0
Deep muscle macrophage infiltration	0	1+	0	0	0	0
Injection site – Right (day 43 recovery necropsy) – 14 days post last injection						
Number examined / Number affected	**3 / 0**	**4 / 2**	**4 / 3**	**4 / 2**	**4 / 2**	**3 / 2**
Intermuscular connective tissue macrophages	0	0	0	0	1+	0
Panniculus muscle fiber degeneration	0	1+	1+ 1++ 1+++	1+	1++	2++
Follicular adnexa deficit	0	0	1++	0	0	0
Dermal hemorrhage	0	1+	0	1+	0	0
Superficial pustular dermatitis	0	1+	0	0	0	0

Findings are presented as number affected followed by severity.
Severity is scored as minimal (+), mild (++), or moderate (+++).

Partial to full reversibility of the findings in the right injection site was also indicated when the main and recovery necropsy animals were compared.

Based on the results of this study, three 0.5 mL intramuscular administrations of FCC/MF59-H5N1 (containing15 µg antigen and 0.25 mL MF59 adjuvant) in NZW rabbits was immunogenic, locally well tolerated, and was not associated with systemic toxicity.

2.6.6.3.1 Supportive Repeat Dose Toxicity Studies

Optaflu

Two dose intramuscular toxicity study of influenza vaccine formulations in New Zealand White rabbits (Study No. 191-44)

The objective of this GLP study was to assess the local and systemic toxicity of Optaflu (called Influenza Cell Culture Subunit Vaccine in the study report) in New Zealand White rabbits after two administrations and to determine the reversibility of findings (section 2.6.7.7, Study No. 191-44). Agrippal, a marketed egg-derived Influenza subunit vaccine, served as the reference article. Phosphate buffered saline (PBS) served as the control article (placebo). The study consisted of three groups of 6 animals/sex/group. Rabbits received an intramuscular injection of 0.5 mL of either the test or reference article or placebo on days 1 and 8 as shown below.

Table 5: Study No. 191-44 – Study Design

Group	No. of Animals	Intramuscular treatment [a]	Dosing day [b]	Necropsy	
				Main [c]	Recovery [d]
1	6/sex	Placebo – 0.5 mL Control (0 µg)	1 & 8	3/sex	3/sex
2	6/sex	Test Article – 0.5 mL Optaflu® (45 µg)	1 & 8	3/sex	3/sex
3	6/sex	Reference Article – 0.5 mL Agrippal® (45 µg)	1 & 8	3/sex	3/sex

[a] Vaccines contained 3 antigens at 15 µg/antigen for a total of 45 µg/dose. The antigens used were A/New Caledonia/20/99 (H1N1), B/Guangdong/120/00 (B), and A/Panama/2007/99 (H3N2).
[b] Rabbits received a 0.5mL IM injection in the left hind limb on day 1and in the right hind limb on day 8.
[c] Main necropsy was performed 2 (males) and 3 (females) days post-last dose.
[d] Recovery necropsy was performed 14 (males) and 15 (females) days post-last dose.

Potential toxicity was evaluated based on clinical signs, dermal scoring of injection sites, body temperature, body weight, food consumption, ophthalmic examination, clinical pathology (hematology, coagulation, and serum chemistry parameters), terminal organ weights, comprehensive macroscopic examination, and microscopic evaluation of selected tissues.

The animals were observed twice daily for mortality and once daily for signs of toxicity. Injection sites were assessed for signs of irritation and scored based on a modified Draize score prior to dosing, one and two days after each injection, and at each necropsy. Rectal body temperatures were taken prior to dosing, one and two days after each injection, and at each necropsy. Body weights were recorded pre-treatment, on days 8 and 15, and at necropsy. Food consumption was measured once weekly. The ophthalmology evaluation was performed pre-treatment and prior to each necropsy. Blood samples for hematology,

serum chemistry, and coagulation (including fibrinogen) analysis were collected pre-treatment, two days after each dose, and on day 22. Additional blood samples were taken prior to each administration, on day 15, and at each necropsy for antibody analysis. At each necropsy, a complete macroscopic examination and collection of tissues was performed. Organ weights were determined as shown below.

Table 6: Study No. 191-44 – Organs Weighed

Adrenals	Heart	Liver	Ovaries	Testes
Brain	Kidneys	Lungs	Spleen	Thymus

Microscopic evaluation of selected tissues was performed as shown below.

Table 7: Study No. 191-44 – Histopathology

Tissues evaluated histopathologically	
Brain (including the medulla oblongata, pons, cerebrum, and cerebellum)	Liver
	Lung (including the bronchus)
Bone marrow	Lymph nodes (including the iliac, mesenteric, and cervical nodes)
Eyes (including the optic nerve and uvea)	
Femur (including the knee joint capsule)	Spleen
Heart	Thymus
Injection sites	Urinary bladder
Kidneys	Macroscopic lesions

There were no deaths, and no treatment-related adverse effects on clinical signs, body temperature, body weights, food consumption, or ophthalmology. There was no erythema or edema observed at the injection sites. There were no changes in hematology, coagulation, or clinical chemistry parameters that were considered related to treatment. Although there were some apparent changes in hematology and clinical chemistry parameters, some of which attained statistical significance, values were always within the range observed prior to commencement of treatment and/or the range historically observed at the test facility for New Zealand White rabbits and, thus, were not considered treatment-related.

Administration of Optaflu did not produce any macroscopic findings. There were no treatment-related effects on organ weights, with the possible exception of thymus in males. Thymus weights were highly variable and a subtle vaccine effect on thymus weights cannot be excluded. The histopathological findings were limited to the expected reactions at the injection sites. These consisted of minimal to slight necrosis in the left injection site and minimal to slight hemorrhage in the right injection site. The incidence

and severity of injection site findings are summarized below. Findings at the injection site were attributed to the trauma caused by the intramuscular injection, occurred in all groups (including control), and were partially to fully resolved by the end of the recovery period.

Table 8: Study No. 191-44 – Histopathology of injection sites

Group and Treatment	**1 - Control**		**2 - Optaflu**		**3 - Agrippal**	
Necropsy	Main	Recovery	Main	Recovery	Main	Recovery
Males						
Left Injection Site [a]						
Necrosis	0	0	0	0	1 (slight)	1 (slight)
Right Injection Site [b]						
Hemorrhage	0	1 (slight)	1 (slight)	0	0	0
Females						
Left Injection Site [a]						
Necrosis	1 (slight)	0	1 (slight)	1 (minimal)	2 (min/slight)	0
Right Injection Site [b]						
Hemorrhage	0	0	0	0	0	0

[a] The left site received the first injection and was evaluated on days 9 (males) or 10 (females) post-injection (main necropsy) and on days 21 (males) or 22 (females) post-injection (recovery necropsy).
[b] The right site received the second injection and was evaluated on days 2 (males) or 3 (females) post-injection (main necropsy) and on days 14 (males) or 15 (females) post-injection (recovery necropsy).

At both the main and recovery necropsy, there was an unusually high incidence of pulmonary and renal findings in all groups (including the controls). Lung findings consisted primarily of slight to moderate interstitial inflammatory foci and were noted at the main and recovery necropsy in more than half of animals (males and females) from all groups, including control. Kidney findings consisted primarily of tubular degeneration and/or dilation, were slight to moderate in severity, and occurred only in females (from all groups, including control) at both the main and recovery necropsy. Pulmonary and renal findings were not considered related to treatment because they occurred in animals from all groups, including control animals. Although the cause of the kidney and lung findings was not determined, these findings were considered spontaneous, and did not compromise the interpretation and/or validity of the study.

Serum samples for antibody analysis were taken prior to each administration on days 1 and 8 (6/sex/group), on day 15 (3/sex/group), and at the main [3/sex/group, day 10(males)/11(females)] and recovery necropsy [3/sex/group, day 22(males)/23(females)]. Results from this analysis demonstrated that the test article was effective in inducing the production of antibodies against the three vaccine influenza virus strains [A/New Caledonia/20/99 (H1N1), B/Guangdong/120/00 (B), and A/Panama/2007/99 (H3N2)]. The immunogenicity data for this study are presented in section 2.6.2.2.2. Briefly, results

for strains A/New Caledonia and B/Guangdong revealed no titer pre-treatment and an increase in titer after the second vaccine dose in the test and reference article-treated animals. There was no titer against these strains in control animals. In contrast, there were background titers against strain A/Panama in control animals and pre-treatment in the test and referenced article-treated animals, which were attributed to nonspecific binding. However, an increase in A/Panama antibody titer was evident after the second vaccine dose.

Under the conditions of the study, two 0.5 mL intramuscular injections of Optaflu, given one week apart, were immunogenic and very well tolerated in male and female New Zealand White rabbits. There were no treatment related adverse effects on clinical observations, dermal scoring, body weights and temperatures, food consumption, clinical pathology (hematology, coagulation, and clinical chemistry), organ weights, or macroscopic evaluations. Histopathological evaluation revealed the expected reactions (necrosis and hemorrhage) at the injection sites, which were seen in all experimental groups, attributed to the intramuscular injection, and partially to fully resolved by the end of the recovery period. The two doses administered to rabbits in this study exceed the intended number (one) proposed for annual interpandemic immunization.

Fluad

4-Week Toxicity Study with Fluad®, Fluad High B, and Fluad High H3+IC31® Influenza Vaccine Formulations by 3 Intramuscular Injections in New Zealand White Rabbits Followed by a 2-week Recovery Period (Study No. 488182)

This GLP toxicology study was performed in rabbits, an accepted animal model for toxicity testing of vaccines. Animals received three injections of saline, or one of three vaccine formulations, two weeks apart.

Table 9: Study No 488182 – Experimental design

Group	No. of Animals	Test Material	Dose Volume	Treatment Days	Necropsy Day	
					Day 17	Day 31
1	8M + 8F	Saline	0.5 mL	1, 15, 29	4M + 4F	4M + 3F
2	8M + 8F	Fluad	0.5 mL	1, 15, 29	4M + 4F	4M + 4F
3	8M + 8F	Fluad High B	0.5 mL	1, 15, 29	4M + 4F	4M + 4F
4	8M + 8F	Fluad High H3+IC31	1.0 mL	1, 15, 29	4M + 4F	4M + 4F

Table 10: Study No. 488182 – Antigen & adjuvant per vaccine dose

Vaccine (dose volume)	B	H1N1	H3N2	MF59	IC31
	µg HA			mL	
Saline (0.5 mL)	–	–	–	–	–
Fluad (0.5 mL)	15	15	15	0.25	–
Fluad High B (0.5 mL)	30	15	15	0.25	–
Fluad High H3N2+IC31 (1.0 mL)	15	15	30	0.25	0.5

Standard toxicology parameters were evaluated including mortality, clinical signs, dermal injection site evaluations after injection and until resolution if necessary, body weights, food and water consumption, ophthalmic examinations, physical examinations (heart rate, respiratory rate and body temperature), and clinical pathology parameters (hematology, and clinical biochemistry). Necropsies were performed on days 31 (main group) and 43 (recovery group) and included terminal organ weights and full macroscopic post-mortem examinations. Microscopic evaluation of selected tissues/organs was performed. In addition, immunogenicity was evaluated on samples collected pretest, prior to dosing on days 15 and 29 and on necropsy day 43. The Hemagglutination Inhibition assay (HI) was used to detect the presence of serum antibodies to influenza virus.

There was no mortality. There were no treatment-related effects on body weights, body weight gain, food consumption, or clinical signs in any group. There were no in-life dermal irritation or ophthalmoscopic findings. There were no treatment-related effects on body temperatures, heart rates, and respiratory rates based on evaluable data. However, due to a technical error (measurements were taken after animals had been placed under a heat lamp to facilitate drawing blood) some of the data were deemed unreliable.

Treatment-related effects on hematology parameters in groups 2, 3 and 4 included increased fibrinogen levels, and slight decreases in prothrombin (PT) times. In females receiving Fluad or Fluad High B, activated partial thromboplastin times (APTT) were slightly decreased. In treated animals, clinical chemistry evaluations showed an increase in total globulin and a corresponding decrease in albumin:globulin ratio; this is expected following administration of an immunologically active substance. All values returned to pretest ranges by the end of recovery. The findings observed in this study are consistent with those seen in other rabbit toxicology studies with MF59-adjuvanted vaccines.

At the main necropsy (day 31), macroscopic observations in treated groups consisted of enlarged iliac lymph nodes in all males and in 3 group 2, 3 group 3, and 1 group 4 female(s). In addition, 2 group 2 females had enlarged popliteal lymph nodes. At the recovery necropsy (day 43), one group 3 male and one group 4 female had enlarged iliac lymph nodes. Effects on draining lymph nodes are consistent with vaccination and all

other findings were considered to be incidental and within the normal range of variation for this species.

There was a slight increase in both absolute and relative adrenal weights in group 3 females at the day 31 necropsy; there were no histopathological changes and no differences from controls on day 43. Liver weights were increased in one male and two females in group 4 on day 31, but statistical significance was not reached and there was no histopathological correlate. There were no differences from controls on day 43.

Histopathology of injection sites showed that administration of the vaccine formulations was associated with a low degree of inflammation at the injection sites and reactive changes in local lymph nodes. There appeared to be no significant differences in the incidence or severity of these changes between the three vaccine formulations. Increasing total antigen levels to 60 µg in Fluad High H3N2+IC31 and Fluad High B groups from the 45 µg in Fluad did not increase local toxicity or reactogenicity in rabbits. No histopathological changes associated with treatment, or indicative of systemic toxicity, were observed in any other tissues.

Under the conditions of the study, all vaccines were immunogenic and well tolerated, and no evidence of toxicity was observed.

30-Day Subacute Toxicity Study in Rabbits by Intramuscular Route (Study No. 940292)

This repeat-dose study was conducted in accordance with the OECD Principles of Good Laboratory Practice (GLP). The systemic toxicity and local tolerability of one to two intramuscular doses of vaccine formulations were studied in male and female New Zealand White rabbits. Treatment groups were Agrippal alone, MF59W.1 adjuvant alone (equivalent to MF59C.1 but without citrate buffer), or Agrippal+MF59W.1 (equivalent to Fluad but without citrate buffer). Two intramuscular injections were administered 14 days apart into alternate hind limbs. The study design is shown below.

Table 11: Experiment No. 940292 - Study Design

4	Number of Animals	Compound Administered	Dose Volume	Treatment Days	Necropsy Day	
					Day 17	Day 31
1	6M + 6F	MF59W.1	0.5 mL	1, 15	3M + 3F	3M + 3F
2	6M + 6F	Agrippal (45 µg)	0.5 mL	1, 15	3M + 3F	3M + 3F
3	6M + 6F	Fluad equivalent (Agrippal (45 µg) + MF59W.1)	0.5 mL	1, 15	3M + 3F	3M + 3F

Parameters evaluated during the study included physical appearance, behavior, clinical signs, Draize scoring of injection site reactions, rectal temperature, body weight gain, ophthalmology, hematology, clinical chemistry, macroscopic pathology and histopathology of selected organs and tissues, including the injection sites. Statistical analyses were performed on body weight gain, body temperature, hematology, clinical chemistry, and organ weights.

No animals died in this study. There were no clinical signs of systemic toxicity after a single dose or detectable reactions at the injection sites in any animal. Following two doses, there were no treatment-related effects on any study parameters indicative of local or systemic toxicity in any treatment group. There were no relevant differences between groups in organ weights at either of the two scheduled necropsies, and individual values were all within the normal ranges. There were no macroscopic observations except for the injection sites. Macroscopic findings at the injection sites treated two days previously indicated an increased frequency of slight focal hemorrhage in the Agrippal+MF59W.1 group compared to the other two groups. There were no macroscopic findings at injection sites treated 16 or 30 days before necropsy.

Histological examination of the injection site 2 days post-injection revealed interstitial inflammation (mainly acute), interstitial hemorrhage, and / or muscle fiber degeneration in almost all animals. These observations were more notable in the Agrippal+MF59W.1 group, followed by the Agrippal group, then the MF59W.1 group. Sixteen days after injection, inflammatory and degenerative changes were still present, but to a lesser extent in most animals. Thirty days after injection, partial to full recovery was evident in most animals. At injection sites 16 and 30 days post-injection, there were no differences between the groups. There were no other tissues with treatment-related findings and any changes seen were comparable in frequency and severity to those commonly found in untreated New Zealand rabbits of similar age in the Test Facility.

In conclusion, no local or systemic adverse effects were seen in rabbits administered two intramuscular injections of MF59W.1 adjuvant, Agrippal, or the Fluad equivalent (Agrippal+MF59W.1).

2.6.6.4 Genotoxicity

In accordance with current international guidelines for vaccines, no studies have been conducted with FCC H1N1sw, FCC/MF59-H5N1, or Optaflu.

MF59 has been evaluated *in vitro* (Ames test) and *in vivo* (mouse micronucleus assay) and was negative in both studies. These studies are discussed in section 2.6.6.8.2.1 and tabular summaries are provided in section 2.6.7.17.2.

2.6.6.5 Carcinogenicity

Not applicable.

2.6.6.6 Reproductive and Developmental Toxicity

No studies have been conducted in animals to investigate the effects of FCC H1N1sw or FCC/MF59-H5N1 on fertility, embryofetal toxicity, or developmental toxicity.

GLP studies in rabbits to evaluate the reproductive and developmental toxicity of Optaflu and the comparable egg-derived MF59-adjuvanted H5N1 vaccine Aflunov have been performed. These studies are described below, and tabular summaries are provided in section 2.6.7.13 and 2.6.7.14. These studies demonstrate that seasonal cell culture-derived antigens at a much higher dose (45 µg per dose, 5 doses administered) and pandemic MF59-adjuvanted egg-derived antigens (15 µg per dose, 5 doses administered) did not affect reproductive or development.

MF59 has been evaluated in rats and rabbits and study summaries are provided in sections 2.6.6.8.2.1 and 2.6.7.17.2. MF59 was not teratogenic, fetotoxic, or a developmental toxicant.

2.6.6.6.1 Supportive Reproductive and Development Toxicity

Intramuscular reproductive and developmental toxicity study of FCC vaccine in rabbits, including a postnatal evaluation (Study No. UBA00037)

This GLP study (section 2.6.7.13 and 2.6.7.14, Study No. UBA00037) was designed to assess the effect of Optaflu (called FCC vaccine in the study report) and its elicited antibodies on reproduction including prenatal and postnatal development in New Zealand White female rabbits. The study encompassed a timeframe which began prior to cohabitation through mating, gestation and lactation.

The study design for this definitive reproductive and developmental toxicity study was developed in consultation with FDA and is designed to comply with FDA Guidance (Considerations for Developmental Toxicity Studies for Preventative Therapeutic Vaccines for Infectious Disease Indications) and ICH Guidelines S5(R2).

The study evaluated ICH Harmonised Tripartite Guideline stages A through E of the reproductive process (with the exception of determination of effects on estrus cycle) and would have detected any effects on tubal transport, implantation, gestation, parturition, lactation and maternal behavior in female rabbits, and on the fetal and postnatal development of the offspring of the treated female rabbits.

Female rabbits were assigned to one of two cohorts (Caesarean-sectioning or natural delivery). In each cohort, there were two groups of twenty-four animals each, which received intramuscular injections of either control article (0.9% sodium chloride [saline]; groups I and III) or Optaflu (groups II and IV). The dose of Optaflu administered was 1× the clinical dose of 45 µg in the clinical dose volume of 0.5 mL (a total of 45 µg antigen

contains 15 µg from each of three different strains). The same volume of saline was administered to control animals. Optaflu or saline was administered as shown.

Table 12: Study No. UBA00037 – Study Design

Dose group	Number of rabbits	Dose (µg) / Injection volume (mL)[a]	
		Study days 1, 15, 29	Gestation days 7 and 20
Caesarean-sectioning			
I	24	0/0.5	0/0.5
II	24	45/0.5	45/0.5
Natural delivery			
III	24	0/0.5	0/0.5
IV	24	45/0.5	45/0.5

[a] On each day of dosing, rabbits were given an intramuscular injection of saline or Optaflu containing purified influenza virus surface antigens (15 µg antigen per strain) from three seasonal influenza strains (A/New Caledonia/20/99 IVR-116 (H1N1), A/New York/55/2004x-157 and B/Jiangsu/10/2003).

Mating occurred on day 36 of study (day 1 of gestation). Three injections were given prior to mating/gestation to ensure the presence of anti-hemagglutinin antibodies during gestation. Two additional injections were administered during gestation in order to expose fetuses to Optaflu and maintain antibody titers. F1 generation offspring from group III and IV rabbits were not given Optaflu but were exposed in utero or through maternal milk (during the lactation period). All animals in groups I and II were Caesarean-sectioned on day 29 of gestation; those in groups III and IV were allowed to deliver their offspring naturally and were then followed through day 29 of lactation (where the day of birth was considered day 1 of lactation).

The following parameters were evaluated in all rabbits: viability, clinical observations and skin reactions, body weight and body weight changes, feed consumption, mating and fertility, and gross lesions. Rabbits assigned to Caesarean-sectioning were also evaluated for pregnancy status, gravid uterine weights, the number and distribution of corpora lutea, implantation sites, and uterine content (live and dead fetuses and early and late resorptions). Caesarean-delivered fetuses were weighed and examined for fetal gross external, soft tissue and skeletal alterations and fetal sex. Placentae were examined for size, color and shape. Rabbits assigned to natural delivery were also evaluated for clinical signs associated with parturition, duration of gestation, number and distribution of implantation sites, litter sizes, offspring viability at birth and maternal behavior.

On day 1 of lactation, the day of birth, all offspring in a litter were individually weighed. Each litter was evaluated for viability at least twice daily, and the offspring in each litter were counted once daily. Clinical observations and offspring body weights were also recorded, and assessments of reflex (i.e. air righting, acoustic startle and pupil

constriction) and physical development (i.e. hair growth and eye opening) were made. F1 generation offspring were necropsied and evaluated for hydrocephaly and gross lesions.

Blood samples for determination of hemagglutinin-inhibiting antibody titers were collected from female rabbits prior to study initiation, prior to dose administration on day 15 and 29 of study and day 7 and 20 of gestation, and at the time of euthanasia on day of gestation 29 (groups I and II) or day 29 of lactation (groups III and IV). Blood samples were collected from each fetus on day 29 of gestation and pooled by litter and from each offspring on day 29 of lactation. Blood samples were processed to serum and a representative subset of available samples was analyzed for the presence of hemagglutinin-inhibiting antibodies to the A/New Caledonia/20/99 strain.

Optaflu did not increase mortality. Cumulative perinatal maternal mortality (including animals euthanized for adverse clinical observations, abortion and total litter loss) was 14.3% (6/42) in control animals and 15.6% (7/45) in Optaflu-treated animals. Any doe that began to deliver prior to scheduled Caesarean sectioning on day 29 was considered to be aborting and was euthanized in accordance with Test Facility SOPs. Total litter loss occurred in one animal in group II and one animal in group IV.

In general, the animals which died, aborted or had total litter loss appeared normal throughout the premating period but then had reduced feed consumption and weight loss beginning on day of gestation 13 to 15. Because reduced feed consumption is an expected observation during the later phase of gestation in rabbits, and there did not appear to be any relationship with the timing of treatment, these events are considered to be likely related to effects of reduced feed consumption and are unrelated to treatment with Optaflu. Necropsy observations did not indicate any relevant findings attributed to Optaflu treatment.

All clinical signs during the premating, gestation and lactation periods were considered unrelated to intramuscular injection of Optaflu. Body weight, body weight changes, gravid uterine weights (in does assigned to Caesarean sectioning) and absolute and relative feed consumption values were unaffected by treatment with Optaflu.

When compared to the respective control groups, mating and female fertility indices (including number of female rabbits that mated, number of female rabbits which mated with the first, second or third male, female fertility index, number of rabbits pregnant) were unaffected by Optaflu.

Pregnancy occurred in 23, 24, 19 and 21 does in groups I, II, III and IV, respectively, of the 24 animals per group. In total, the number of animals available for evaluation on day 29 of gestation (groups I and II) and day 2 of lactation (groups III and IV) were 21 and 20 and 14 and 15, respectively. The low number of rabbits available at the end of the lactation period for evaluation is the result of the lower pregnancy rate in group III and IV animals combined with the loss of some animals/litters in the perinatal period. Nonetheless, 14 and 15 litters is sufficient to detect potential significant effects of Optaflu

on postnatal development and interpret potential effects of the vaccine on natural delivery and litter parameters.

Caesarean-sectioning parameters and litter parameters (including corpora lutea, implantations, litter sizes, live fetuses, early and later resorptions, fetal body weights, percent resorbed conceptuses and percent live male fetuses) were not affected by Optaflu treatment. There were no gross external, soft tissue or skeletal fetal alterations caused by injection of does with Optaflu. The average numbers of ossification sites per fetus per litter were comparable between groups and consistent with the historical database at the Test Facility. There were no gross lesions in any animal which were attributed to treatment with Optaflu.

Optaflu was immunogenic in rabbits (section 2.6.2.2.2). Anti-HA antibodies were measured on day 29 of the study following two injections of Optaflu. Antibody levels remained elevated through day 29 of gestation in does and were comparable in fetuses of these animals. Antibodies were still detectable on day 29 of lactation in both does and their offspring, although the levels were reduced compared to those measured in animals at the time of Caesarean-sectioning.

There were no signs of overt systemic toxicity, no adverse effects on mating or fertility, no effects on Caesarean-sectioning or litter parameters in fetuses and offspring, respectively, or effects on natural delivery, when female rabbits were given multiple injections of Optaflu at approximately 20× and 4× the clinical exposure anticipated for an adult (60 kg) and child (12 kg), respectively. Optaflu was immunogenic in female rabbits and anti-HA antibodies were passed to fetuses through placental transfer. Optaflu is not a developmental toxicant, as there were no adverse findings noted in any parameter evaluated in fetuses on day 29 of gestation or in offspring on day 29 of lactation.

Intramuscular reproductive and developmental toxicity of Fluad H5N1 in rabbits, including a postnatal evaluation (Study No. UBA00021)

The objective of this GLP study was to assess the potential effects of Aflunov (called Fluad H5N1 in the study report) on reproductive and developmental toxicity in female rabbits and their fetuses or offspring when administered by intramuscular injection at 2× the clinical dose of 7.5 µg, on each of five occasions, before mating and during gestation (section 2.6.7.13 and 2.6.7.14, Study No. UBA00021). The study design is summarized below.

Table 13: Study No. UBA00021 – Study Design

Group	Number of rabbits	Dose (μg) / Injection volume (mL)[a]		Evaluations
		Study days 1, 15 and 29	Gestation days 7 and 20	
Caesarean-sectioning (day 29 of gestation)				Maternal and embryofetal toxicity and teratogenic potential
I	20	0/0.5	0/0.5	
II	20	15/0.5	15/0.5	
Natural delivery (day 29 of lactation)				
III	20	0/0.5	0/0.5	
IV	20	15/0.5	15/0.5	

[a] Group I and III animals were administered saline as a control article. Group II and IV animals were administered Aflunov (total volume 0.5 mL containing 15 μg antigen and 0.25 mL MF59 adjuvant). After 35 days on study, rabbits were mated (gestation day 0).

Twenty female New Zealand White rabbits were assigned to each of four treatment groups. Animals received the same dosage and volume of control (groups I and III) or test (groups II and IV) article on each dosing occasion. Group I and II animals were Caesarean-sectioned on day 29 of gestation; group III and IV animals delivered offspring and were followed through day 29 of lactation. Treatment of does with Aflunov during the 5 weeks before mating ensured exposure to influenza antibodies at critical reproductive and developmental timepoints, from implantation through closure of the hard palate. Treatment of the does during gestation ensured exposure of the fetuses to the vaccine itself (in addition to any circulating anti-influenza antibodies) through maternal placental transfer.

The following parameters were evaluated: viability, clinical observations and skin reactions, body weight and body weight changes, feed consumption, mating, fertility and gross lesions of the thoracic, abdominal and pelvic viscera. Additionally, rabbits assigned to Caesarean-sectioning were also evaluated for pregnancy status, gravid uterine weights, the number and distribution of corpora lutea, implantation sites, and uterine content (live and dead fetuses and early and late resorptions). Caesarean-delivered fetuses were weighted and examined for fetal gross external, soft tissue, and skeletal alternations and fetal sex. Placentae were examined for size, color and shape. Rabbits assigned to natural delivery were also evaluated for clinical signs associated with parturition, duration of gestation, number and distribution of implantation sites, litter sizes, offspring viability at birth and maternal behavior. Offspring from does which were assigned to the natural delivery groups were evaluated for viability, clinical observations, body weight, reflexes (air righting, acoustic startle and pupil constriction) and physical development (hair growth and eye opening). At necropsy, offspring were evaluated for hydrocephaly and gross lesions.

No deaths were caused by intramuscular injection of female rabbits with Aflunov vaccine; all rabbits survived to their scheduled euthanasia with the exception of a single group IV doe (vaccine-treated, natural delivery) that was euthanized according to protocol directive on day 1 of lactation (the day of birth) after delivering only stillborn offspring.

There were no clinical observations or skin reactions, effects on body weight or gravid uterine weights (Caesarean-sectioned rabbits only), or effects on absolute and relative feed consumption during the pre-mating, gestation or lactation periods which were considered to be related to treatment with the vaccine.

Mating and fertility indices were unaffected by treatment with Aflunov. There was no effect of maternal treatment with the vaccine on Caesarean-sectioning parameters (group II) or litter parameters (group IV) in fetuses and offspring, respectively, compared to control.

Subset analysis of serum samples taken from does throughout the study confirmed the presence of antibodies using the hemagglutinin inhibition (HI) assay. Antibody titers were measurable beginning on day 15 of study (after one injection of Aflunov) in all animals assayed. Titers increased and/or remained elevated over the duration of the study (either day 29 of gestation for group II, or day 29 of lactation for group IV), demonstrating continued immune response to the vaccine. At the time of Caesarean-sectioning, anti-influenza antibodies were detected in all pooled fetal samples of group II does at levels comparable to those of the respective maternal sample. At the time of euthanasia, 29 days after birth, antibodies remained elevated in all group IV offspring tested, although titers were lower than those of group II fetuses.

The planned clinical use of this vaccine involves two intramuscular injections approximately three weeks apart. The vaccine contains 7.5 µg of HA in a volume of 0.5 mL, which includes 0.25 mL of MF59 adjuvant to enhance the immune response. This study tested relatively high multiples of human doses for a vaccine product. Rabbits were injected on five occasions with 2× the clinical dose, which represents five exposures to levels approximately 30-fold the expected clinical exposure to 7.5 µg on a body weight basis (assuming a human body weight of 60 kg and a rabbit body weight of 4 kg). Under the conditions of this study, Aflunov was well tolerated, did not cause maternal or embryofetal toxicity, was not teratogenic, and had no effects on post-natal development. Additionally, the vaccine was immunogenic in maternal rabbits, developing fetuses had comparable titers, and antibodies persisted through the first 4 weeks of life in F1 offspring.

2.6.6.7 Local Tolerance

The local tolerability of FCC/MF59-H5N1 was evaluated as part of the GLP repeat dose toxicology study. For details see sections 2.6.6.3 and 2.6.7.7.

2.6.6.8 Other Toxicity Studies

There are no other studies with FCC H1N1sw or FCC/MF59-H5N1.

Tumorigenicity and oncogenicity studies were performed with the MDCK cell line used to produce virus for cell-culture derived influenza vaccines. These studies are discussed briefly below. A sensitization study was performed with Fluad in Guinea pigs, for details see below.

Pivotal toxicology studies performed with MF59 include the evaluation of repeat-dose toxicity (including local tolerability), genotoxicity, sensitization, and embryofetal and developmental toxicity. Studies with MF59 are discussed in section 2.6.6.8.2.1, and tabular summaries are provided in section 2.6.7.17.2.

Studies have also been conducted with vaccine formulations composed of antigens combined with MF59 with or without an MF59-alone group. In these studies, the antigen+MF59 and MF59-alone formulations were well tolerated. Based on the parameters evaluated, no treatment-related safety issues were identified. Findings in these studies were attributed to the adjuvant, with no additional adverse effects being noted with the combined products. These studies are described in section 2.6.6.8.2.2.

2.6.6.8.1 Other Supportive Studies

Tumorigenicity/oncogenicity studies

The antigens contained in FCC/MF59-H5N1 are produced using a Madin Darby Canine Kidney (MDCK) cell line. A series of *in vivo* studies was performed to characterize the tumorigenicity of the MDCK cell line, and the oncogenicity of process intermediates (cell lysates and purified MDCK cell DNA).

These studies were designed to characterize the tumorigenic or oncogenic potential of materials from the manufacturing process at progressive stages: intact cells, lysates prepared from these cells, and purified DNA from influenza virus infected and uninfected MDCK cells. Very sensitive species were selected to optimize the detection of possibly very rare events leading to tumorigenicity or oncogenicity: adult nude mice (a well-known immunocompromised model for investigating tumorigenicity of various cell lines) and neonatal animals (<4 days old) of three rodent species (nude mice, rats, and hamsters) with immature immune systems at the time of dosing. Each study had a 150-day duration and was designed to allow time for any potential adverse effects to emerge.

Only intact MDCK cells were tumorigenic in immunocompromised adult (nude) mice. Cell lysates or purified MDCK cell DNA were not oncogenic in infant mice, rats and hamsters. Since intact MDCK cells are reliably excluded from the vaccine during multiple steps of the manufacturing process, and because studies with MDCK cell lysates and DNA demonstrated no oncogenicity in three species of very sensitive animal models,

Novartis concludes that the theoretical safety risk is exceedingly low and the vaccine is safe for use in humans.

Biocine FLU/MF59C.1 Magnusson-Kligman Maximization Test in Guinea Pigs (Project No. 564110, Report No. 14430)

This GLP study was conducted in Guinea pigs to investigate the delayed contact hypersensitivity potential of Fluad®. Fluad is referred to as Biocine® Flu/MF59C.1 in the study report.

Female Dunkin-Hartley Guinea pigs (10/group) were used in this study (section 2.6.7.17.3, Study No. 14430 / 564110). Based on range finding, Fluad was used without dilution. For intradermal induction in test animals, duplicate 0.1 mL injections of the following solutions were administered to each of 20 test animals into scapular skin: 50% aqueous Freund's Complete Adjuvant (FCA), a 1:1 (v/v) mixture of Fluad and 0.9% saline, and a 1:1 (v/v) mixture of Fluad and 50% aqueous FCA. A group of 10 control animals each received duplicate 0.1 mL intradermal injections of the following solutions: 50% aqueous FCA, 0.9% saline, and 1:1 (v/v) mixture of 0.9% saline and 50% aqueous FCA.

Six days later, the scapular region was clipped free of hair and the injection sites observed for signs of irritation. On the following day (day 7) a second induction was performed via the topical route in the same animals. The test animals were treated with 0.5 mL of Fluad, and controls were treated with 0.5 mL of 0.9% saline. The site was covered with an occlusive dressing for 48 hours, and then examined for signs of irritation.

Thirteen days after the topical application, the scapular region was again clipped free of hair, and the following day (day 21), both test and control animals were treated with 0.5 mL of 0.9% saline, and 0.5 mL of Fluad applied topically to the upper left and upper right flanks respectively (challenge). The test site was covered with an occlusive dressing for 24 hours, after which it was again clipped, and observed for signs of irritation. All animals were examined for clinical signs twice daily, and body weights were recorded at the start and end of the study.

During intradermal induction, slight erythema was observed in 5/20 test animals at 1 hour after injection, and in 3 different test animals 24 hours after injection. There was no reaction in any of the control animals at any time.

During the topical induction phase, which followed a week later, mainly slight to moderate erythema was observed in 17/20 test animals at 1 hour post treatment, and in 7/20 animals at 24 hours post-treatment. In control animals, slight erythema was observed in 5/10 animals at the 1-hour observation time only.

On challenge, there was no reaction in either the test or control groups following topical treatment with Fluad. There was slight erythema in 2/20 of the test animals challenged topically with 0.9% saline.

Apart from injection site reactions, no other clinical signs were noted, and body weight gain was normal. Fluad was not a skin sensitizer in Guinea pigs in this study.

2.6.6.8.2 MF59 Studies

MF59 has been extensively tested in nonclinical toxicology studies. MF59 is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen and minor inflammatory and degenerative changes at the injection site are consistent with the effects of intramuscular injections of an immunological adjuvant. These findings are readily reversible within days to 1 to 2 weeks. In repeat-dose toxicology studies in dogs, there were no effects on cardiovascular or central nervous system (safety pharmacology) parameters. MF59 is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat). MF59 has been demonstrated to be an effective adjuvant with many antigens in many species, including man. The safety/tolerability profile established in nonclinical studies has been confirmed in clinical trials.

Studies considered pivotal in the nonclinical safety evaluation of MF59 alone include repeat dose toxicity (rabbits), genotoxicity (in vitro and in vivo), sensitization (Guinea pigs) and reproductive and developmental toxicity (rats and rabbits) studies. These studies are described in section 2.6.6.8.2.1 and additional data are provided in the tabular summaries in section 2.6.7.17.2.

Nonpivotal studies are described in sections 2.6.6.8.2.2 and tabular summaries are provided 2.6.7.17.3.

2.6.6.8.2.1 Pivotal MF59 Studies

14-Day Intramuscular Study in Rabbits (Study No. 90-6081)

In this GLP study, New Zealand White rabbits received daily 0.5 mL intramuscular injections of saline or MF59 for 14 days (2.6.7.17.2, Study No. 90-6081). Half the animals were necropsied on day 15, the remainder on day 22, following a 7-day recovery period. The study design is shown below:

Table 14: Study No. 90-6081 – Study Design

Group*	Number of animals	Compound administered	Dose volume	Treatment days	Necropsy day	
					Day 15	Day 22
1	8M + 8F	0.45% saline	0.5 mL	1 - 14	4M + 4F	4M + 4F
5	8M + 8F	MF59 (water)	0.5 mL	1 - 14	4M + 4F	4M + 4F

* Other groups received a second adjuvant + MF59, for clarity only relevant treatment groups are shown

Study parameters included clinical observations, body weight, food consumption, ophthalmology, clinical chemistry, hematology, urinalysis, and body temperatures. Comprehensive macroscopic examinations were performed and selected organs were weighed. A complete tissue list was collected and selected organs were weighed as shown below:

Table 15: Study No. 90-6081 – Evaluated Organs

Organs weighed			
Adrenal glands*	Liver	Prostate	Thymus
Brain	Lung*	Seminal vesicles*	Thyroid with parathyroid glands
Epididymides*	Ovaries*	Spleen	
Heart	Pituitary gland	Submandibular glands	Uterus
Kidneys*	Popliteal lymph nodes*	Testes*	Vesicular gland

* Paired organs weighed together

Tissues were evaluated microscopically as shown below.

Table 16: Study No. 90-6081 – Tissues Evaluated

Tissues examined histopathologically	
Heart with aorta	Thymus
Kidneys	Spleen
Lung	Eyes
Liver with gallbladder	Pituitary gland
Knee joint capsules	Bone marrow (of rib)
Iliac and para-aortic lymph nodes	All selected injection sites
Thymus	All gross lesions

There was no mortality and no clinical signs of toxicity. There was no effect on bodyweight, food consumption, body temperature, ophthalmology, clinical chemistry, or

urinalysis. Fibrinogen levels were slightly elevated in MF59-treated females at the end of the dosing period but returned to normal during the recovery period.

There were no macroscopic observations consistent with systemic toxicity. Microscopically, slight, reversible thymic atrophy, slight increase in neutrophils and precursor cells in splenic red pulp (more marked in females) and slight bone marrow hypercellularity were observed in the MF59 group.

Macroscopic findings at the MF59 injection sites included hemorrhage, edema and discoloration of sub-fascial and subcutaneous tissues. These findings were observed more frequently after the last 4 injections, with incidence and severity reduced in recovery animals, indicating resolution.

Six of the 14 injection sites on each animal were evaluated microscopically. In MF59-treated animals, findings 1 day post-injection were generally minimal to mild and included neutrophil and macrophage infiltration, edema, hemorrhage, and muscle cell necrosis. Observations 7 days post-injection included inflammatory cell infiltrates, macrophages and fibroblasts with occasional muscle cell necrosis, regeneration and calcification. By 12 days post-injection, observations consisted of minimal foci of macrophages and mononuclear cells accompanied by muscle cell regeneration.

Daily intramuscular administration of MF59 for 14 days was well tolerated in rabbits. There were no observations consistent with systemic toxicity, and local reactogenicity was of a low order of magnitude. Injection site findings were mild and reversible. The treatment schedule in this study was much more intense than that envisioned for any human vaccine usage.

Genotoxicity: in vitro and in vivo

Because MF59 contains a natural product with inherent contaminants (squalene), mutagenicity testing of the adjuvant was considered to be judicious. MF59 adjuvant, both water and citrate formulations, was investigated using the mouse micronucleus cytogenetic assay and the bacterial reverse mutation assay (Ames test) using standard study designs. The program of genotoxicity studies is shown below:

Table 17: Genotoxicity Studies with MF59

Study type	Table number, study number	MF59 formulation
Bacterial Reverse Mutation Assay	2.6.7.17.2, G96AQ62.502	Water
Bacterial Reverse Mutation Assay	2.6.7.17.2, G96AQ61.502	Citrate
Micronucleus Cytogenetic Assay in Mice	2.6.7.17.2, G96AQ62.122	Water
Micronucleus Cytogenetic Assay in Mice	2.6.7.17.2, G96AQ61.122	Citrate

Bacterial Reverse Mutation Assay (Study Nos. G96AQ61.502 and G96AQ62.502)

The purpose of these studies (Ames Test) was to evaluate the mutagenic potential of MF59 (both the water and citrate formulations) and/or its metabolites by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium and one strain of E. coli in the presence and absence of S9 activation. The assay was conducted according to standard, published protocols.

MF59C.1 and MF59W.1 were tested using S. typhimurium tester strains TA98, TA100, TA1535, and TA1537 as well as E. coli tester strain WP2 uvrA both in the presence and absence of Aroclor 1254-induced rat liver, S9 (as the metabolic activation system). The assay was performed using the plate incorporation method.

Doses of 100, 333, 1000, 3333, and 5000 µg MF59/plate were used. Saline was used as the negative control and positive controls were included for each bacterial strain. The positive controls used were as follows: with S9 activation, 2-aminoanthracene; without S9 activation, TA98 = 2-nitrofluorene; TA100 and TA1535 = sodium azide; TA1537 = 9-aminoacridine; and WP2 uvrA = methyl methanesulfonate. No positive response was observed, and neither precipitate nor appreciable toxicity was observed. Under the conditions of this study, both MF59C.1 and MF59W.1 formulations were negative in the Bacterial Reverse Mutation Assay.

Micronucleus Cytogenetic Assay in Mice (Study Nos. G96AQ61.122 and G96AQ62.122)

The purpose of these studies was to evaluate the clastogenic potential of MF59C.1 and MF59W.1 formulations, as measured by their ability to induce micronucleated, polychromatic erythrocytes in the bone marrow of male and female mice. The assay was conducted according to standard, published protocols.

In the micronucleus assay, male and female ICR mice were dosed via intraperitoneal injection with vehicle or 1250, 2500, or 5000 mg/kg of MF59 (MF59C.1 or MF59W.1) in a constant volume of 20 mL/kg. Saline was used as the negative control and cyclophosphamide as the positive control. Animals (5/sex/group) were sacrificed at 24, 48, and 72 hours after dose administration except for positive controls where five animals per sex were sacrificed 24 hours after dose administration.

Table 18: Study No. G96AQ61.122 and – Q62.122 - Study Design

Test materials	Number per sex used for bone marrow collection after dose administration		
	24 hours	48 hours	72 hours
Vehicle Control	5	5	5
Test Article			
Low Dose 1250 mg/kg	5	5	5
Mid Dose 2500 mg/kg	5	5	5
High Dose 5000 mg/kg	5	5	5
Positive Control	5	none	none

There was no mortality. Clinical signs following dose administration included lethargy in male and female mice at 5000 mg/kg. Slides of bone marrow cells, collected at 24, 48, and 72 hours after treatment were prepared and stained with May-Grunwald-Giemsa stain and read microscopically for micronucleated polychromatic erythrocytes. Slight reductions (up to 11% for MF59 citrate formulation, and up to 13% for the water formulation) in the ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the test article-treated groups relative to the respective vehicle controls.

There was no significant increase in the number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in test article treated groups, relative to the vehicle control group at any time point. Cyclophosphamide induced a significant increase in micronucleated polychromatic erythrocytes in both female and male mice. In this study both formulations of MF59 were negative in the mouse micronucleus assay.

Biocine® MF59C.1 and Biocine® MF59W.1 Magnusson-Kligman Maximisation Test in Guinea Pigs (Project No. 564278, Report No. 14465)

The potential for MF59C.1 and MF59W.1 to cause delayed contact hypersensitivity was investigated using a Magnusson-Kligman Maximization Test.

Female Dunkin-Hartley Guinea pigs were used in this GLP study (2.6.7.17.2, Study No. 14465 / 564278). Each MF59 formulation was tested in a group of 20 animals; 10 control animals received saline. MF59 concentrations for use in each phase of the study were determined by range finding; for the intradermal induction phase, 2% MF59 was used, and for the topical induction and challenge phases, 100% MF59 was used. Skin reactions were graded after each procedure by a standardized scoring system.

For intradermal induction in test groups, duplicate 0.1 mL injections of the following solutions were administered into scapular skin: 50% aqueous Freund's Complete

Adjuvant (FCA), a 1:1 (v/v) 2% MF59 and 0.9% saline, and a 1:1 (v/v) mixture of 2% MF59 and 50% aqueous FCA. Control animals received duplicate 0.1 mL intradermal injections of the following solutions: 50% aqueous FCA, 0.9% saline, and 1:1 (v/v) mixture of 0.9% saline and 50% aqueous FCA.

Six days later, the scapular region was clipped free of hair and the injection sites observed for signs of irritation. On the following day (day 7) a second induction was performed via the topical route in the same animals. The test animals were treated with 0.5 mL of 100% MF59, and controls were treated with 0.5 mL of 0.9% saline. The site was covered with an occlusive dressing for 48 hours, and then examined for signs of irritation.

Thirteen days after the topical application, the scapular region was again clipped free of hair, and the following day (day 21), both test and control animals were treated with 0.5 mL of 0.9% saline, and 0.5 mL of 100% MF59 applied topically (challenge). The test site was covered with an occlusive dressing for 24 hours, after which it was again clipped, and observed for signs of irritation. All animals were examined for clinical signs twice daily, and body weights were recorded at the start and end of the study.

During intradermal induction, slight reactions were noted in all test animals (both MF59C.1 and MF59W.1). There was no reaction in any of the control animals.

During the topical induction phase, slight reactions were seen in 4/20 MF59C.1 and 10/20 MF59W.1 animals. In the control group, 2/10 animals had slight reactions.

On challenge, 3/20 MF59C.1 animals had a positive reaction at 24 hours; in 1 animal the reaction was still present at 48 hours. There was no positive reaction in any MF59W.1 or control animal.

Apart from injection site reactions, no other clinical signs were noted, and body weight gain was normal. Under the conditions of the assay, MF59C.1 and MF59W.1 were not considered to be sensitizers in guinea pigs.

Developmental Toxicity (Embryo-Foetal and Teratogenic Potential) Study of a Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to Crl:CD®BR VAF/Plus® Female Rats (Study No. 1303-002)

The purpose of this study was to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of a vaccine. The focus of this section is MF59, therefore only data pertaining to the saline and MF59-alone groups will be presented here (2.6.7.17.2, Study No. 1303-002).

Forty-five Crl:CD BR VAF/Plus (Sprague-Dawley) female rats were randomly assigned to each group. Rats were injected intramuscularly with either saline or MF59 on day 1 of the study (three weeks before cohabitation) and on days 0, 6, 8 and 10 of presumed

gestation (rats assigned to Caesarean-sectioning) or on days 0, 6, 8, 10 and 20 of presumed gestation (rats assigned to natural delivery).

The test and control articles administered are shown in the table below:

Table 19: Study No. 1303-002 – Test and Control Articles

Group	Number of females	Intramuscular injection
I	45	1.0 mL of saline (control) (0.5 mL in each of two separate sites)
II	45	0.5 mL of MF590 (2× clinical dose)*

* The clinical dose of MF59 is 0.25 mL

Body weight and feed consumption values were recorded weekly before cohabitation, on day 0 of presumed gestation and daily until necropsy on day 20 of presumed gestation (rats assigned to Caesarean-sectioning), day 25 of presumed gestation (rats assigned to natural delivery that did not deliver a litter) or day 21 postpartum (rats that delivered litters). Day 0 of presumed gestation was defined as the day spermatozoa were observed in a smear of vaginal contents or the presence of a copulatory plug in situ was noted.

Mating performance was evaluated daily during the cohabitation period and confirmed by observation of pregnancy (implantation sites present in utero on either day 20 or 25 of presumed gestation or natural delivery of a litter).

Twenty dams from each group underwent Caesarean sectioning on day 20 of presumed gestation. Parameters evaluated included number of corpora lutea, number and placement of implantation sites, early and late resorptions, and number of live and dead fetuses. Fetuses were weighed and examined to identify sex and gross external alterations. Approximately half the fetuses in each litter were evaluated for soft tissue alterations using a variation of Wilson's sectioning technique. The remaining fetuses were eviscerated, cleared, stained using alizarin red S, and examined for skeletal alterations and ossification sites.

The remaining pregnant dams were allowed to deliver naturally. Dams were evaluated for delivery complications, litter size and pup viability. Maternal behavior was recorded on days 1, 4, 7, 14 and 21 postpartum and observed on all days postpartum. Pup sex, bodyweight, gross external anomalies and mortality were evaluated. The pups in each litter were counted once each day and clinical signs recorded. Two pups per litter were euthanized and blood collected for antibody analysis on days 1, 4, 7, 14 and 21. Litters were culled to approximately 10 pups per litter on day 7. On day 21 all dams and surviving pups were euthanized; all dams were examined for gross lesions and number and placement of implantation sites. Pups were necropsied and examined for macroscopic lesions.

Two animals in the MF59-treated group died. One animal, number 10951, had a swollen hind limb (associated with intramuscular injection) on day 1 of presumed gestation and was found dead on day 4 of presumed gestation. Feed consumption was reduced on days 1-2 and 3-4 of presumed gestation, and bodyweight was decreased on day 4. Necropsy observations included a fluid-filled bladder, green substance in the stomach, and large kidneys with tan spots and slight dilation of the pelvis. Pregnancy status could not be determined, as implantation had not yet occurred. This death was not considered to be related to innate toxicity of MF59, but rather to an individual susceptibility to the multiple administrations of a large dose volume (2× the clinical dosage) or a 'peculiarity' of MF59 administration in this animal (Consultant's Report – available on request).

A second animal, number 10984, had a swollen hind limb on days 1, 11 and 21 of presumed gestation, and was found dead on day 11 of lactation. Body weight and feed consumption were unremarkable. Necropsy findings included a large liver and spleen, and red fluid in the abdominal cavity. This animal had delivered a litter of 14 live pups that appeared normal at the time of maternal death. This death was not considered to be related to innate toxicity of MF59 (see Consultant's Report – available on request).

One control group rat delivered on day 20 of presumed gestation (the mating date was incorrectly identified) and was euthanized on day 6 of lactation. No other deaths occurred, and there were no abortions or premature deliveries.

Clinical observations related to treatment consisted of hind limb swelling. Swelling was associated with intramuscular injection, and occurred during gestation in all animals receiving MF59 alone or with antigens. Injection of 0.9% saline was not associated with swelling in the hind limb.

Maternal body weights and body weight gains were unaffected throughout the pre-mating, gestation and lactation periods. Significant reductions ($p<0.05$ to $p<0.01$) in absolute and relative feed consumption values on days 8 to 10 of gestation were possibly caused by MF59; all other time points were comparable to the saline control.

There were no treatment-related macroscopic observations in dams caesarean-sectioned on gestation day 20. Litter averages for corpora lutea, implantations, litter sizes, early resorptions, percent live male fetuses, percent resorbed conceptuses, and number of dams with any resorptions was not affected by treatment.

There were no soft tissue or external alterations in fetuses. There was an increase in the litter and fetal incidences of incompletely ossified sternebrae, pubes and/or ischia in fetuses from MF59-treated dams. This finding was possibly related to treatment as the incidence exceeded historical ranges at the test facility.

Natural delivery was unaffected by treatment. There was no effect on litter parameters, pup sex ratios, pup body weights or clinical signs. Maternal behavior was unaffected, and there were no noteworthy necropsy findings for dams or pups.

In this study, MF59 was administered either 5 (Caesarean section group) or six (natural delivery group) times. Two maternal deaths (not considered related to innate toxicity) and a brief reduction in feed consumption were probably related to treatment. In fetuses an increased incidence of incomplete ossification of the sternebrae, pubes and/or ischia was seen. The MF59 dose used in this study was equivalent to twice the usual clinical dose of 0.25 mL. On a bodyweight basis, a 2× clinical dose in a 0.3 kg rat is approximately 200 times higher than the same dose in a 60 kg human. MF59 was not considered to be teratogenic or fetotoxic in this study.

Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to New Zealand White Rabbits (Study No. 1303-001P)

In this pilot GLP study, developmental toxicity (embryo-fetal toxicity and teratogenic potential) in female New Zealand White (NZW) rabbits was evaluated following intramuscular administration of MF59 alone or in combination with antigens (2.6.7.17.2, Study No. 1303-001P). This study was used to select doses for a subsequent definitive study. The definitive study did not contain an MF59-alone group, so is not summarized here.

Five artificially inseminated and presumed pregnant New Zealand White [Hra:(NZW)SPF] rabbits were assigned to each dose group. Saline (control) or MF59 was injected intramuscularly on days 6 through 28 of presumed gestation. Dose levels were as follows:

Table 20: Study No. 1303-001P – Test and Control Articles

Group*	Number of females	Clinical dose equivalent	Test Material
I	5	—	0.5 mL of saline (control)
III	5	0.25 ×	MF59-0 diluted with saline
IV	5	0.50 ×	MF59-0 diluted with saline

* Groups II and V received antigen and are therefore not presented here

Body weights were recorded weekly before study assignment and days 0 and 6 through 29 of presumed gestation. Feed consumption values were recorded daily during the acclimation and study periods. Rabbits were observed on days 0 and 6 through 29 of presumed gestation for clinical signs, abortions and premature deliveries; viability was recorded at least twice daily. Ophthalmological evaluations were conducted during the acclimation period and on day 29 of presumed gestation.

On day 29 of presumed gestation all surviving rabbits were euthanized. A gross necropsy of the thoracic and abdominal viscera was performed. Uteri of apparently nonpregnant does were stained with 10% ammonium sulphide to confirm the absence of implantation

sites. Lymphoid tissues from three sites (cervical, mesenteric and popliteal lymph nodes) and tissues with gross lesions were preserved in neutral buffered 10% formalin for possible histological evaluation. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses, and early and late resorptions. The number of corpora lutea present in each ovary was recorded. Each fetus was weighed and examined for sex and macroscopic external alterations.

No deaths occurred during the conduct of this study. One group IV (0.5 × MF59) doe prematurely delivered on day 29 of gestation. This was considered unrelated to the test article because it was a single occurrence and common in this strain of rabbit.

There were no treatment-related effects on clinical observations, body weight, feed consumption or necropsy observations. No Caesarean-sectioning or litter parameters were affected. The litter averages for corpora lutea, implantations, litter sizes, resorptions, percent male fetuses and percent resorbed conceptuses were comparable among groups. There were no dead fetuses, and no litter consisted of only resorbed conceptuses. One late resorption occurred in a litter in group III (0.25 × MF59). There were no macroscopic external alterations in fetuses.

This study was performed to select doses for a definitive study. The definitive study did not have an MF59-alone group, therefore the data is not presented here, however the same dosing schedule with 0.5× and 1.0× MF59 combined with antigens had no effect on litter parameters and was not teratogenic in rabbits.

2.6.6.8.2.2 Nonpivotal MF59 Studies

Rabbits and dogs received single and repeated doses of MF59 alone as a control for various MF59-adjuvanted antigens. These studies are described below and in section 2.6.7.17.3. Study reports are available on request.

A Single Dose Intramuscular Toxicity Study of Rabies Vaccine Formulations in New Zealand White Rabbits (Project No. 501464, Report No. 20717)

The objective of this GLP study was to assess the local and systemic toxicity of vaccine formulations in New Zealand White rabbits (2.6.7.17.3, Study No. 20717 / 501464). The study consisted of five groups of 4 animals/sex/group. The MF59-treated group received a single dose of 0.5 mL containing 1:1 MF59:saline by intramuscular injection.

Potential toxicity was evaluated based on clinical and injection site observations, body weights, food consumption, body temperature, ophthalmic examinations, clinical pathology (hematology, coagulation, and serum chemistry parameters), terminal organ weights, comprehensive macroscopic examination, and microscopic evaluation of selected tissues.

There were no deaths, and no treatment-related adverse effects on clinical signs, body weights, food consumption, body temperature, hematology, or ophthalmology. There was no erythema or edema observed at the injection sites. On day 3, mean fibrinogen levels were slightly elevated in males dosed with MF59. By day 15, levels had decreased to pre-test levels, indicating complete reversal. There were no relevant changes in terminal organ weights. Macroscopic postmortem findings at injection sites on day 3 (reddening) were similar across groups. By day 15, there were no findings at the injection, indicating complete resolution.

Microscopic findings of minimal-mild inflammation, hemorrhage, or cellular infiltrates at the injection site were seen only on day 3. With the exception of injection sites, there were no microscopic alterations that could be attributed to treatment.

Under the conditions of the study, a single intramuscular injection of MF59 was well tolerated in male and female New Zealand White rabbits.

A Single Dose Safety and Tolerability Study of Recombinant HCV Proteins and MF59 in Rabbits with a 15-Day Recovery Period (Study No. 00-2672)

The purpose of this study was to assess the local and systemic effects of a single dose of antigens and/or MF59 adjuvant (2.6.7.17.3, Study No. 00-2672). New Zealand White rabbits (4 per sex) received a single intramuscular dose 1.0 mL of 1:1 MF59:saline.

In-life evaluations included clinical signs, dermal injection-site observations, body weights, food consumption, body temperatures, ophthalmoscopy, hematology, coagulation parameters and clinical chemistry. Two animals per sex were necropsied 48 hours after dose administration (day 2); the remaining animals were necropsied 15 days after dose administration (day 15). Organ weights were obtained and complete macroscopic pathology examinations were performed. Histopathological evaluation of selected tissues, including injection sites, was conducted.

There were no test article-related clinical observations or ophthalmic findings. No treatment-related effects on body weight, food consumption, body temperature, or hematology parameters were observed. Fibrinogen levels were slightly elevated on day 2 when compared to pretest values but returned to baseline by the end of the recovery period. Creatine kinase values was transiently increased 48 hours after dosing and returned to baseline by day 15. The increase in creatine kinase is likely due to animal restraint and/or administration of a relatively large volume by the intramuscular route.

Occasional slight desquamation or erythema was observed, as was discoloration of the injection sites, including saline sites. At the day 2 necropsy, minimal to moderate edema, inflammation (presence of mixed inflammatory infiltrate), and/or hemorrhage was observed in injection sites of most animals across all groups. Local muscle necrosis with mineral deposition was observed less frequently. At the end of the recovery period (day

15), there were only a few incidences of injection site findings. There were no treatment-related findings in other tissues and no organ weight changes.

The local effects at the injection sites were of minimal to moderate severity and were partially to fully reversible. Systemic findings of transient increased fibrinogen and creatine kinase levels were consistent with the intramuscular administration of an adjuvant.

Comparative Intramuscular Toxicity in Rabbits (Study No. 89-6280)

Groups of 5 per sex New Zealand White rabbits received intramuscular injections of saline or MF59 on days 1 and 8 (2.6.7.17.3, Study No. 89-6280). The 0.5 mL dose volume per dosing occasion was split into two 0.25 mL injections (one per leg). Animals were necropsied 7-8 days after the second injection.

There was no mortality and no evidence of systemic toxicity. Injection site reactions were limited to small areas of erythema or scabbing, consistent with the physical introduction of a needle and reactions, where they occurred, had generally regressed within 72 hours of dosing.

Fourteen-Day Intramuscular Toxicity Study of Connaught Fluzone/MF59 Vaccine in Rabbits (Study No. 2777-102)

This study was designed to evaluate the local and systemic toxicity of Connaught Fluzone® Vaccine in combination with MF59 adjuvant when administered via intramuscular injection into alternating hind limbs of male and female adult rabbits (2.6.7.17.3, Study No. 2777-102). Injections were administered on days 1 and 15, and treatment was followed by a 14-day recovery period. Twenty-four Hra:(NZW)SPF/HRP rabbits were assigned to the study as follows:

Table 21: Study No. 2777-102 – Study Design

Group and treatment	Number of animals		Dose volume mL
	Male	Female	
1 (MF59 Control)	6	6	0.5
2 (Vaccine)	6	6	0.5

Control animals received 1:1 MF59:saline. Three animals/sex/group were necropsied approximately 48 hours after the dose on day 15. All remaining animals were necropsied on day 29 following a non-treatment recovery period.

Study parameters included survival, clinical signs, ophthalmology, injection-site irritation, body weight data, rectal temperatures, clinical pathology data, gross pathology

observations, organ weight data, and microscopic examination of selected tissues from all animals.

All of the rabbits survived until their scheduled sacrifice. No treatment-related clinical signs were observed during the study, except for mild local irritation at the injection sites of some animals in both groups. Observations at the injection sites included very slight erythema on days 3, 4, 15, 16, 17, and 18 in a few animals of both sexes from each group; well-defined erythema in a Group 2 male on day 15; very slight edema in some Group 2 males on days 15, 16, 17, and 18, and in Group 1 females on days 15 and 17; slight edema in a Group 1 female on day 16; and moderate edema in a Group 1 female on day 15. The dermal findings appeared to resolve within a few days of dosing.

All animals gained weight during the study. There was no evidence of treatment-related effects in the ophthalmoscopic examinations, rectal temperature, hematology, serum chemistry, gross pathology, and organ weight data. Inflammation, hemorrhage, and focal skeletal muscle and collagen degeneration seen histologically at the intramuscular injection sites of some animals were similar between both groups. With the exception of focal skeletal muscle degeneration, these microscopic findings were reversible following the 14-day non-treatment recovery period.

Other microscopic findings in various tissues were generally inflammatory in nature, and were considered incidental to treatment with the Fluzone® Vaccine plus MF59 adjuvant. There were no histomorphological findings suggestive of adverse systemic effects associated with the administration of the test material.

Based upon the results of this study, Fluzone® Vaccine in combination with MF59 was associated with localized tissue reactions when administered in two separate doses approximately 2 weeks apart via intramuscular injection to New Zealand White rabbits.

Intramuscular Study in Chinchilla Rabbits (Study No. 89-6192)

Chinchilla rabbits received three courses of two separate intramuscular injections of 0.25 mL of saline or MF59, administered at 2 week intervals, with dosing on days 1, 15 and 29 (2.6.7.17.3, Study No. 89-6192). There were no deaths and clinical/laboratory investigations indicated no adverse reactions. Postmortem examination showed no target-organ toxicity, however, a slight increase in fibrinogen and body temperature was noted in some MF59-treated animals. Injection sites indicated a slight inflammatory response that was also noted for the saline animals. MF59 emulsion was well tolerated under the conditions of the study.

Comparative Intramuscular Tolerability in Rabbits (Study No. 90-6230)

New Zealand White rabbits (5 per sex) received two 0.25 mL intramuscular injections of MF59 (1:1 MF59:vehicle equivalent to 1× human dose) on days 1, 15 and 29 (2.6.7.17.3, Study No. 90-6230). At least 7 days after the third dose the animals were necropsied.

There was no evidence of systemic toxicity or dermal irritation at the injection sites. Injection site findings were generally minor in severity, and included chronic inflammation, degeneration of muscle fibers, multinucleate giant cell aggregation, and mineralization and aggregation of vacuolated macrophages.

28-Day Intramuscular Study in Rabbits (Study No. HWA 2670-101)

New Zealand White rabbits received 0.5 mL intramuscular injections of MF59 (1:1 MF59:saline equivalent to 1× human dose) on days 1, 15 and 29 followed by a 14-day recovery period (2.6.7.17.3, Study No. HWA 2670-101).

There was no evidence of systemic toxicity or dermal irritation at the injection sites. There was focal hemorrhage and/or residual inflammation of minimal severity at injection sites, which mostly resolved after the recovery period.

Subchronic Intramuscular Toxicity Study of Biocine® HPV-6 Vaccines in Rabbits (HPV-6 E7, HPV-6 L1 Antigens and MF59C.1 Adjuvant) (Study No. 759-002)

This study was conducted to evaluate the toxicity of vaccines administered intramuscularly in rabbits (2.6.7.17.3, Study No. 759-002). Six New Zealand White (Hra: (NZW) SPF) rabbits per sex received injections of 1:1 saline:MF59. There was no saline control for comparison. Doses were administered by intramuscular injection into the hind limb. Animals were injected on study days 1, 15 and 29 into alternating hind limbs. The injection volume was 0.5 mL. Three animals per sex per group were necropsied on day 31 and the remaining animals were necropsied on day 43.

Criteria evaluated for treatment effect included mortality, clinical signs, injection-site scores, body weights, body temperatures, ophthalmoscopic examinations, clinical pathology laboratory tests, macroscopic observations, organ weights, and histopathologic examinations.

All animals survived to terminal or recovery necropsy. There were no significant clinical findings noted. There were no test article-related effects on body weights, body temperatures or ophthalmic findings. Bruising was noted at the injection site for 1 female 24 hours post-second injection and also on study days 17 and 18; the injection sites of all other animals at all time points had no evidence of erythema or edema.

Macroscopic pathology findings at the terminal necropsy consisted of red discoloration of the third injection site for 1 out of 3 males. In females, red discoloration of the third injection site was seen in 2 of 3 animals. This red discoloration correlated with the microscopic findings of hemorrhage, subacute inflammation, and/or muscle fiber degeneration. Other macroscopic findings noted at the terminal or recovery necropsies were considered incidental and not related to test article administration.

A decrease in incidence and severity of these lesions was noted in the first (day 1) and second (day 15) injection sites compared to the third injection site (day 29). No other MF59-related changes were evident at either the terminal or recovery necropsy in the organs evaluated histologically. Injection-site findings present at the terminal necropsy had resolved by the end of the recovery period.

Hepa Bio Vax B 6-Week Subacute Toxicity Study in New Zealand White Rabbits by Intramuscular Route (Study No. 950031)

The purpose of the study was to evaluate the toxicity and local tolerability of Hepa Bio Vax B with MF59 adjuvant administered by the intramuscular route to rabbits (6/sex/group) once every two weeks for 6 weeks (section 2.6.7.17.3, Study No. 950031). The control group was MF59 (0.5 mL of a 1:1 dilution with Tris buffer). Half the animals (3/sex/group) were necropsied 48 hours post-last dose. The remaining 3/sex/group were necropsied 2 weeks after the last dose.

Toxicity was evaluated based on clinical signs and injection site observations, body weights and temperatures, clinical pathology, ophthalmoscopy, macroscopic postmortem observations, selected organ weights, and histopathology of the injection sites.

There were no deaths and no systemic toxicity. The only treatment-related effects were mildly elevated lactic dehydrogenase and creatine kinase, both of which are related to intramuscular injection. Findings at the injection site consisted of reversible slight inflammatory and degenerative changes in the muscle.

Subchronic Intramuscular Toxicity Study of Biocine HIVp24 Vaccine in Rabbits (Project No. 656583, Report No. 14160)

The purpose of this study was to evaluate local and systemic effects of the recombinant antigen combined with MF59C.1.

New Zealand White rabbits received 4 doses (0.5 mL on days 1, 15, 29 and 43) of 1:1 saline:MF59 via intramuscular injection into alternate hind limbs (section 2.6.7.17.3, Study No. 14160 / 656583). In-life toxicological evaluations included clinical signs, injection-site scoring (Draize), body weights, body temperatures, ophthalmoscopy, hematology, and clinical chemistry. Three animals per sex were necropsied 48 hours after the fourth dose; the remaining animals were necropsied after a 14-day treatment-free period. Organ weights were obtained and macroscopic examinations performed.

There was no mortality. There were no treatment-related effects on body weights, ophthalmoscopy, or organ weights. Local reactions at injection sites were minimal in severity and of low incidence. Body temperatures increased slightly over time; 48 hours after the fourth dose the mean increase compared to pre-trial and pre-dose values was 0.69ºC for males and 0.14ºC for females. Enlarged popliteal lymph nodes were observed

macroscopically on day 45 in 1 male and 2 females. On day 57 reddened lymph nodes were noted in 1 male and 1 female.

Microscopic evaluation of injection sites at day 45 showed inflammatory cell infiltrates; the severity was graded 'very mild' or 'mild'. On day 57 the incidence and severity of findings was decreased, indicating reversibility.

A Multiple Dose Intramuscular Toxicity and Local Tolerability Study of Rabies Vaccine Formulations in New Zealand White Rabbits (Project No. 501438, Report No. 20611)

The objective of this GLP study was to evaluate the systemic toxicity and local tolerability of multiple (5) doses of rabies vaccine formulations administered once every one to two weeks by intramuscular injection to New Zealand White rabbits (section 2.6.7.17.3, Study No. 20611 / 501438).

The study consisted of five groups of 6/sex/group. The MF59 dose volume was 0.5 mL injected alternately into the quadriceps or posterior thigh muscle on days 1, 8, 15, 29, and 43 of the study. A comprehensive macroscopic examination and tissue collection was performed on 3 animals/sex/group on days 45 and 57.

Potential toxicity was evaluated based on the following parameters: clinical signs, dermal injection site observations (0, 24, and 48 hours after each administration), body weights, ophthalmic examinations, food consumption, body temperatures, clinical pathology (hematology, coagulation, and serum chemistry parameters), terminal organ weights, full macroscopic postmortem examination, and microscopic evaluation of selected tissues.

There were no deaths or treatment-related adverse effects on any antemortem study parameters. There were no adverse clinical signs and no edema or erythema at the injection site at any of the observation time points. Globulin levels were slightly elevated on days 17 and 45 in males given MF59. By the end of the recovery period (day 57), levels were comparable to control values.

Macroscopic postmortem findings at the injection site consisted of reddening. The incidence of injection site findings was lower on day 57 than on day 45, indicating partial to full resolution. Microscopic findings at the injection consisted of inflammatory cell infiltrate in muscle and subcutaneous layer.

Under the conditions of the study, administration of five 0.5 mL intramuscular injections of MF59 once every one to two weeks was well tolerated.

A Multiple Dose Safety and Tolerability Study of Recombinant HCV Proteins and MF59 in Rabbits with a 14-Day Recovery Period (Study No. 00-2673)

The purpose of this study was to assess the local and systemic effects of multiple doses of HCV antigens combined with MF59 adjuvant (section 2.6.7.17.3, Study No. 00-2673). New Zealand White rabbits received six intramuscular doses of 1 mL of 1:1 MF59:saline administered at 2-week intervals (days 0, 14, 28, 42, 56, and 70).

In-life evaluations included clinical signs, dermal injection site observations, body weights, food consumption, body temperatures, ophthalmoscopy, hematology, coagulation parameters, and clinical chemistry. Three animals per sex were necropsied 48 hours after the final dose administration (day 72); the remaining animals were necropsied 14 days after the final dose administration (day 84). Organ weights were obtained and macroscopic pathology examinations were performed. Histopathological evaluation of selected tissues, including injection sites, was conducted.

There were no MF59-related clinical observations or ophthalmic findings. No treatment-related effects on body weight, food consumption, body temperature, or hematology parameters were observed. Fibrinogen levels were generally elevated after treatment but returned to pretest levels after the recovery period.

Injection sites were examined at 24 and 48 hours after each injection. Very slight erythema and edema were seen sporadically.

There were no treatment-related effects on organ weights. Microscopic observations included minimal to slight to moderate edema, inflammation (presence of mixed inflammatory infiltrate), and hemorrhage in many of the animals at day 72. There were no microscopic findings other than those observed at the injection sites.

The administration of six intramuscular doses of MF59 given at 2-week intervals was well tolerated. Local effects at the injection sites were reversible.

8-Month Intramuscular Study in Rabbits (Study No. HWA 2670-100)

New Zealand White rabbits received a 0.5 mL intramuscular injection of saline, or 0.5 mL MF59:saline (1:1) mixture, administered into alternate hind limbs approximately once every 3 weeks for 8 months (section 2.6.7.17.3, Study No. HWA 2670-100).

There were no deaths and no treatment-related clinical signs, with the exception of slight dermal irritation at the injection sites of most animals, the incidence being slightly more frequent with MF59 than in the control group.

A slight decrease in prothrombin times, slight increase in mean fibrinogen values, and an increase in creatine kinase values were consistent with inflammation at injection sites. In conjunction with antigen, there was also a slight increase in germinal centre activity

within the white pulp of the spleen in a few animals (considered a normal immunological response).

Comparative Intramuscular Tolerability Study in Beagle Dogs (Report No. 89-6281)

Beagle dogs were dosed with saline or MF59 on days 1 and 8 (section 2.6.7.17.3, Study No. 89-6281). One animal from each sex per group was euthanized on day 15 of the study, with remaining animals euthanized on either day 19 or 20.

Treatment-related findings were limited to local reactions of erythema or scabbing consistent with needle trauma.

Intramuscular Tolerability Study in Dogs (Report No. 89-6193)

Beagle dogs received intramuscular injections of 0.5 mL of saline or MF59 on days 1, 16 and 29 (2.6.7.17.3, Study No. 89-6193). Some animals showed a transient, non-recurring pain reaction after the first dose of MF59. There was no evidence of systemic toxicity.

Safety pharmacology (cardiovascular and neurological) parameters were also assessed in this study. The summary results are presented in section 2.6.2.4; there were no effects on either organ system.

Comparative Intramuscular Tolerability in Beagle Dogs (Study No. 90-6231)

Beagle dogs (2 per sex) received 0.5 mL intramuscular injections of MF59 (1:1 MF59:vehicle, equivalent to 1× human dose) on days 1, 15 and 29 (section 2.6.7.17.3, Study No. 90-6231). Seven days after the third dose the animals were necropsied.

There was no evidence of systemic toxicity. Injection site reactions were acute and minor in severity, and consisted of a needle mark with erythema 1-3 mm in diameter that regressed 1-2 days following dosing. There were no notable histological changes related to treatment.

Safety pharmacology (cardiovascular and neurological) parameters were also assessed in this study. The summary results are presented in section 2.6.2.4; there were no effects on either organ system.

Supportive Studies

The safety of MF59 can also be inferred from efficacy studies. These studies were conducted with vaccine formulations composed of antigens combined with MF59 (no MF59-alone group). Reports for these studies are available on request. In these studies, the antigen/MF59 formulations were generally well tolerated. Based on the parameters evaluated, no treatment-related safety issues were identified. Adverse events were limited to inflammatory responses at the injection site. These were of a low grade of severity and

partially to fully resolved by the end of the recovery period. Consistent systemic treatment-related findings consisted of increases in fibrinogen levels and slight increases in globulin. These findings are consistent with administration of adjuvanted vaccine formulations.

Table 22: Summary of Supportive Studies with MF59

Study Title	Study Number
Immunogenicity Study of a Vaccine (Antigen and Adjuvant Components) in Rabbits	1303-003
Intramuscular Dosage-Range Developmental Toxicity Study of Biocine Vaccine (HSV gD2 and gB2dTM Antigens) in Rabbits	1303-004P
A 10 Week Intramuscular Vaccine Safety Study in New Zealand White Rabbits With HCV E1E2 Antigen, CpG and MF59 Adjuvant	500757
Intramuscular Toxicity Study of HCV (E2+Core) Vaccine in Rabbits	3-K84
Intramuscular Toxicity Study in Rabbits with HCV E2 Vaccine	N002833A
Subchronic Intramuscular Toxicity Study of BIOCINE HIV Thai E gp120/SF2 gp120 Vaccine in Rabbits	759-001
12-week vaccine toxicity study in female New Zealand White rabbits with intranasal gp140 and LT-K63 adjuvant priming and intramuscular gp140 and MF59 adjuvant boosting	433665
Administration of Woodchuck Hepatitis Virus Surface Antigen (WHsAg) to Woodchucks with and without Chronic Woodchuck Hepatitis Virus (WHV) Infection	98-07-263
HCV E2 and E2/Core Vaccines Adjuvanted with MF59-0 and Iscomatrix in Baboons	ACR 381-PC-0
Safety and Efficacy of Chiron HBV PreS2+S/MF59C.1 Vaccine in an Adult Chimpanzee with Chronic Hepatitis B Infection	420-PT-0

The MF59-adjuvanted vaccine formulations tested in these studies were well tolerated and immunogenic.

2.6.6.9 Discussion and Conclusions

The primary nonclinical data supporting the use of FCC H1N1sw is a GLP rabbit toxicology study (Study No. 466122) performed with the comparable vaccine formulation FCC/MF59-H5N1. Based on the results of this study, three 0.5 mL intramuscular administrations of FCC/MF59-H5N1 (containing15 µg antigen and 0.25 mL MF59 adjuvant) in NZW rabbits was immunogenic, locally well tolerated, and was not associated with systemic toxicity. In this study, the doses of antigen and adjuvant in the FCC/MF59-H5N1 vaccine tested exceeded those proposed for the FCC H1N1sw vaccine. Each of the three doses administered to rabbits (~3.5 kg) contained 15 µg of antigen and

0.25 mL adjuvant, resulting in a cumulative dose of 45 µg antigen and 0.75 mL adjuvant. In comparison, the proposed 2-dose clinical regimen of FCC H1N1sw will contain 3.75 µg of antigen and 0.125 mL adjuvant per dose, for a cumulative dose of 7.5 µg antigen and 0.25 mL adjuvant. Therefore, comparing on a body weight basis, rabbits received approximately 17× more antigen than a 10 kg child will receive, and approximately 8.5× more adjuvant.

No dedicated secondary or safety pharmacology addressing potential effects on cardiovascular, respiratory and CNS parameters were performed with FCC H1N1sw. However, based on the cardiovascular and neurological evaluations performed in dogs that received repeated intramuscular injections of MF59 adjuvant, the risk of unanticipated secondary or safety pharmacological effects in subjects receiving FCC H1N1sw is considered extremely low.

Nonclinical studies performed for the parent vaccine, Optaflu (repeat-dose and reproductive and developmental toxicity), and the related egg-derived MF59-adjuvanted vaccines, Aflunov (reproductive and developmental toxicity), and Fluad (repeat-dose toxicity and sensitization), all provide strong support for the safety and immunogenicity of the candidate vaccine.

A comprehensive toxicology program was conducted for MF59 adjuvant. Pivotal toxicology studies performed with MF59 include the evaluation of repeat-dose toxicity (including local tolerability), genotoxicity, sensitization, and embryofetal and developmental toxicity. Its use is not associated with systemic toxicity and MF59 has a low order of local reactogenicity. In multiple injection rabbit studies, clinical pathology findings of increased fibrinogen, globulin, and creatine kinase and minor inflammatory and degenerative changes at the injection site are reversible within 2 days to 2 weeks and are consistent with the administration of immunologically active materials in animals and in man (Cayol 1995). The local reactogenicity of MF59 compares favorably with a well-accepted adjuvant like alum. MF59 is not genotoxic, teratogenic, or a developmental toxicant. MF59 does not cause sensitization.

Potential safety concerns with respect to the use of FCC H1N1sw in humans include hypersensitivity reactions (to vaccine ingredients or residuals from the manufacturing process) and interactions with ongoing therapy that could diminish antibody response to active immunization. These concerns will be addressed in the label.

2.6.6.10 References

Cayol M, Tauveron I, Rambourdin F, Prugnaud J, Gachon P, Thieblot P, et al. Whole-body protein turnover and hepatic protein synthesis are increased by vaccination in man. Clin Sci (Lond). 1995 Oct;89(4):389-96.

2.6.6 Toxicology Written Summary

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GLOSSARY OF TERMS AND ABBREVIATIONS

BPL	Beta-propiolactone
EoP	End of Production
FCC	Flu Cell Culture
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HA	Hemagglutinin
HAU	Hemagglutination Units
HI	Hemagglutination Inhibition
MDCK	Madin Darby Canine Kidney
NA	Neuraminidase
PBS	Phosphate Buffered Saline
Ph. Eur.	European Pharmacopoeia
PDL	Population Doubling Level
ppm	parts per million
qPCR	Quantitative Polymerase Chain Reaction
TCID	Tissue Culture Infecting Dose
WHO	World Health Organization

2.6.6 Toxicology Written Summary

2.6.6.1 Brief Summary

Chiron's Flu Cell Culture (FCC) vaccine is a trivalent, surface antigen, inactivated, influenza vaccine. This vaccine is intended for interpandemic use. FCC vaccine contains purified hemagglutinin (HA) and neuraminidase (NA) antigens from the surface of the three influenza virus strains, type A and type B, recommended annually for immunization by the WHO (World Health Organization) and the EMEA. The influenza virus strains are individually grown in MDCK cells and inactivated by beta-propiolactone (BPL) before purification of the surface antigens and final formulation.

The drug product, FCC vaccine, has undergone nonclinical testing in different animal species to assess toxicity, immunogenicity, and protection. As discussed in the Pharmacology section 2.6.2, the FCC vaccine is immunogenic in mice, rabbits and ferrets. Nonclinical results, expressed as HI antibody titers, indicate that FCC vaccine is comparable to a conventional egg-derived vaccine (section 2.6.2.2). Although safety was not the primary goal of these immunogenicity or challenge studies, the animals tolerated the vaccine based on viability and absence of overt clinical signs.

The Abnormal Toxicity Test is performed as part of the release criteria (section 3.2.P.5.1). In accordance with Ph. Eur., mice and Guinea pigs are dosed once and observed for seven days. This *in vivo* test confirms the safety and quality of each trivalent vaccine lot (Drug Product).

Toxicological testing of the vaccine product itself, as per applicable guidelines for influenza vaccines and vaccines in general, consisted of one GLP study in rabbits that addressed the major safety concerns with a novel vaccine. The study design addressed the important safety issues related to vaccines: local tolerability, systemic toxicity, potential hypersensitivity, effects on immune system tissues, and the reversibility of any observed effects or any delayed effects. In addition, the clinical route and dose level was studied, the expected number of clinical doses (one) was exceeded by one dose, and safety margins (on a body weight basis) of approximately 20× (for a 60 kg adult) were achieved. Toxicity was evaluated based on clinical signs, dermal scoring, body weights and temperatures, food consumption, ophthalmoscopy, clinical pathology (hematology, serum chemistry, and coagulation including fibrinogen), organ weights, macroscopic post-mortem examinations, and histopathology of selected tissues. The short-term safety of the vaccine itself, any inadvertent contaminants, intentional additives, and the elicited antibodies were, therefore, evaluated.

The results demonstrated that the administration of two intramuscular injections of FCC vaccine, given one week apart, was immunogenic and systemically well tolerated in male and female New Zealand White rabbits (section 2.6.6.3). Local tolerability (injection site reactions) in vaccinated animals was comparable to saline-treated controls.

Studies that are considered standard for therapeutic small molecules or some biologics (safety pharmacology, pharmacokinetics, genotoxicity, reproductive toxicity, carcinogenicity) were, appropriately, not performed with FCC vaccine. However, because the vaccine is manufactured in a novel mammalian cell substrate, an extensive panel of studies was performed to characterize the MDCK cell line (section 3.2.S.2.3.2).

Chiron's MDCK cells have been adapted to grow in serum-free and protein-free medium in suspension (section 3.2.S.2.2.3). Because the ability to grow in suspension in protein-free media is often associated with tumorigenicity *in vivo*, standard tumorigenicity testing of viable end-of-production MDCK cells was performed in adult nude mice. In addition, because the precise mechanism responsible for the tumorigenicity of Chiron's MDCK cells is not known, oncogenicity studies with cell lysates and purified DNA were also performed in rodents. Although these tumorigenicity/oncogenicity studies do not test the FCC vaccine product, *per se*, they are pertinent to the overall safety assessment of the vaccine and are presented in section 2.6.6.8.7. Intact MDCK cells were tumorigenic in adult nude mice, but cell lysates or purified DNA were not oncogenic in neonatal nude mice, rats or hamsters. This demonstrated lack of oncogenicity *in vivo* is consistent with the profiling for adventitious agents presented in section 3.2.A.2.

In summary, FCC vaccine and the antibodies elicited by the antigens were well tolerated in rabbits. In addition to the influenza antigens, rabbits were also exposed to formulation excipients, manufacturing residuals, and any unintentional contaminants. On a body weight basis, rabbits (~3 kg) received approximately 20 times the dose of any vaccine component that would be administered in a clinical dose to a human subject (~60 kg). Together, the GLP rabbit toxicology study, the product quality testing, and the *in vivo* rodent tumorigenicity/oncogenicity studies comprise a data package that supports the safety of the FCC vaccine.

Toxicology Overview

The studies performed with FCC vaccine are outlined below.

Study Type and Duration	Route of Administration	Species	Compound Administered
Abnormal toxicity; non-GLP Single dose	Intraperitoneal	Mice, Guinea pigs	FCC vaccine
Toxicity; GLP Repeat dose	Intramuscular	Rabbits	FCC vaccine

The FCC vaccine formulations tested in animals are summarized in Table 2.6.7.4.1. Process intermediates testing in tumorigenicity and oncogenicity studies are summarized in Table 2.6.7.4.2.

2.6.6.2 Single Dose Toxicity

Abnormal toxicity testing (Table 2.6.7.5, no study number assigned)

Abnormal Toxicity (single-dose) studies in mice and Guinea pigs via the intraperitoneal route are performed routinely as lot release tests for FCC vaccine (Ph. Eur. Test for Abnormal Toxicity). Although these tests are not toxicological studies *per se*, they do provide information on the safety of the product. This is particularly important during the development of influenza vaccines, since the composition of the active ingredients changes every year. These single dose data in mice and Guinea pigs with FCC vaccine administered by the intraperitoneal route confirmed the absence of abnormal toxicity. Studies performed with selected lots are listed below.

Type of Study	Description
Abnormal Toxicity (single dose)	FCC vaccine Lot Number: [redacted]08[redacted]12 Ph. Eur. Abnormal Toxicity
	FCC vaccine Lot Number: [redacted]09[redacted]11 Ph. Eur. Abnormal Toxicity
	FCC vaccine Lot Number: [redacted]11[redacted]11 Ph. Eur Abnormal Toxicity

Rabbit toxicology study (Table 2.6.7.5, Study No. 191-44)

Single-dose toxicity and local tolerability of FCC vaccine was evaluated in rabbits following the administration of the first dose in the repeat-dose toxicology study (section 2.6.6.3). Two intramuscular injections were given 7 days apart into alternate hind limbs. Each site, therefore, received a 'single-dose' of FCC vaccine.

No animals died in this study. There was no evidence of systemic toxicity after a single dose based on in-life evaluations. No detectable erythema or edema was observed at the injection sites in any animal. Histopathological findings at the injection site indicated that the vaccine was well tolerated (see results below).

2.6.6.3 Repeat Dose Toxicity

Two dose intramuscular toxicity study of Influenza vaccine formulations in New Zealand White rabbits (Table 2.6.7.7, Study No. 191-44)

The objective of this GLP study was to assess the local and systemic toxicity of FCC vaccine in New Zealand White rabbits after two administrations and to determine the reversibility of findings. Agrippal™, a marketed egg-derived Influenza subunit vaccine formulation, served as the reference article. Phosphate buffered saline (PBS) served as the control article (placebo). The study consisted of three groups of 6 animals/sex/group.

Rabbits received an intramuscular injection of 0.5 mL of either the test or reference article or placebo on Days 1 and 8 as shown below.

Table 2.6.6.3-1 Study design – Study No. 191-44

Group	No. of Animals	Test Materials (Dose)[a]	Dosing Day[b]	Necropsy	
				Main[c]	Recovery[d]
1	6M + 6F	Placebo - PBS Control (0 µg)	1 & 8	3M + 3F	3M + 3F
2	6M + 6F	Test Article - FCC vaccine[e] (45 µg)	1 & 8	3M + 3F	3M + 3F
3	6M + 6F	Reference Article - Agrippal™ Influenza Subunit vaccine formulation (45 µg)	1 & 8	3M + 3F	3M + 3F

[a] Each vaccine formulation contained 3 antigens at 15 µg/antigen for a total of 45 µg/dose. The antigens used were A/New Caledonia/20/99 (H1N1), B/Guangdong/120/00 (B), and A/Panama/2007/99 (H3N2).
[b] Rabbits received a 0.5mL IM injection in the left hind limb on day 1 and in the right hind limb on day 8.
[c] Main necropsy was performed 2 (M) and 3 (F) days post-last dose.
[d] Recovery necropsy was performed 14(M) and 15(F) days post-last dose.
[e] Referred to as Influenza Cell Culture Subunit Vaccine in the study report
M = Males; F = Females

Potential toxicity was evaluated based on clinical signs, dermal scoring of injection sites, body temperature, body weight, food consumption, ophthalmic examination, clinical pathology (hematology, coagulation, and serum chemistry parameters), terminal organ weights, comprehensive macroscopic examination, and microscopic evaluation of selected tissues.

The animals were observed twice daily for mortality and once daily for signs of toxicity. Injection sites were assessed for signs of irritation and scored based on a modified Draize score prior to dosing, one and two days after each injection, and at each necropsy. Rectal body temperatures were taken prior to dosing, one and two days after each injection, and at each necropsy. Body weights were recorded pre-treatment, on days 8 and 15, and at necropsy. Food consumption was measured once weekly. The ophthalmology evaluation was performed pre-treatment and prior to each necropsy. Blood samples for hematology, serum chemistry, and coagulation (including fibrinogen) analysis were collected pre-treatment, two days after each dose, and on day 22. Additional blood samples were taken prior to each administration, on day 15, and at each necropsy for antibody analysis. At each necropsy, a complete macroscopic examination and collection of tissues was performed. Organ weights were determined as shown below.

Table 2.6.6.3-2 Organs weighed – Study No. 191-44

Adrenals	Heart	Liver	Ovaries	Testes
Brain	Kidneys	Lungs	Spleen	Thymus

Microscopic evaluation of selected tissues was performed as shown below.

Table 2.6.6.3-3 Histopathology – Study No. 191-44

Tissues evaluated histopathologically	
Brain (including the medulla oblongata, pons, cerebrum, and cerebellum)	Liver
	Lung (including the bronchus)
Bone marrow	Lymph nodes (including the iliac, mesenteric, and cervical nodes)
Eyes (including the optic nerve and uvea)	
Femur (including the knee joint capsule)	Spleen
Heart	Thymus
Injection sites	Urinary bladder
Kidneys	Macroscopic lesions

There were no deaths, and no treatment-related adverse effects on clinical signs, body temperature, body weights, food consumption, or ophthalmology. There was no erythema or edema observed at the injection sites. There were no changes in hematology, coagulation, or clinical chemistry parameters that were considered related to treatment. Although there were some apparent changes in hematology and clinical chemistry parameters, some of which attained statistical significance, values were always within the range observed prior to commencement of treatment and/or the range historically observed at the test facility for New Zealand White rabbits and, thus, were not considered treatment-related.

Administration of the test article did not produce any macroscopic findings. There were no treatment-related effects on organ weights, with the possible exception of thymus in males. Thymus weights were highly variable and a subtle vaccine effect on thymus weights cannot be excluded. The histopathological findings were limited to the expected reactions at the injection sites. These consisted of minimal to slight necrosis in the left injection site and minimal to slight hemorrhage in the right injection site. The incidence and severity of injection site findings are summarized below (Table 2.6.6.3-4). Findings at the injection site were attributed to the trauma caused by the intramuscular injection, occurred in all groups (including control), and were partially to fully resolved by the end of the recovery period.

Table 2.6.6.3-4 Histopathology of injection sites – Study No. 191-44

Group and Treatment	1 - Control		2 - FCC vaccine		3 - Agrippal™	
Necropsy	Main	Recovery	Main	Recovery	Main	Recovery
Males						
Left Injection Site [a]						
Necrosis	0	0	0	0	1 (slight)	1 (slight)
Right Injection Site [b]						
Hemorrhage	0	1 (slight)	1 (slight)	0	0	0
Females						
Left Injection Site [a]						
Necrosis	1 (slight)	0	1 (slight)	1 (minimal)	2 (min/slight)	0
Right Injection Site [b]						
Hemorrhage	0	0	0	0	0	0

[a] The left site received the first injection and was evaluated on days 9 (males) or 10 (females) post-injection (main necropsy) and on days 21 (males) or 22 (females) post-injection (recovery necropsy).
[b] The right site received the second injection and was evaluated on days 2 (males) or 3 (females) post-injection (main necropsy) and on days 14 (males) or 15 (females) post-injection (recovery necropsy).

At both the main and recovery necropsy, there was an unusually high incidence of pulmonary and renal findings in all groups (including the controls). Lung findings consisted primarily of slight to moderate interstitial inflammatory foci and were noted at the main and recovery necropsy in more than half of animals (males and females) from all groups, including control. Kidney findings consisted primarily of tubular degeneration and/or dilation, were slight to moderate in severity, and occurred only in females (from all groups, including control) at both the main and recovery necropsy. Pulmonary and renal findings were not considered related to treatment because they occurred in animals from all groups, including control animals. Although the cause of the kidney and lung findings was not determined, these findings were considered spontaneous, and did not compromise the interpretation and/or validity of the study.

Serum samples for antibody analysis were taken prior to each administration on days 1 and 8 (6/sex/group), on day 15 (3/sex/group), and at the main [3/sex/group, day 10(males)/11(females)] and recovery necropsy [3/sex/group, day 22(males)/23(females)]. Results from this analysis demonstrated that the test article was effective in inducing the production of antibodies against the three vaccine influenza virus strains [A/New Caledonia/20/99 (H1N1), B/Guangdong/120/00 (B), and A/Panama/2007/99 (H3N2)]. The immunogenicity results for this study are presented in section 2.6.2.2.2. Briefly, results for strains A/New Caledonia and B/Guangdong revealed no titer pre-treatment and an increase in titer after the second vaccine dose in the test and reference article-treated animals. There was no titer against these strains in control animals. In contrast, there

were background titers against strain A/Panama in control animals and pre-treatment in the test and referenced article-treated animals, which were attributed to nonspecific binding. However, an increase in A/Panama antibody titer was evident after the second vaccine dose.

Under the conditions of the study, two 0.5 mL intramuscular injections of the test article, FCC vaccine, given one week apart, were immunogenic and very well tolerated in male and female New Zealand White rabbits. There were no treatment related adverse effects on clinical observations, dermal scoring, body weights and temperatures, food consumption, clinical pathology (hematology, coagulation, and clinical chemistry), organ weights, or macroscopic evaluations. Histopathological evaluation revealed the expected reactions (necrosis and hemorrhage) at the injection sites, which were seen in all experimental groups, attributed to the intramuscular injection, and partially to fully resolved by the end of the recovery period. The two doses administered to rabbits in this study exceed the intended number (one) proposed for annual interpandemic immunization.

2.6.6.4 Genotoxicity

Genotoxicity studies were not performed with FCC vaccine due to the nature of the product. There are no known formulation components or impurities in the final product expected to be of concern.

2.6.6.5 Carcinogenicity

Carcinogenicity studies were not performed. The final product does not contain any known formulation components or impurities at dose levels that would be expected to be of concern.

2.6.6.6 Reproductive and Developmental Toxicity

Reproductive and developmental toxicity studies have not been performed with FCC vaccine. The final product does not contain any known formulation components or impurities at dose levels that would be expected to be of concern.

2.6.6.7 Local Tolerance

Rabbit toxicology study (Table 2.6.7.16, Study No. 191-44)

Local tolerability was evaluated during the repeat-dose toxicology study in rabbits (section 2.6.6.3). There were no in-life observations of irritation and no macroscopic injection site findings.

Microscopic findings were seen in all groups, including controls. Findings consisted of minimal to slight necrosis and hemorrhage, and were partially to fully resolved by the end of the recovery period.

2.6.6.8 Other Toxicity Studies

2.6.6.8.1 Antigenicity

Not applicable.

2.6.6.8.2 Immunotoxicity

Not applicable.

2.6.6.8.3 Mechanistic Studies

Not applicable.

2.6.6.8.4 Dependence

Not applicable.

2.6.6.8.5 Metabolites

Not applicable.

2.6.6.8.6 Impurities

As discussed in ICH M4S, the focus for biologic products is the comparability of the vaccine lots used in nonclinical and clinical testing and the relevance of these lots to commercial lots (section 3.2.P.2.2). The comparability of lots used in nonclinical and clinical studies is presented in Table 2.4.4-6. As shown below in Table 2.6.6.8.6-1, there are no novel excipients in the Drug Product.

Table 2.6.6.8.6-1 Composition of FCC vaccine

Ingredients	Quantity per dose
Strain A/(H1N1)	15 µg
Strain A/(H3N2)	15 µg
Strain B/(B)	15 µg
Phosphate buffered saline (PBS)*	up to 0.5 mL

* 1 mL PBS, pH 7.2, contains: 8 mg NaCl, 0.2 mg KCl, 0.1 mg $MgCl_26H_2O$, 1.29 mg $Na_2HPO_42H_2O$, 0.37 mg KH_2PO_4, and water for injection.

However, there are three manufacturing residuals that were identified as warranting a literature-based toxicological evaluation: cetyl trimethyl ammonium bromide (CTAB), polysorbate 80 (Tween 80), and beta-propriolactone (BPL). An extensive review of the available toxicity literature was performed. Detailed information on CTAB, polysorbate 80, and BPL, including a full listing of references for each compound, is provided in 4.2.3.7.6. The relevant findings for each residual are briefly summarized below.

Cetyl trimethyl ammonium bromide (CTAB, 4.2.3.7.6-1)

Cetyl trimethyl ammonium bromide (CTAB, CAS No. 57-09-0) is approved for use as an inactive ingredient in drug products in Canada and in topical therapeutic products at levels of up to 0.2% in the United States (Health Canada, 1996; FDA, 2003). No specific European limits were identified in literature searches performed.

The intravenous LD_{50} values in rats and mice were 44 and 32 mg/kg, respectively. Other parenteral (intraperitoneal and subcutaneous) LD_{50} values range from 100 to 125 mg/kg in rodents, guinea pigs, and rabbits. The oral LD_{50} value in rats was 410 mg/kg. CTAB, CTAC, and STAC are considered to be dermal and ocular irritants at high concentrations.

CTAB was negative in an Ames Assay with and without metabolic activation, not mutagenic. There was evidence of embryotoxicity and teratogenicity in rodents following oral and intraperitoneal administration of CTAB, respectively. A developmental NOAEL of 50 mg/kg/day was determined following oral administration and a developmental LOAEL of 10.5 mg/kg was identified following intraperitoneal administration.

Based on the assay limits of detection for CTAB (section 3.2.S.4.3), the highest theoretical amount of CTAB per dose of FCC vaccine is 54 μg/dose. CTAB is nontoxic at this level. With the exception of a rare allergic response (that could be expected at a very low incidence based on the use of CTAB in cosmetics and topical drugs), there are no safety concerns anticipated with the theoretical content of ≤54 μg contained in an annual dose of the FCC vaccine.

Polysorbate 80 (Tween 80, 4.2.3.7.6-2)

Polysorbate 80 (CAS No. 9005-65-6) is one of the ethoxylated sorbitan and sorbitol esters of fatty acids that function as general purpose, hydrophilic, nonionic surfactants in cosmetic and pharmaceutical formulations. Polysorbate 80 is present in FDA-approved intravenous and intramuscular therapeutic formulations at concentrations of up to 10 and 12%, respectively. In Europe at least two licensed products contain >1 mg/mL polysorbate 80: one oncolytic drug containing a final concentration of 0.8 – 1.6 mg/mL in an intravenous therapeutic formulation; and Chiron's MF59-adjuvanted influenza vaccine, Fluad, which contains 2.35 mg/mL polysorbate 80.

Acute toxicity studies have shown Polysorbate 80 to be of low acute oral and parenteral toxicity. The minimum lethal dose following intravenous administration of Polysorbate 80 to rats and dogs was 1,000 and 500 mg/kg, respectively. Intravenous administration of Polysorbate 80 has been shown to result in the release of histamine in the dog, but not in other laboratory animals or in man.

Polysorbate 80 was not genotoxic when tested in bacteria, Chinese hamster ovary cells, hamster lung fibroblasts, rat bone marrow, in silk worms, or in the mouse micronucleus tests.

There was no evidence of developmental toxicity with Polysorbate 80 CD-1 mice, Sprague-Dawley rats, or Japanese white rabbits.

Polysorbate 80, in a quantity of 150 mg, was determined to be mild eye irritant in the rabbit.

Polysorbate 80 was administered orally for 14-days and 13-weeks to $B_6C_3F_1$ mice and Fisher 344/N rats. In these studies, animals received up to 50,000 ppm Polysorbate 80 in their diet [approximately 7,500 and 2,500 mg/kg body weight/day for mice and rats, respectively. In mice, there were no significant changes compared to controls. With the exception of lower body weight gain in male rats administered 50,000 ppm Polysorbate 80 for 14 days, there were no other adverse. No adverse effects were observed in a 17-month oral toxicity study in which 2 monkeys were administered 1 g Polysorbate 80/day.

Two-year bioassays have been conducted with Polysorbate 80 in $B_6C_3F_1$ mice and Fisher 344/N rats. The NOAEL was 25,000 ppm in female mice (3,750 mg/kg/day). In rats, based on the decreased survival rate in female rats and a marginal increase in the incidence of adrenal medulla pheochromocytoma in male rats at higher doses, the NOAEL was 25,000 ppm (1,250 mg/kg/day).

Based on the assay limits of detection for Polysorbate 80 (section 3.2.S.4.3) the highest theoretical amount per dose of FCC vaccine is 1125 μg/dose. Polysorbate 80 is considered nontoxic at this level; therefore, there are no safety concerns anticipated with the theoretical content of ≤1125 μg contained in a dose of the FCC vaccine.

Beta-Propiolactone (BPL, 4.2.3.7.6-3)

BPL (CASRN: 57-57-8) is used as a chemical intermediate in organic synthesis and has been used in sterilization of blood plasma, vaccines, tissue grafts, surgical instruments, enzymes, water, milk, and nutrient broth. In Chiron's FCC vaccine, BPL is used in the manufacturing process to inactivate virus/cellular macromolecules. The mechanism of inactivation is via covalent interaction with RNA, DNA, and proteins, and these macromolecular interactions are also the basis for BPL toxicity.

BPL readily hydrolyzes to 3-hydroxypropionic acid. This product of BPL hydrolysis is inactive and of low toxicity. In an aqueous formulation, such as the FCC vaccine, hydrolysis of theoretical residual levels of BPL to inactive 3-hydroxypropionic acid would occur.

Based on the assay limits of detection for BPL (section 3.2.S.4.3), the highest theoretical amount per dose of FCC vaccine is 0.5 µg/dose. Unreacted BPL is a potent mutagen and carcinogen, however, under the conditions of use in manufacturing, and given the very high reactivity/hydrolysis in aqueous solutions, no unreacted BPL is present in the final product that is administered to humans. The hydrolysis product of BPL is considered nontoxic. Therefore the BPL used during vaccine manufacturing is concluded not to be a risk for general toxicity, genotoxicity, or carcinogenicity

Theoretical risk of exposure to manufacturing residuals

Based on the toxicology information presented above, it is considered that the amounts of CTAB, Polysorbate 80, and BPL contained in a single annual dose of FCC vaccine pose no risk to the human populations that would receive the product (Table 2.4.4-8).

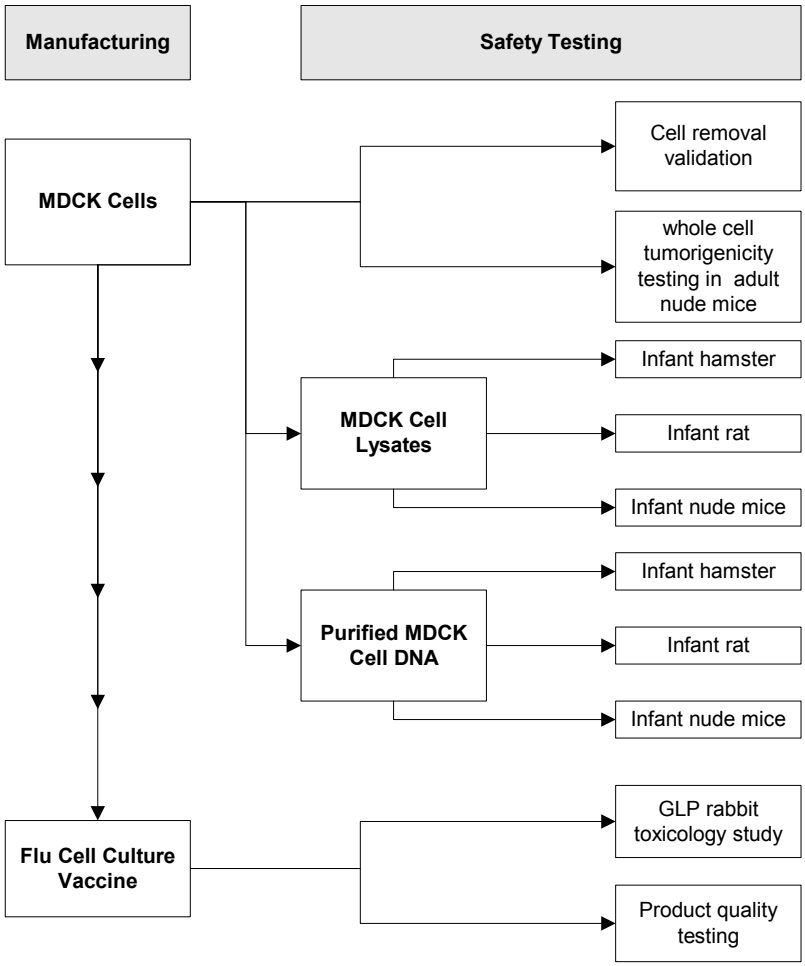
In the GLP toxicology study, rabbits received two clinical doses within a one-week period. Based on body weights, these doses to rabbits (~3 kg) represented approximately a 4× to 20× multiple of a single dose to a child (12 kg) or an adult (60 kg). The safety or tolerability of any intentional or unintentional residuals would, therefore, have been tested. The FCC vaccine was very well tolerated in rabbits, both locally at the injection sites and systemically. The clinical data support these conclusions.

2.6.6.8.7 Other Studies

Tumorigenicity/oncogenicity studies

Consistent with current international guidelines for the qualification of a new cell line as a platform for vaccine production, Chiron performed studies with intact MDCK cells, lysates of MDCK cells, and purified DNA obtained from MDCK cells. The program of animal studies is summarized in Figure 2.6.6.8.7.1-1 and study summaries are provided in section 2.6.6.8.1.

Figure 2.6.6.8.7.1-1 Synopsis of product quality-related safety studies



These studies were designed to characterize the tumorigenic or oncogenic potential of materials from the manufacturing process at progressive stages: intact cells, lysates prepared from these cells, and purified DNA from influenza virus infected and uninfected MDCK cells. Very sensitive species were selected to optimize the detection of possibly very rare events leading to tumorigenicity or oncogenicity: adult nude mice (a well-known immunocompromised model for investigating tumorigenicity of various cell lines) and infant animals (<4 days old) of three rodent species (nude mice, rats, and hamsters) with immature immune systems at the time of dosing. Each study had a 150-day duration and was designed to allow time for any potential adverse effects to emerge.

Only intact MDCK cells were tumorigenic. MDCK cell lysates and purified DNA were not oncogenic in three species of infant rodents.

Two tumorigenicity studies in adult nude mice were conducted with related MDCK cells (adapted to serum-free but not protein free growth) early in the development process. These supportive studies are described in detail in section 2.6.6.8.7.4. In the first study (Study No. B96YG21.001), MDCK cells were tumorigenic when injected subcutaneously into female nude mice. In the second study, (Study No. B012888/02) intact (unlysed) MDCK cells were tumorigenic and metastatic in the infant rat anti-thymocyte model, whereas lysed or BPL-treated MDCK cells were not. Because no MDCK cell titration was performed in these studies, and lysates were assessed minimally and DNA not at all, the program described below was initiated.

The recent program of tumorigenicity and oncogenicity studies was performed with the serum-free and protein-free growth adapted MDCK cells currently used for vaccine production. The study designs reflect international guidance concerning cell characterization, with the exception that the study duration was increased from the standard 120 days to 150 days. The program (summarized in Table 2.6.6.8.7-1 and Table 2.6.7.17.7) consisted of seven studies that were performed by [redacted] formerly [redacted]).

Table 2.6.6.8.7-1 Program of tumorigenicity and oncogenicity studies

Study Number	Age/Species	Test Article(s) [a]	Control Article(s)
48329	Adult nude mice	1×10^{1} - 1×10^{7} MDCK cells	1×10^{7} MRC-5 (negative) 1×10^{7} HeLa cells (positive)
48330	Infant nude mice	MDCK cell lysates [b]	Tris/BPL buffer
48332	Infant rats	MDCK cell lysates [b]	Tris/BPL buffer
48331	Infant hamsters	MDCK cell lysates [b]	Tris/BPL buffer
48333	Infant nude mice	MDCK DNA [c]	Murine DNA
48335	Infant rats	MDCK DNA [c]	Murine DNA
48334	Infant hamsters	MDCK DNA [c]	Murine DNA

[a] End of production cells were used to prepare all test articles in this program of studies
[b] Test articles were clarified MDCK cell lysate and BPL-treated MDCK cell lysate
[c] Test articles were MDCK cell DNA, influenza-infected MDCK cell DNA and BPL-treated influenza-infected MDCK cell DNA

The individual studies are described below.

2.6.6.8.7.1 Tumorigenicity of intact MDCK cells

Custom *in vivo* tumorigenicity assay of the sponsor's MDCK cells using adult nude mice (Table 2.6.7.17.7, Study No. 48329)

The purpose of this study was to evaluate the tumorigenic potential of MDCK cells injected subcutaneously in adult nude mice. Mice (13/sex/group) received MRC-5, HeLa, or MDCK cells as a single subcutaneous injection of 0.2 mL to the flank of the right hindlimb on day 0. Animals were approximately 4 weeks old at the time of dosing. The in-life portion of the study was 150-151 days in duration.

Table 2.6.6.8.7.1-1 Study design 48329 - Intact MDCK cells in adult nude mice

Group	Treatment [a] (0.2 mL subcutaneous injection)	M/F [b] dosed
A (negative control)	1×10^7 MRC-5 cells (5×10^7 cells/mL)	13/13
B	1×10^1 MDCK cells (5×10^1 cells/mL)	12/14[c]
C	1×10^3 MDCK cells (5×10^3 cells/mL)	13/13
D	1×10^5 MDCK cells (5×10^5 cells/mL)	13/13
E	1×10^7 MDCK cells (5×10^7 cells/mL)	13/13
F (positive control)	1×10^7 HeLa cells (5×10^7 cells/mL)	13/13

[a] Necropsy performed on day 150 (groups A, B and C) or day 151 (groups D, E and F)
[b] M – males; F – females
[c] One animal counted as male was female – error noted day 91

MDCK cells for injection were prepared from Chiron's population doubling level (PDL) of ~35 as follows: Cells were thawed, pooled and placed into culture for 8 days using standard cell culture SOPs. The PDL after this culture period represents the end-of-production PDL (~40). MRC-5 and HeLa cells were also propagated in culture. Cells were centrifuged and resuspended and/or diluted to a final volume of 6 mL of fresh media containing from 5×10^1-5×10^7 viable cells per mL for MDCK cells and 5×10^7 viable cells per mL for MRC-5 and HeLa cells. The injection volume was 0.2 mL per animal containing the appropriate number of cells.

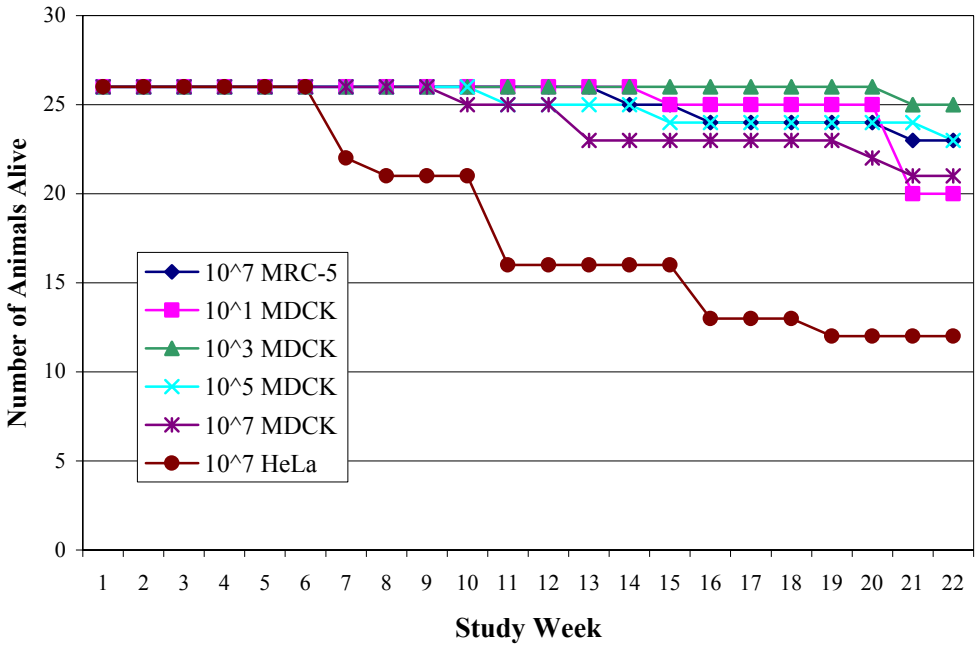
The study parameters evaluated included clinical signs, palpation of injection sites, measurements of nodules, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated/measured weekly to monitor the presence and size of nodules. HeLa cell-treated animals were euthanized when injection site lesions reached approximately 100 mm^2. Macroscopic evaluation of all animals included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the site of inoculation, lungs and any lesions were examined microscopically in all MDCK-treated animals and in 2/sex in the positive and negative control groups.

Results

Mortality

The mortality/euthanasia data for are summarized in Figure 2.6.6.8.7.1-2. Three MRC-5-treated mice died prior to the scheduled necropsy on day 150. Most (77 to 96%) MDCK-treated animals survived to the scheduled necropsy. Euthanasia of HeLa-treated animals (due to lesion size) began on day 48 and 7 males (54%) and 5 females (38%) survived to day 151.

Figure 2.6.6.8.7.1-2 Adult nude mouse mortality



Clinical Observations (in-life) and Postmortem Macroscopic Correlates

Data are summarized in Table 2.6.6.8.7.1-2 and Table 2.6.6.8.7.1-3. There were no treatment-related findings in any negative control animals injected with 1×10^7 MRC-5 cells. In the group injected with 1×10^7 HeLa cells, with the exception of animals F10 and M20, all animals (24 of 26) had palpable/measurable tumors at the site of injection, regardless of the date of death. There were no fully regressed HeLa nodules in this study. As per protocol, based on these criteria, the study was valid (no negative control animals with tumors; >90% of positive control animals with tumors in-life).

No nodules at the site of injection were palpable/measurable in animals receiving MRC-5 cells or 1×10^1 MDCK cells. At 1×10^3 cells, one animal (F1) had a palpable/measurable

nodule at the site of injection from days 80-143 but had no macroscopic observation of a nodule at necropsy; this animal did have an in-life observation of 'distorted hindlimb' and had the postmortem observation of a flat nodular mass. In the in-life study records, the flat nodular masses are described as 'tumors'. A second animal in this group had no in-life findings but an abscess was noted at the site of injection at necropsy; there were no histological findings at the injection site in this animal. At 1×10^5 cells, nodules were palpable/measurable beginning on day 73. With 1×10^7 MDCK cells or 1×10^7 HeLa cells, nodules were first seen on day 38. MDCK cell nodules were regressive in a few animals, relatively constant in size once established in some animals, or progressed in size in some animals.

A few animals had injection site nodules at necropsy that were not detected by palpation (or measured). Similarly, a few animals had palpable/measurable injection site nodules in-life that were not apparent at necropsy. Although the reason for these apparent inconsistencies is not clear in all cases, the interpretation of the study is not impacted.

In addition to the palpable/measurable nodules at the injection site, clinical observations of twisted or distorted/swollen limbs were reported for a few animals in each of the four MDCK-treated groups. There were no other notable treatment-related signs.

The clinical observations of distorted hindlimb correlated, in most animals, with macroscopic postmortem observations of a flat nodular mass in the region of the injection site on the flank. The incidence and correlations are shown below.

Table 2.6.6.8.7.1-2 Correlation of clinical signs (distorted hindlimb) with macroscopic observations (flat nodular mass) in MDCK-treated mice (N=26 per group)

Number of MDCK cells injected	Animal number and sex	Initial clinical observation [a]	Mortality [b]	Flat nodular mass
1×10^1	F1 or F6 F6 or F1 M22	Day 88 none Day 88	Day 145 Found dead Day 145 Found dead Day 144 Culled	yes yes yes
1×10^3	F1 F3 M16	Day 88 Day 88 Day 136	Day 145 Found dead Day 150 Scheduled necropsy Day 150 Scheduled necropsy	- [c] yes yes
1×10^5	F10 M16 M17	Day 88 none Day 146	Day 151 Found dead Day 151 Scheduled necropsy Day 151 Scheduled necropsy	yes yes yes
1×10^7	F12 M16 M24 M25	Day 140 none none none	Day 140 Culled Day 151 Scheduled necropsy Day 151 Scheduled necropsy Day 151 Scheduled necropsy	yes yes yes yes

[a] Twisted and/or distorted limb was the clinical observation for all except M17 (1×10^5 MDCK cells) where 'swollen body' was the only in-life observation.
[b] No cause of death was determined
[c] No tissues were collected from this animal because of autolysis of tissues

Table 2.6.6.8.7.1-3 Macroscopic postmortem incidence of nodules and flat nodular masses in MDCK-treated mice

Number of MDCK cells injected	Number of animals evaluated	Macroscopic lesions			Total lesions
		Animals with injection site nodules	Animals with flat nodular masses	Animals with both observations	
1×10^1	24	0	3	not applicable	3
1×10^3	25	0 [a]	2 (1) [b]	not applicable	3
1×10^5	24	12	3	0	15
1×10^7	24	13	4	2	17

[a] An injection site abscess was noted in 1 animal, however there was no in-life or microscopic correlate.
[b] Animal F1was found dead and tissues were autolyzed; this animal had in-life observations of a distorted hindlimb beginning on day 88 so is presumed to have had a lesion.

As per protocol, the species of origin of selected lesions (flat nodular masses) was indicated. Initially, immunohistochemistry was performed, but subsequently invalidated, because the primary antibody selected for the determination of canine origin was not of

appropriate specificity. Therefore, species of origin was determined using quantitative polymerase chain reaction (qPCR) under a separate protocol ([redacted] Study No. 1053629.800210). The PCR analysis is considered the definitive proof of species of origin (see section on PCR following histopathology).

Macroscopic and Microscopic Correlations

Nodules and flat nodular masses were examined microscopically for the presence of neoplastic cells. As shown in Table 2.6.6.8.7.1-4, in most cases, nodules were confirmed to consist of neoplastic cells but in some cases, consisted of non-neoplastic tissues. In 3 animals there was no macroscopic correlate to the microscopic finding of neoplastic cells.

Table 2.6.6.8.7.1-4 Correlation between macroscopic postmortem hindlimb observation and microscopic observations of neoplastic cells in hindlimb tissues

Group	Animals examined (N)	Mice w/ flat nodular masses or nodules	Microscopic correlates: Incidence (finding)
10^7 MRC-5	4	None	None
10^1 MDCK	24	3	3 (neoplastic cells)
10^3 MDCK	25	2	2 (neoplastic cells)
10^5 MDCK	24	14	9 (neoplastic cells) 2 (no significant findings) 1 (normal mammary gland) 1 (reactive lymph node) 1 (myositis) 1 (neoplastic cells without corresponding macroscopic post mortem observation)
10^7 MDCK	24	15	9 (neoplastic cells) 3 (no significant findings) 1 (normal mammary gland) 1 (abscess) 1 (no tissues examined - no block) 2 (neoplastic cells without corresponding macroscopic post mortem observations)
10^7 HeLa [a]	4	4	4 (neoplastic cells)

[a] 2 animals per sex were evaluated microscopically; total with macroscopic tumors was 24/26

Other protocol-specified tissues (muscle at site of injection, lung, other tissues with macroscopic lesions) were examined microscopically for the presence of neoplastic cells. Missing tissues were identified for 7 animals as shown below.

Table 2.6.6.8.7.1-5 List of missing tissues

Group	Animal	Missing tissue
10^1 MDCK	7F 25M	muscle muscle
10^3 MDCK	3F 16M 28M	lung lung lung
10^5 MDCK	20M	muscle, lung
10^7 MDCK	22M	muscle, lung

These missing tissues are not considered to have impacted the interpretation of the study overall because sufficient evidence of tumorigenicity and metastasis was collected.

As shown in Table 2.6.6.8.7.1-6, a subset of animals with neoplastic cells in the hindlimb also had metastases/invasion to other tissues. With the exception of 6F in the group given 1×10^5 MDCK, metastases/invasion was seen in animals with the macroscopic observation of a flat nodular mass.

Table 2.6.6.8.7.1-6 Histopathological correlation of site of injection lesions with metastases

Group	Animals examined (N)	Neoplastic cells at SOI/tissues in hindlimb [a]	Neoplastic cells in tissues other than hindlimb
		Animal number and sex	Animal number and sex (affected tissues)
10^7 MRC-5	4	None	None
10^1 MDCK	24	1F, 6F, 22M	22M (kidney)
10^3 MDCK	25	3F, 16M	16M (kidney)
10^5 MDCK	24	1F, 3F, 5F, 6F, 10F, 16M, 17M, 19M, 25M, 27M	6F (lung) 10F (not determined - autolysis) 17M (testes, lymph node, lung, kidney, rectal wall, pelvic tissues)
10^7 MDCK	24	3F, 5F, 6F, 9F, 12F, 16M, 18M, 20M, 21M, 24M, 25M	12F (lung, lymph node, kidney) 16M (lymph node)
10^7 HeLa	4	5F, 13F, 19M, 23M	None

[a] SOI: site of inoculation

The microscopic description of hindlimb lesions (nodules or flat nodular masses) was similar and consistent with the known morphology of MDCK cells injected into nude mice: epithelial cells forming tubular structures. This conclusion is supported by the PCR data on selected flat nodular masses, which confirmed MDCK cell origin (see below).

Study No. 1053629.800210: Quantitative PCR (qPCR) for species of origin

The qPCR determination of species of origin was performed on a cryopreserved segment of the flat nodular mass removed from one animal per MDCK cell-treated group in Study 48329. The final report for this study is included as an appendix of Study Report 48329. Validated methods were used as described in [redacted] Draft Validation Report 33019.02 - Detection and qualification of contaminating DNA in biological samples (report provided upon request). Canine DNA was detected using primers directed at the canine SINE repeat (conserved regions of the *C. familiaris* short interspersed nuclear element) as the target region. Detection of murine DNA relied on primers directed at the murine B2 repeat as the target region. The appropriate control reactions were included in the experiment and are described in Table 2.6.6.8.7.1-7.

Table 2.6.6.8.7.1-7 Summary of PCR assay controls

Controls	Description
Assay target positive	mouse: murine B2 repeat DNA
	MDCK: canine SINE repeat DNA
Post-extraction spike	triplicates containing test article nucleic acid spiked with 10^2 and 10^3 copies of MDCK DNA (included to monitor possible test sample inhibition)
Specificity	minimum of 3 concentrations of DNA extracted from negative control murine tissue (for MDCK PCR assay)
	minimum of 3 concentrations of DNA extracted from MDCK cells (for murine PCR assay)
MDCK calibration	100 ng per reaction of murine negative control DNA spiked with 10^2 or 10^3 copies of MDCK DNA
Exogenous internal positive	TaqMan® control reagents (to confirm that negative results were truly negative and not due to failed amplification)
No template	triplicates consisting of PCR reaction mix only
Negative	triplicates consisting of the PCR reaction mix with negative control – DNA from muscle of an untreated mouse
Sentinel extraction	triplicates consisting of the PCR reaction mix in sentinel extraction tubes

For the test samples, PCR was performed on DNA extracted from untreated murine muscle (negative control), DNA extracted from MDCK stock cells passaged for use in the titration study (positive control), and DNA extracted from a flat nodular mass harvested from one animal in each MDCK cell-treated group as described in Table 2.6.6.8.7.1-8.

Table 2.6.6.8.7.1-8 Selection of samples for qPCR analysis

48329 Study Group	Number of MDCK cells injected	Mice [a] with flat nodular masses	Selected for qPCR analysis
B	1×10^1	F1, F6, M22	M22
C	1×10^3	F1, F3, M16	M16
D	1×10^5	F10, M16, M17	M16
E	1×10^7	F12, M16, M24, M25	F12

[a] F – female; M – male. Animals were numbered sequentially in each dose group; females 1-13 and males 16-28.

The results of the qPCR analysis are summarized in Table 2.6.6.8.7.1-9:

Table 2.6.6.8.7.1-9 Species of origin PCR experimental design and results

Samples Tested [animal number and sex, dose group]	Amount of DNA tested per replicate	PCR result using B2 repeat (murine) primer	PCR result using MDCK primer
		Quantity (pg/reaction ± SD)	Quantity (copies/reaction ± SD)
Sentinel Extraction	-	4.26 ± 0.78 [a]	7.28 ± 2.74 [a]
Negative tissue control (mouse muscle DNA)	100.43 ng	$2.71 \times 10^4 \pm 3.64 \times 10^4$ [b]	24.36 ± 12.55 [a]
Sample 22B (TA 4) [M22, 1×10^1 MDCK]	100.43 ng	116.98 ± 8.38	$1.51 \times 10^6 \pm 7.97 \times 10^4$
Sample 16C (TA 3) [M16, 1×10^3 MDCK]	70.50 ng	4.22 ± 0.60 [a]	$2.17 \times 10^4 \pm 4.90 \times 10^3$
Sample 16D (TA 1) [M16, 1×10^5 MDCK]	100.46 ng	4.39 ± 0.31 [a]	$1.02 \times 10^5 \pm 3.83 \times 10^4$
Sample 12E (TA 2) [F12, 1×10^7 MDCK]	100.28 ng	25.73 ± 3.37	$9.00 \times 10^4 \pm 1.68 \times 10^4$
MDCK DNA positive control	5 pg	0.41 ± 0.72 [a]	$2.18 \times 10^4 \pm 4.84 \times 10^3$

[a] Signals detected were equivalent to the assay background

[b] For the B2 Repeat assay the quantity of target present in the negative tissue control fell outside the upper range of the standard curve (1 ng/reaction) and therefore the quantity of target detected within this reaction cannot be measured with accuracy.

The result for each test sample for each PCR target (B2 Repeat or MDCK) was compared to the result obtained for the negative tissue control (murine) and/or the MDCK DNA (5 pg) positive control (Table 2.6.6.8.7.1-9). The data from the B2 Repeat assay (murine) demonstrated a decrease in quantity of murine DNA in the test samples when compared to the negative tissue control (murine).

The data from the MDCK assay demonstrated an increase in the number of target copies detected in the test samples when compared to the negative tissue control (murine) and detected MDCK target sequences comparable with or greater than those observed in the MDCK DNA (5 pg) positive control. The results indicate that each test sample assessed was predominantly of MDCK origin. The presence of a low murine signal is consistent with intratumoral murine endothelial and stromal elements that are known to be present in tumor xenografts in nude mice and/or mouse tissue that was not completely removed at the time of dissection. Therefore, the flat nodular masses seen in the region of the injection site in mice treated with MDCK cells were of MDCK cell origin.

The tumorigenicity results in this current study are consistent with previous findings with Chiron's MDCK cells in which tumorigenicity and metastasis were seen at 1×10^7 cells. However, the earlier studies did not examine the tumorigenicity of lower MDCK cell numbers (1×10^1, 1×10^3, 1×10^5) in adult nude mice. The current end-of-production MDCK cells were tumorigenic in 3 of 24, 2 of 25, 10 of 24, and 11 of 24 animals at 1×10^1, 1×10^3, 1×10^5 and 1×10^7, respectively (based on histopathology).

The intended site of injection, per protocol, was subcutaneous into the hindlimb. Both nodules (at 1×10^5 and 1×10^7) and flat nodular masses (1×10^1, 1×10^3, 1×10^5 and 1×10^7) were seen in injected hindlimbs and had comparable microscopic morphology. The anatomical location of the flat nodular masses extended, in various animals, from the thigh to the spine. If flat nodular masses were outgrowths from nodules, the location could be related to variability in injections, to the lymphatic anatomy in the mouse hindlimb in the region of the injection site, subcutaneous spreading of the injected cells, or other unknown factors, including variability in individual nude mice.

Host-dependent factors could include the growth factor milieu, extent of angiogenesis, inflammatory responses to the injected cells (e.g. cytokines, natural killer cells) in individual animals. The literature indicates that MDCK cells can exhibit very different phenotypes depending upon stimulation with various growth factors.

The ability to establish a tumor in nude mice can be a threshold phenomenon, and a clear dose-response may not always be seen. In this study, a dose-response relationship, based on the number of animals (~3 per group) developing flat nodular masses at the injection site was not established. However, based on the date of onset of nodule formation and the incidence of hindlimb nodules and flat nodular masses per group, a dose-response was evident comparing animals injected with low versus high cell numbers. Tumorigenicity at lower cell numbers is consistent with the behavior of cells grown in suspension, and is

not unexpected, based on the fact that these cells are less susceptible to anoikis, which is defined as apoptosis of cells that have lost contact with extracellular matrix.

Although tumorigenic at low cell numbers, MDCK cells are completely removed during the manufacturing process and, therefore, whole cells do not pose a risk in the final product.

2.6.6.8.7.2 Oncogenicity studies with MDCK cell lysates

These studies assessed the potential oncogenicity of cell-free MDCK cell components such as chromatin, host cell proteins, etc. Indirectly, these studies provide evidence that MDCK cells do not contain tumorigenic agents such as polyomavirus or herpesvirus.

The test and control articles used in the infant nude mouse, rat and hamster studies were prepared at Chiron Vaccines (Marburg). The preparation of the test and control articles was well documented and controlled while applying the principle rules of cGMP. End of production (EoP) MDCK cells were used to prepare both cell-free and BPL-treated cell lysates. EoP cells are at the limit of the *in vitro* age used for production and have a population doubling level (PDL) of approximately 40 from the working cell bank (50 from the master cell bank).

Test and control articles

MDCK cell-free lysate

EoP cells were subjected to 5 freeze / 4 thaw cycles. Tubes containing ~5×10^7 lysed cells/mL in cell culture media were frozen and shipped to BioReliance Stirling. At Stirling the lysate was thawed for the fifth time and centrifuged at 1600 rpm (500 g) for 15 minutes to remove any remaining whole cells. The supernatant was transferred to a fresh tube and frozen for transport to the study sites.

BPL-treated MDCK cell lysate

EoP cells were suspended in 20 mmol/l Tris buffer. Cells were lysed by addition of BPL (1:2000; 0.05% v/v) with incubation for at least 18 hours at 5-8°C. The lysed cells were incubated at 37°C for at least 2 hours to convert residual BPL to nontoxic beta-hydroxypropionic acid (3-hydroxypropionic acid). The lysate (~5×10^7 lysed cells/mL) was frozen and shipped to the study sites, where it was thawed and injected into animals.

BPL/Tris (negative control)

BPL was added to 20 mmol/l Tris buffer (1:2000; 0.05% v/v) and incubated at 37°C for at least 2 hours, sterile filtered (0.2µ), frozen and shipped to the study sites, where it was thawed and injected into animals.

The test and control articles used in the studies with MDCK cell lysates are summarized below in Table 2.6.6.8.7.2-1.

Table 2.6.6.8.7.2-1 Test and control articles used in cell lysate studies

Lot Number	Test Article	Test Article Information
Negative control	BPL/Tris	BPL/Tris (1:2000). Hydrolyzed for >2 hours at 37°C. Sterile filtered (0.2µ) Tris buffer: pH 8.0, 20 mmol/l
0[redacted]0-b	MDCK cell-free lysate	Cells lysed using at least 5 freeze/thaw cycles and concentrated in cell culture medium. Endotoxin: 0.168 EU/mL (Specification: <1 EU/mL)
0[redacted]0-c	BPL-treated MDCK cells	Cells lysed using BPL (1:2000 dilution; 18 hr at 5±3°C, hydrolysis of BPL for 2h at 37°C) and concentrated in Tris buffer. Tris buffer: pH 8.0, 20 mmol/l Endotoxin: 0.0858 EU/mL (Specification: <1 EU/mL) Residual BPL: 0.3 ppm (Specification: <1 ppm)

The volumes of cell lysate administered to the animals (0.2 mL to infant rats and hamsters and 0.1 mL to infant nude mice due to their small size) were the equivalent of 1×10^7 and 5×10^6 cells, respectively. 1×10^7 cells are considered to be the average number of cells needed to produce a single clinical dose of trivalent vaccine (lower limit is 9×10^6 and upper limit is 4×10^7 cells).

Custom *in vivo* oncogenicity assay of the sponsor's MDCK cell lysate and BPL treated MDCK cell lysate using new born nude mice (Table 2.6.7.17.7, Study No. 48330)

The purpose of this study was to evaluate the oncogenic potential of MDCK cell lysates injected subcutaneously in infant nude mice. Lysates were prepared from untreated/uninfected MDCK cells and BPL-treated MDCK cells. Control animals received Tris buffer containing BPL. Mice (at least 15/sex/group) received MDCK cell lysate, BPL-treated MDCK cell lysate, or control article as a single subcutaneous injection of 0.1 mL to the right flank on day 0. Animals were 1-3 days old at the time of dosing. The study duration was 150 days.

Table 2.6.6.8.7.2-2 Study design 48330 - cell lysates in infant nude mice

Group	Treatment) [a] (subcutaneous injection of 0.1 mL)	M/F (Intended)	Total Number Dosed	M/F (Day 150)	Observations
A	BPL-Tris buffer	15/15	31	5/9	Mortality seen primarily during weeks 1 and 2 Stable from ~day 14
B	MDCK cell lysate in media	15/15	33	7/4	
C	BPL-treated MDCK cell lysate in Tris buffer	15/15	30	5/7	

[a] Necropsy: day 150

Due to the large number of infant nude mice required for this study, dams were bred, delivered, and litters culled and housed at [redacted] the supplier for [redacted] nude mice. Staff from [redacted] inoculated the animals at [redacted]. On day 52 the dosed mice were shipped to BioReliance for the remainder of the study and for necropsy and histopathology.

Evaluations included clinical signs, palpation of injection sites, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated weekly to monitor the presence and size of nodules. Macroscopic evaluation of all animals included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the site of inoculation, lungs and any lesions from all test-article treated animals and one animal per sex from the control group were examined microscopically.

Mortality in the first two weeks of this study was high in all groups, including animals injected with the control article (BPL-Tris buffer, see Table 2.6.6.8.7.2-2). Pups were cannibalized by the dams and could not be examined. Since similar mortality was seen in Study 48333 where the control article was murine DNA in phosphate buffered saline, the likely explanation for the mortality is due not to the injection materials, but rather to the fragility of these very young animals when subjected to numerous interventions, including disturbing the nest, culling 'hairy' pups, sexing the pups and injecting a large volume of foreign material. The mice were Balb C, a strain considered to be among the most cannibalistic, a tendency which may also have contributed to the high incidence of mortality.

There were no clinical signs observed in any group. Animals were palpated weekly to check for signs of nodule or tumor formation at the site of injection. There were no injection site observations in any group.

On day 150, all surviving animals were euthanized and examined macroscopically. There were no notable findings at the injection sites, or in brain, kidneys, liver, spleen, lungs, or

lymph nodes in any group. The muscle at the site of injection and lungs were collected for microscopic examination from one animal per sex in the control group and all test article-treated animals. No evidence of tumor formation was seen in any group.

Under the conditions of the study, a single subcutaneous administration of 0.1 mL of MDCK cell lysate or BPL-treated MDCK cell lysate followed by a 150-day observation period was not oncogenic in infant nude mice.

Custom *in vivo* oncogenicity assay of the sponsor's MDCK cell lysate and BPL treated MDCK cell lysate using new born rats less than four days old (Table 2.6.7.17.7, Study No. 48332)

The purpose of this study was to evaluate the oncogenic potential of MDCK cell lysates administered via subcutaneous injection to infant rats. Lysates were prepared from untreated/uninfected MDCK cells and BPL-treated MDCK cells. Control animals received Tris buffer containing BPL. Rats received MDCK cell lysate (15/sex/group), BPL-treated MDCK cell lysate (14/sex/group), or control article (15/sex/group) as a single subcutaneous injection of 0.2 mL to the right flank on day 0. Animals were 1-3 days old at the time of dosing. The study duration was 151-152 days.

Table 2.6.6.8.7.2-3 Study design 48332 - cell lysates in infant rats

Group	Treatment (subcutaneous injection of 0.2 mL)	M/F (Intended) [b]	Total Number Dosed	M/F Day 151/152 [a]
A	BPL-Tris buffer	15/15	30	13/16
B	MDCK cell lysate in media	15/15	30	16/14
C	BPL-treated MDCK cell lysate in Tris buffer	14/14	28	12/16

[a] Necropsy: day 151 (control group) and day 152 (test article-treated groups)
[b] On day 26 animals were weaned and sex confirmed, see last column for actual numbers dosed.

Evaluations included clinical signs, palpation of injection sites, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated weekly to monitor the presence and size of nodules. Macroscopic evaluation included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the site of inoculation, lungs and any lesions were examined microscopically.

One male from the control group died on day 1. There were no clinical signs prior to death, and the body was cannibalized so could not be examined. All other animals survived to the scheduled necropsy.

On day 26 animals were weaned and sex confirmed. The intended and actual numbers of males and females dosed is shown above in Table 2.6.6.8.7.2-3.

There were no clinical signs observed in any group. Animals were palpated weekly to check for signs of nodule or tumor formation at the site of injection. There were no injection site observations in any group.

On day 151 (control animals) and day 152 (MDCK cell lysate and BPL-treated MDCK cell lysate treated animals) all animals were euthanized and examined macroscopically. There were no notable findings at the injection sites, or in brain, kidneys, liver, spleen, lungs, or lymph nodes in any group. The muscle at the site of injection and lungs were collected for microscopic examination from one animal per sex in the control group and all test article-treated animals. No evidence of tumor formation was seen in any group.

Under the conditions of the study, a single subcutaneous administration of 0.2 mL of MDCK cell lysate or BPL-treated MDCK cell lysate followed by a 152-day observation period was not oncogenic in infant rats.

Custom *in vivo* oncogenicity assay of the sponsor's MDCK cell lysate and BPL treated MDCK cell lysate using new born hamsters (Table 2.6.7.17.7, Study No. 48331)

The purpose of this study was to evaluate the oncogenic potential of MDCK cell lysates injected subcutaneously in infant hamsters. Lysates were prepared from untreated/uninfected MDCK cells and BPL-treated MDCK cells. Control animals received Tris buffer containing BPL. Hamsters received MDCK cell lysate (14/sex/group), BPL-treated MDCK cell lysate (15/sex/group), or control article (15/sex/roup) as a single subcutaneous injection of 0.2 mL to the right flank on day 0. Animals were 1-3 days old at the time of dosing. The study duration was 150 days.

Table 2.6.6.8.7.2-4 Study design 48331 - cell lysates in infant hamsters

Group	Treatment (subcutaneous injection of 0.2 mL)	M/F (Intended) [b]	Total Number Dosed	M/F Day 150 [a]
A	BPL-Tris buffer	15/15	30	14/14
B	MDCK cell lysate in media	14/14	28	13/15
C	BPL-treated MDCK cell lysate in Tris buffer	15/15	30	21/9

[a] Necropsy: day 150
[b] On day 29 animals were weaned and sex confirmed, see last column for actual numbers dosed.

Evaluations included clinical signs, palpation of injection sites, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated weekly to monitor the presence and size of nodules. Macroscopic evaluation of all animals included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the site of inoculation, lungs and any lesions from all test article-treated and one animal per sex from the control group were examined microscopically.

One male and one female from the control group died on days 1 and 29, respectively. No clinical signs were observed prior to death, and the bodies were cannibalized so could not be examined. All other animals survived to the scheduled necropsy.

On day 29 animals were weaned and sex confirmed. The intended and actual numbers of males and females dosed is shown above in Table 2.6.6.8.7.2-4.

There were no clinical signs observed in any group. Animals were palpated weekly to check for signs of nodule or tumor formation at the site of injection. There were no injection site observations in any group.

On day 150, all animals were euthanized and examined macroscopically. There were no notable findings at the injection sites, or in brain, kidneys, liver, spleen, lungs, or lymph nodes in any group. The muscle at the site of injection and lungs were collected for microscopic examination from one animal per sex in the control group and all test article-treated animals. No evidence of tumor formation was seen in any group.

Under the conditions of the study, a single subcutaneous administration of 0.2 mL of MDCK cell lysate or BPL-treated MDCK cell lysate followed by a 150-day observation period was not oncogenic in infant hamsters.

In conclusion, lysates were not oncogenic in neonatal nude mice, rats, or hamsters. The amount of lysate administered to neonatal nude mice, rats and hamsters represents an extremely large exposure to cellular macromolecules or theoretical adventitious agents versus a human receiving a dose of FCC vaccine. Neonatal animal weights were in the low grams when lysates were administered. Taking the conservative approach of using a young adult body weight estimate for nude mice (25 g), rats (300 g), and hamsters (300 g) versus a most-vulnerable human subject, an infant (8 kg), mice received approximately 160 times (320×0.5) and rats and hamsters received 27 times what a human infant would receive if there were no downstream host cell protein removal/inactivation steps. In contrast, even if a seasonal vaccine required 4×10^7 cells for a trivalent dose, the downstream removal/inactivation steps provide additional safety factors of several orders of magnitude. Although the number of cells needed to produce a dose of vaccine may be up to 4 times higher than 1×10^7 cells used to prepare a lysate dose, the assessment of oncogenicity was adequately addressed.

2.6.6.8.7.3 Oncogenicity studies with DNA

These studies assessed the potential oncogenicity of MDCK cell DNA preparations and provide evidence that MDCK cell DNA does not contain oncogenic elements. In addition, flu infection of MDCK cells does not alter the oncogenic potential of the DNA.

The test and control articles used in the infant nude mouse, rat and hamster studies were prepared at Chiron Vaccines (Marburg). The preparation of the test and control articles was well documented and controlled while applying the principal rules of cGMP. End of production (EoP) MDCK cells were used to prepare DNA from MDCK cells, influenza-infected MDCK cells, influenza-infected BPL-treated MDCK cells and murine tissue. EoP cells are at the limit of the *in vitro* age used for production and have a PDL (population doubling level) of approximately 40 from the working cell bank (50 from the master cell bank).

The concentration of ~70 µg DNA per 200 µL dose is approximately equivalent to 1×10^7 MDCK cells. 1×10^7 cells are considered to be the average number of cells needed to produce a single clinical dose of trivalent vaccine (lower limit is 9×10^6 and upper limit is 4×10^7 cells).

Test and control articles

Murine DNA (negative control)

[redacted] supplied liver, spleen, heart, kidney and lung tissue from SPF (specified pathogen-free) mice for preparation of the negative control article. DNA was extracted as described above and resuspended in sterile PBS. The final concentration of ~70 µg DNA per 200 µL dose administered to hamsters and rats is equivalent to 1×10^7 MDCK cells. Mice received half of the volume (0.1 mL) due to their small size.

MDCK cell DNA

Cells were centrifuged, washed, and resuspended in Tris buffer. DNA was extracted using a Qiagen Blood & Cell Culture Maxi Kit, incubated with protease and purified using a Qiagen Genomic-tip 500/G column. DNA was precipitated, centrifuged and resuspended in sterile PBS. The final concentration of ~70 µg DNA per 200 µL dose administered to hamsters and rats is equivalent to 1×10^7 MDCK cells. Mice received half of the volume (0.1 mL) due to their small size.

Flu-infected MDCK cell DNA

EoP MDCK cells were infected with A/Panama 2007/99 virus. HA titer was measured to confirm infection. Approximately 47 hours post-infection, cells were centrifuged, washed, and resuspended in Tris buffer. The cell suspension was tested for mycoplasma. DNA was extracted as described above and resuspended in sterile PBS. The final

concentration of ~70 µg DNA per 200 µL dose administered to hamsters and rats is equivalent to 1×10^7 MDCK cells. Mice received half of the volume (0.1 mL) due to their small size.

Flu-infected BPL-treated MDCK cell DNA

EoP MDCK cells were infected with A/Panama 2007/99 virus. HA titer was measured to confirm infection. Approximately 47 hours post-infection, BPL was added (1:2000; 0.05% v/v) and cells incubated for 16 hours at 2-8ºC. Residual BPL was hydrolyzed to beta-hydroxypropionic acid (3-hydroxypropionic acid) during a second incubation for 2 hours at 37ºC. Cells were centrifuged, the pellet was collected and DNA extracted as described above. The final concentration of ~55 µg DNA per 200 µL dose administered to hamsters and rats is equivalent to 1×10^7 MDCK cells. Mice received half of the volume (0.1 mL) due to their small size. The final concentration of DNA was lower than for MDCK cell DNA or flu-infected MDCK cell DNA; this was attributed to destruction of some of the DNA (during BPL treatment) and incomplete recovery of lysed cells by centrifugation.

The test and control articles used in the studies with MDCK cell DNA are summarized in Table 2.6.6.8.7.3-1.

Table 2.6.6.8.7.3-1 Test and control articles used in DNA studies

Lot Number	Test Article	Test Article Information
Negative control	Murine DNA	DNA extracted from primary murine tissue (SPF mice) and concentrated in PBS buffer PBS buffer: pH 7.2, 230-330 mOs/kg DNA concentration: ~70 µg/200 µL
0[redacted]0-a	Untreated MDCK cell DNA	DNA extracted from native MDCK PF cell line and concentrated in PBS buffer PBS buffer: pH 7.2, 230-330 mOs/kg DNA concentration: ~70 µg/200 µL
0[redacted]0-d	Infected MDCK cell DNA	DNA extracted from flu-infected MDCK cells and concentrated in PBS buffer PBS buffer: pH 7.2, 230-330 mOs/kg DNA concentration: ~70 µg/200 µL
0[redacted]0-e	Infected BPL-treated MDCK cell DNA	DNA extracted from flu-infected BPL-treated MDCK cells and concentrated in PBS buffer PBS buffer: pH 7.2, 230-330 mOs/kg DNA concentration: ~55 µg/200 µL

Comparing the WHO limit of 10 ng of host cell DNA per vaccine dose to the 55-70 µg of DNA injected into infant rats or hamsters (0.2 mL injected) or 28-35 µg of DNA injected into infant nude mice (0.1 mL injected due to very small size), animals received high multiples of the amount of DNA that would be in a dose of vaccine.

Custom *in vivo* oncogenicity assay of the sponsor's MDCK cell DNA using new born nude mice (Table 2.6.7.17.7, Study No. 48333)

The purpose of this study was to evaluate the oncogenic potential of high molecular weight MDCK cell DNA when injected subcutaneously into infant nude mice. DNA was purified from three sources; 1) untreated uninfected MDCK cells, 2) influenza-infected untreated MDCK cells and 3) influenza-infected BPL-treated MDCK cells. Control animals received murine DNA. Mice (at least 15/sex/group) received a single 0.1 mL subcutaneous injection to the right flank on day 0. The study duration was 150 days.

Table 2.6.6.8.7.3-2 Study design 48333 - DNA in infant nude mice

Group	Treatment [a] (subcutaneous injection of 0.1 mL)	M/F (Intended)	Total Number Dosed	M/F (Day 150)	Observations
Negative control	Murine DNA in PBS	15/15	34	8/3	38 mis-phenotyped ('hairy') spread across groups Mortality seen primarily during weeks 1 and 2 Stable from ~day 14
1	Untreated MDCK cell DNA	15/15	39	0/4	
2	Flu infected MDCK cell DNA	15/15	38	5/11	
3	BPL-treated flu-infected MDCK cell DNA	15/15	47	14/15	

[a] Necropsy: day 150

Due to the large number of infant nude mice required for this study, dams were bred, delivered, and litters culled and housed at [redacted] the supplier for [redacted] nude mice. Staff from [redacted] inoculated the animals at [redacted] On day 59, the dosed mice were shipped to [redacted] for the remainder of the study and for necropsy and histopathology.

Evaluations included clinical signs, palpation of injection sites, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated weekly to monitor the presence and size of nodules. Macroscopic evaluation of all animals included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the

site of inoculation, lungs and any lesions from all test-article treated and one animal per sex from the control group were examined microscopically.

As described above for Study 48330, survival in the first two weeks of this study was also considerably lower than expected (Table 2.6.6.8.7.3-2) due to cannibalism of infants.

There were no clinical signs observed in any group. Animals were palpated weekly to check for signs of nodule or tumor formation at the site of injection. There were no injection site observations in any group.

On day 150, all animals were euthanized and examined macroscopically. There were no notable findings in brain, kidneys, liver, spleen, lungs, or lymph nodes in any group. Two females from group 2 (influenza-infected MDCK cell DNA) had small (2mm × 2mm) macroscopic lesions in the region of the injection site. These lesions were evaluated microscopically and found to be normal mammary/lymph node tissue, which in the mouse can extend from ventrum to dorsum, especially during the estrus cycle. One female (group 2) had a macroscopic lesion (1.5mm × 1.5mm) removed from the thoracic wall. This lesion was evaluated microscopically and found to be a subcutaneous abscess. There were no other macroscopic observations in any group.

The muscle at the site of injection and lungs were collected for microscopic examination from one animal per sex in the control group and all test article-treated animals. No evidence of tumor formation was seen in any group.

Under the conditions of the study, a single subcutaneous administration of 0.1 mL of DNA from untreated, uninfected MDCK cells, influenza-infected, untreated MDCK cells and influenza-infected, BPL-treated MDCK cells followed by a 150-day observation period was not oncogenic in infant nude mice.

Custom *in vivo* oncogenicity assay of the sponsor's MDCK cell DNA using new born rats (Table 2.6.7.17.7, Study No. 48335)

The purpose of this study was to evaluate the oncogenic potential of high molecular weight MDCK cell DNA when injected subcutaneously into infant rats. DNA was purified from three sources; 1) untreated uninfected MDCK cells, 2) influenza-infected untreated MDCK cells and 3) influenza-infected BPL-treated MDCK cells. Control animals received murine DNA. Rats (15/sex/group) received a single 0.2 mL subcutaneous injection to the right flank on day 0. The study duration was 152 days.

Table 2.6.6.8.7.3-3 Study design 48335 - DNA in infant rats

Group	Treatment (subcutaneous injection of 0.2 mL)	DNA (µg per 0.2 mL)	M/F (Intended) [b]	Total Number Dosed	M/F Day 152 [a]
Negative control	Murine DNA	70	15/15	30	12/16
1	Untreated MDCK cell DNA	70	15/15	30	15/14
2	Flu infected MDCK cell DNA	70	15/15	30	14/14
3	BPL-treated flu infected MDCK cell DNA	55	15/15	30	14/16

[a] Necropsy: day 152
[b] On day 27 animals were weaned and sex confirmed. See last column for actual numbers dosed.

Evaluations included clinical signs, palpation of injection sites, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated weekly to monitor the presence and size of nodules. Macroscopic evaluation of all animals included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the site of inoculation, lungs and any lesions from all test article-treated and one animal per sex from the control group were examined microscopically.

Three animals died during the study: 2 negative control group females (day 2) and 1 group 2 female (day 27). No clinical signs were observed prior to death, and the bodies were cannibalized so could not be examined. Two females were euthanized: 1 group 1 female (day 98) and 1 group 2 female (day 63). Both animals had difficulty eating due to jaw misalignment, a congenital condition. All other animals survived to the scheduled necropsy.

On day 27 animals were weaned and sex confirmed. Actual numbers of males and females dosed are shown above in Table 2.6.6.8.7.3-3.

There were no clinical signs observed in any group. Animals were palpated weekly to check for signs of nodule or tumor formation at the site of injection. There were no injection site observations in any group. On day 152 all animals were euthanized and examined macroscopically. There were no notable findings at the injection sites or in brain, kidneys, liver, spleen, lungs or lymph nodes in any group. The muscle at the site of injection and lungs were collected for microscopic examination from one animal per sex in the control group and all test article-treated animals. No evidence of tumor formation was seen in any group.

Under the conditions of the study, a single subcutaneous administration of 0.2 mL of DNA from untreated, uninfected MDCK cells, influenza-infected, untreated MDCK cells and influenza-infected, BPL-treated MDCK cells followed by a 152-day observation period was not oncogenic in infant rats.

Custom *in vivo* oncogenicity assay of the sponsor's MDCK cell DNA using new born hamsters (Table 2.6.7.17.7, Study No. 48334)

The purpose of this study was to evaluate the oncogenic potential of high molecular weight MDCK cell DNA when injected subcutaneously into infant hamsters. DNA was purified from three sources; 1) untreated uninfected MDCK cells, 2) influenza-infected untreated MDCK cells and 3) influenza-infected BPL-treated MDCK cells. Control animals received murine DNA. Hamsters (15/sex/group) received a single 0.2 mL subcutaneous injection to the right flank on day 0. The study duration was 150 days.

Table 2.6.6.8.7.3-4 Study design 48334 - DNA in infant hamsters

Group	Treatment (subcutaneous injection of 0.2 mL)	DNA (µg per 0.2 mL)	M/F (Intended) [b]	Total Number Dosed	M/F Day 150 [a]
Negative control	Murine DNA	70	15/15	30	12/16
1	Untreated MDCK cell DNA	70	15/15	30	16/14
2	Flu infected MDCK cell DNA	70	15/15	30	12/15
3	BPL-treated flu infected MDCK cell DNA	55	15/15	30	17/13

[a] Necropsy: day 150
[b] On day 29 animals were weaned and sex confirmed. See last column for actual numbers dosed.

Evaluations included clinical signs, palpation of injection sites, macroscopic examination, and microscopic evaluation of selected tissues. Animals were observed daily for mortality and clinical signs. Injection sites were palpated weekly to monitor the presence and size of nodules. Macroscopic evaluation of all animals included examination of the liver, spleen, kidneys, lungs, brain, lymph nodes and site of inoculation. Muscle from the site of inoculation, lungs and any lesions from all test article-treated animals and one animal per sex from the control group were examined microscopically.

Five animals died during the study: 2 negative control females (days 1 and 8) and 3 group 2 animals (1 male and 1 female on day 15; 1 male on day 16). No clinical signs were observed prior to death, and the bodies were cannibalized so could not be examined. All other animals survived to the scheduled necropsy.

On day 29 animals were weaned and sex confirmed. Actual numbers of males and females dosed are shown above in Table 2.6.6.8.7.3-4.

There were no clinical signs observed in any group. Animals were palpated weekly to check for signs of nodule or tumor formation at the site of injection. There were no injection site observations in any group. On day 150 all animals were euthanized and examined macroscopically. There were no notable findings at the injection sites or in brain, kidneys, liver, spleen, lungs or lymph nodes in any group. The muscle at the site of injection and lungs were collected for microscopic examination from one animal per sex in the control group and all test article-treated animals. No evidence of tumor formation was seen in any group.

Under the conditions of the study, a single subcutaneous administration of 0.2 mL of DNA from untreated, uninfected MDCK cells, influenza-infected, untreated MDCK cells and influenza-infected, BPL-treated MDCK cells followed by a 150-day observation period was not oncogenic in infant hamsters.

2.6.6.8.7.4 Supportive studies

MDCK cells test for tumorigenicity (Table 2.6.7.17.7, Study No. B012888/02)

The purpose of this non-GLP study was to examine the tumorigenic potential of the MDCK cell line. The study was conducted according to the WHO technical report No. 878 (1998): Requirements for the use of animal cells as in vitro substrates for the production of biologicals - Part B. Requirements for continuous-cell-line substrates (B.2.3.7 Tests for Tumorigenicity).

The MDCK cells tested in this study were adapted to serum-free (but not protein-free) growth. Infant rats (10 per group, <24 hours old) received MDCK cells, MDCK cell lysate, or BPL-treated MDCK cell lysate as a single 0.2 mL subcutaneous injection to the ventral region. Control animals received HeLa cells. Anti-thymocyte serum was administered as a 0.1 mL subcutaneous injection under the skin of the neck on days 0, 2, 7 and 14. Five rats from each of the three treatment groups and all control rats were necropsied on day 21. The remaining five rats from each treatment group were necropsied on day 84.

Animals were observed each working day for morbidity and mortality. Injection site nodules were measured on days 7, 9, 14, 16 and 21 for all animals, and once weekly from day 28 for remaining animals. Macroscopic postmortem evaluations included examination of the site of inoculation, lymph nodes, lungs, brain, spleen, kidneys and liver. Tissues collected for microscopic evaluation included the site of inoculation (ventral wall), axillary lymph nodes, lungs and any other lesions.

The test was valid based on the criterion that HeLa cell tumors arose in 9/10 animals. The study results are summarized below.

Table 2.6.6.8.7.4-1 Results - Study No. B 012888/02

Group	N [a]	Treatment [b] (1×10^7 cells)	Results at Necropsy		
			Postmortem finding	Day 21 [c]	Day 84
1	10	MDCK cells	injection site tumor	5/5	0/4
			lung/lymph node metastases	4/5	0/4
2	10	lysed MDCK cells	injection site tumor	0/5	0/5
			lung/lymph node metastases	0/5	0/5
3	10	BPL-treated MDCK cells	injection site tumor	0/5	0/5
			lung/lymph node metastases	0/5	0/5
4	10	HeLa cells [d]	injection site tumor	10/10	-
			lung/lymph node metastases	7/10	-

[a] Rats (10 per group) were <24 hours old at the time of dosing. Sex was not specified.
[b] Animals were dosed via subcutaneous injection (0.2 mL) to the ventral region. Anti-thymocyte rat serum was administered subcutaneously (0.1 mL) on days 0, 2, 7 and 14.
[c] Three group 1 rats died on day 14. Two were necropsied, the third was cannibalized and could not be evaluated.
[d] All HeLa-treated animals were necropsied on day 21.

Only intact MDCK cells were tumorigenic and/or metastatic.

Evaluation of tumor formation in nude (nu/nu) athymic mice after subcutaneous inoculation of cell suspension (Table 2.6.7.17.7, Study No. B96YG21.001)

This GLP study was conducted in 1997 at [redacted] USA. The MDCK cells tested in this study were adapted to serum-free (but not protein-free) growth. Four-week old female nude mice (10/group) received 1×10^7 MDCK cells, 18C1-10T cells (positive control) or SHE cells (negative control producing non-progressing nodules) as a single 0.2 mL injection subcutaneously between the scapulae. The study duration was 57 days.

The results of this study are shown in the table below.

Table 2.6.6.8.7.4-2 Results - Study No. B96YG21.001

Organ/Lesion	MDCK	18C1-10T	SHE
Skin injection site (number examined)	(10)	(10)	(10)
- Fibrosarcoma	0	10	0
- Sarcoma (not otherwise specified)	8	0	0
- Osseous proliferation	0	0	9
- Hematopoietic cell proliferation	0	0	8
- Inflammation, subacute	1	0	0
- Ulceration, epidermis	5	0	0
Scapular lymph node (number examined)	(10)	(10)	(10)
- Sarcoma (not otherwise specified), metastatic	2	0	0

As shown above, MDCK cells were tumorigenic and metastatic in female nude mice. No cell number titration was performed in either of these supportive studies, however the observation of tumorigenicity at 1×10^7 MDCK cells is consistent across all studies.

Based on this program of tumorigenicity and oncogenicity studies, Chiron's end-of-production MDCK cells (~population doubling = 40 from the working cell bank) were tumorigenic at all levels tested in adult nude mice (1×10^1, 1×10^3, 1×10^5, 1×10^7; Study 48329), and as such, may be considered highly tumorigenic. Therefore, a robust manufacturing process has been developed and a stringent testing program has been instituted (section 3.2.S.2.3.2.).

Lysed cells, whether BPL-treated or untreated, were not oncogenic in three neonatal rodent species. In the three infant rodent species, the dose of lysate administered represents a high multiple of the amount of theoretical adventitious agents or host cell proteins prior to any downstream manufacturing inactivation/removal steps. Given that the manufacturing process removes cellular elements to a great extent, the safety factors are very high (see discussion in section 2.4.4).

The experiments with cell lysates also provide indirect evidence that MDCK cells do not contain oncogenic agents, such as polyoma or herpesvirus. The absence of oncogenic viruses was confirmed in the degenerate PCR studies (section 3.2.S.2.3.2).

Injection of high molecular weight DNA (prepared from MDCK cells, flu-infected MDCK cells, or BPL-treated flu-infected MDCK cells) was not oncogenic in infant nude mice, rats, or hamsters. Animals received very high multiples of the amount of DNA that would be in a dose of vaccine (based on 10 ng residual DNA limit).

Based on the results of the studies with DNA, and the robust manufacturing process and testing program, the theoretical risk to a vaccinee receiving an annual dose of FCC vaccine is exceedingly low.

2.6.6.9 Discussion and Conclusion

The FCC vaccine has undergone nonclinical testing in mice (immunogenicity and abnormal toxicity), Guinea pigs (abnormal toxicity), rabbits (repeat-dose toxicity), and ferrets (immunogenicity and efficacy). Although assessments for tolerability are general in immunogenicity and abnormal toxicity studies, FCC vaccine appeared to be well tolerated. The pivotal toxicology study showed that administration of two intramuscular injections of FCC vaccine, given one week apart, was well tolerated both locally and systemically in male and female New Zealand White rabbits. As observed with the conventional egg-derived vaccine, the FCC vaccine produced no treatment-related adverse effects. Injection site reactions in vaccinated animals were comparable to controls (PBS).

The testing of manufacturing 'process intermediates' demonstrated that only intact MDCK cells were tumorigenic in immunocompromised adult (nude) mice. Cell lysates or purified MDCK cell DNA were not oncogenic in the three infant rodent species tested. Since whole MDCK cells are reliably excluded from the vaccine during multiple steps of the manufacturing process (section 3.2.S.2.2.), and because studies with MDCK cell lysates and DNA demonstrated no oncogenicity in sensitive animal models, Chiron concludes that the theoretical safety risk to a vaccinee receiving an annual dose of FCC vaccine is exceedingly low and the vaccine is safe for use in humans.

The nonclinical immunogenicity and local and systemic tolerability have been confirmed in clinical safety, immunogenicity, and comparability trials (section 5.3.5).

2.6.6.10 Tables and Figures

Not applicable.

2.6.6 Toxicology Written Summary

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2.6.6 Toxicology Written Summary

MF59 adjuvant formulations have undergone extensive nonclinical toxicology testing. During the development of MF59, various formulations were tested. A water-based formulation of MF59 (referred to as MF59 water, MF59-0, or MF59W.1) was later optimized by the addition of citrate buffer to provide increased stability, and is designated MF59C.1. Safety information pertaining to both formulations is relevant because citrate is a common, well-tolerated excipient, and immunogenicity and toxicology studies have identified no notable differences between the two formulations.

MF59 administered alone and in combination with a variety of antigens has been tested in a number of animal models including mice, Guinea pigs, rabbits, ferrets, dogs, goats and several non-human primates, including chimpanzees. Antigens adjuvanted with MF59 include recombinant proteins or glycoproteins from herpes simplex virus (HSV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), cytomegalovirus (CMV), hepatitis B virus (HBV), human papilloma virus (HPV), and malaria, as well as natural glycoproteins from influenza virus. In all cases, the antigen + MF59 combinations generated high antigen-specific antibody titers and, where tested, high virus neutralizing titers.

Pivotal toxicology studies performed with MF59 include the evaluation of single- and repeat-dose toxicity (including local tolerability), genotoxicity, sensitization, and embryofetal and developmental toxicity. These studies are described in detail in the following sections. MF59 is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen and minor inflammatory and degenerative changes at the injection site are consistent with the effects of intramuscular injections of an immunological adjuvant. These findings are readily reversible within days to 1 to 2 weeks. In repeat-dose toxicology studies in dogs, there were no effects on cardiovascular or central nervous system (safety pharmacology) parameters. MF59 is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat).

Non-pivotal studies conducted with vaccine formulations composed of MF59-adjuvanted antigens with an MF59-alone group are also presented. In these studies, the antigen + MF59 and MF59-alone formulations were well tolerated. Based on the parameters evaluated, no treatment-related safety issues were identified. Findings were generally limited to inflammatory responses at the injection site. These were of low severity and were partially to fully resolved by the end of a 7- to 14-day recovery period. Consistent systemic treatment-related findings in animals treated with antigen + MF59 included increases in fibrinogen levels and slight increases in globulin. These findings are consistent with administration of adjuvanted vaccine formulations.

Supportive studies performed with MF59-adjuvanted antigen formulations but without an MF59-alone group are listed in section 2.6.6.8.2. These studies are not summarized, however the study reports are provided in Module 4.

2.6.6.1 Brief Summary

Toxicology Program

Study type and duration	Route of administration	Species	Compound administered [a]
Single dose toxicity	Intramuscular	Rabbit	1-2× MF59
Repeat dose toxicity (2-14 doses)	Intramuscular	Rabbit	1-2× MF59
Genotoxicity	In vitro	N/A	N/A
	Intraperitoneal	Mouse	Up to 5000 mg/kg
Reproductive toxicity (5-23 doses)	Intramuscular	Rat, rabbit	0.25-2× MF59
Local tolerability (2-3 doses)	Intramuscular	Rabbit, dog	1-2× MF59
Other toxicity (3 doses)	Intradermal and topical	Guinea pig	0.25-2× MF59
Other (Other) studies (2-6 doses)	Intramuscular, intradermal and topical	Rat, Guinea pig, rabbit, woodchuck, chimpanzee	Various MF59-adjuvanted antigens

[a] The standard clinical dose of MF59 is 0.25 mL (combined with 0.25 mL antigen). In nonclinical studies, a 1× or 2× dose of MF59 is 0.25 or 0.5 mL, respectively.

2.6.6.2 Single Dose Toxicity

Study No. 501464 A Single Dose Intramuscular Toxicity Study of Rabies Vaccine Formulations in New Zealand White Rabbits

The objective of this GLP study was to assess the local and systemic toxicity of vaccine formulations in New Zealand White rabbits. The study consisted of five groups of 4 animals/sex/group. The MF59-treated group received a single dose of 0.5 mL containing 1:1 MF59:saline by intramuscular injection.

Potential toxicity was evaluated based on clinical and injection site observations, body weights, food consumption, body temperature, ophthalmic examinations, clinical pathology (hematology, coagulation, and serum chemistry parameters), terminal organ

weights, comprehensive macroscopic examination, and microscopic evaluation of selected tissues.

There were no deaths, and no treatment-related adverse effects on clinical signs, body weights, food consumption, body temperature, hematology, or ophthalmology. There was no erythema or edema observed at the injection sites. On day 3, mean fibrinogen levels were slightly elevated in males and females dosed with MF59. By day 15, levels had decreased to pre-test levels, indicating complete reversal. There were no relevant changes in terminal organ weights. Macroscopic postmortem findings at injection sites on day 3 (reddening) were similar across groups. By day 15, there were no findings at the injection, indicating complete resolution.

Microscopic findings of minimal-mild inflammation, hemorrhage, or cellular infiltrates at the injection site were seen only on day 3. With the exception of injection sites, there were no microscopic alterations that could be attributed to treatment.

Under the conditions of the study, a single intramuscular injection of MF59 was well tolerated in male and female New Zealand White rabbits.

Study No. 00-2672 A Single Dose Safety and Tolerability Study of Recombinant HCV Proteins and MF59 in Rabbits with a 15-Day Recovery Period

The purpose of this GLP study was to assess the local and systemic effects of a single dose of antigens and/or MF59 adjuvant. New Zealand White rabbits (4 per sex) received a single intramuscular dose 1.0 mL of 1:1 MF59:saline.

In-life evaluations included clinical signs, dermal injection-site observations, body weights, food consumption, body temperatures, ophthalmoscopy, hematology, coagulation parameters and clinical chemistry. Two animals per sex were necropsied 48 hours after dose administration (day 2); the remaining animals were necropsied 15 days after dose administration (day 15). Organ weights were obtained and complete macroscopic pathology examinations were performed. Histopathological evaluation of selected tissues, including injection sites, was conducted.

There were no test article-related clinical observations or ophthalmic findings. No treatment-related effects on body weight, food consumption, body temperature, or hematology parameters were observed. Fibrinogen levels were slightly elevated on day 2 when compared to pretest values but returned to baseline by the end of the recovery period. Creatine kinase values was transiently increased 48 hours after dosing and returned to baseline by day 15. The increase in creatine kinase is likely due to animal restraint and/or administration of a relatively large volume by the intramuscular route.

Occasional slight desquamation or erythema was observed, as was discoloration of the injection sites, including saline sites. At the day 2 necropsy, minimal to moderate edema, inflammation (presence of mixed inflammatory infiltrate), and/or hemorrhage was

observed in injection sites of most animals across all groups. Local muscle necrosis with mineral deposition was observed less frequently. At the end of the recovery period (day 15), there were only a few incidences of injection site findings. There were no treatment-related findings in other tissues and no organ weight changes.

The local effects at the injection sites were of minimal to moderate severity and were partially to fully reversible. Systemic findings of transient increased fibrinogen and creatine kinase levels were consistent with the intramuscular administration of an adjuvant.

2.6.6.3 Repeat Dose Toxicity

2.6.6.3.1 Pivotal Studies

A subset of repeat-dose rabbit toxicology studies was selected as pivotal because of the parameters evaluated, the dose administered, or the study duration. These studies were also used to calculate safety margins based on body weight and body surface area (discussed in section 2.4). The studies designated as pivotal are shown below.

Pivotal MF59 studies

Study Number	Regimen	Comments
466122	3 doses 2 weeks apart	Histopathology performed on complete WHO tissue list; 1× the clinical dose and a saline control group
00-2673	6 doses 2 weeks apart	Each dose contained 0.5 mL MF59, 2× the clinical dose; no saline control group
2670-100	12 doses ~3 weeks apart	Animals were dosed over an 8-month period, comprehensive histopathological evaluation; 1× the clinical dose and a saline control group
90-6081	14 doses, once daily	Animals received a total of 3.5 mL MF59 in 14 days; 1× the clinical dose and a saline control group

Study No. 466122 6-Week Vaccine Toxicity Study with H5N1 FCC + MF59 + Thiomersal Vaccine by 3 Intramuscular Injections in NZW Rabbits

This GLP rabbit toxicity study was performed to assess immunogenicity and any local or systemic effects following three intramuscular administrations of aCCD-H5N1-ivv (adjuvanted cell culture-derived H5N1 influenza vaccine; called H5N1 FCC+MF59+Thiomersal in the study report) at a dose of 15 µg of antigen per administration. The formulation used in this study is equivalent to the highest proposed clinical dose of antigen and MF59. The study design is shown below.

Study No. 466122 – Experimental Design

Group	Number of animals	Treatment 0.5 mL per intramuscular dose Days 1, 15 and 29	Main necropsy	Recovery necropsy
			Day 31	Day 43
1	8/sex	Phosphate buffered saline (PBS) (saline control, 0.5 mL)	4/sex	4/sex
2	8/sex	MF59C.1 + PBS (adjuvant control, 0.25 mL MF59C.1)	4/sex	4/sex
3	8/sex	aCCD-H5N1-ivv (15µg antigen, 0.25 mL MF59C.1)	4/sex	4/sex

Note: all test and control materials contained 100 µg/mL Thiomersal

New Zealand White (NZW) rabbits received three intramuscular injections in alternating hind legs, starting with the right leg. The following parameters were evaluated during the treatment and recovery periods: clinical signs (at least once daily; twice daily on dosing days), skin reactions at the intramuscular sites of injection (24 and 48 hours post-dose), body weights (weekly), and food consumption (twice weekly). Ophthalmoscopy was performed pre-study for all animals, on day 30 for main necropsy animals, and on day 42 for recovery animals. Heart rate, respiratory rate and rectal body temperature were evaluated pre-study, prior to dosing, and approximately 2 hours post-dose. Hematology and clinical biochemistry were evaluated pre-study and on days 8, 17 and 31 for all animals, and on day 43 for recovery animals. Blood samples were collected for antibody analyses pre-study, prior to dosing on days 15 and 29, on day 31 (main necropsy animals), and on day 43 (recovery necropsy animals).

A comprehensive macroscopic evaluation was performed at termination. Selected organs (adrenals, brain including brainstem, heart, kidneys, liver, ovaries, spleen, testes and thymus) were weighed. Histopathology was performed on the complete WHO tissue list.

Due to a laboratory error, data from one male in recovery group 1 and one female in recovery group 3 had to be excluded from day 29 onward. Therefore, only three males in group 1 and three females in group 3 (instead of the planned 4 per sex per group) were evaluated during the recovery period. The available data was considered sufficient for toxicological evaluation.

There was no treatment-related mortality and no adverse dermal reactions at the injection sites were observed in-life. There were no toxicologically relevant changes in clinical signs, body weights, food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, clinical biochemistry, macroscopic observations, or organ weights.

Increased fibrinogen levels and shortened prothrombin times were observed in males and females treated with MF59 alone or aCCD-H5N1-ivv on days 17 and 31 compared to the PBS control group. Both parameters returned to control values following the recovery

period. The increased fibrinogen levels (and the related effects on prothrombin time) reflect a temporary immune/inflammatory response to adjuvant and vaccine; these findings are consistent with other nonclinical studies where MF59 was administered alone or with other antigens.

Slightly higher rectal body temperatures (mean increase +0.4°C) were measured only in males 2 hours after the third dose of aCCD-H5N1-ivv (day 29).

An increase in globulin levels (resulting in increased total protein levels and a reduced albumin/globulin ratio) was noted after the second and third dose with either MF59 alone or aCCD-H5N1-ivv and reflects an anticipated effect of an immunological response. aCCD-H5N1-ivv was immunogenic in rabbits.

There were no adverse treatment-related macroscopic observations or effects on organ weights. There were no adverse treatment-related microscopic findings in the systemic organs that were examined. There were no notable histopathological findings, except at the injection sites. Findings at the injection sites consisted of the expected inflammatory changes associated with intramuscular injections. The findings in all groups included inflammation, infiltration and hemorrhage as shown in the tables below. The first and third injections were into the right hind limb; the second injection was into the left hind limb. The findings in the left injection site (single administration) are summarized in the table below.

Study No. 466122 – Incidence/severity of histopathology findings at the left injection site (injection on day 15)

Group	**1 (PBS)**		**2 (MF59)**		**3 (Vaccine)**	
Finding	**M**	**F**	**M**	**F**	**M**	**F**
Injection site – Left (day 31 main necropsy) – 16 days post last injection						
Number examined / Number affected	**4 / 0**	**4 / 1**	**4 / 4**	**4 / 3**	**4 / 3**	**4 / 1**
Intermuscular connective tissue macrophages	0	0	0	0	2+	0
Dermal hemorrhage	0	0	1+	0	0	1++
Panniculus muscle fiber degeneration	0	1+	2++	3+	2++	0
Deep muscle focal degeneration	0	0	1++ 1+++	0	0	0
Deep muscle hemorrhage	0	0	1++	0	0	0
Injection site – Left (day 43 recovery necropsy) – 28 days post last injection						
Number examined / Number affected	**3 / 0**	**4 / 1**	**4 / 0**	**4 / 0**	**4 / 1**	**3 / 1**
Muscle fiber degeneration	0	1+	0	0	1++	1++

Note: findings are presented as number affected followed by severity.
Severity is scored as minimal (+), mild (++), or moderate (+++).

Comparison of the left injection sites (one injection on day 15) from animals necropsied on day 31 to the injection sites from the recovery animals necropsied on day 43 indicates reversibility. By day 43 both the incidence and severity of findings had markedly decreased.

The findings in the right injections site are summarized in the table below.

Study No. 466122 – Incidence/severity of histopathology findings at the right injection site (injections on days 1 and 29)

Group	**1 (PBS)**		**2 (MF59)**		**3 (Vaccine)**	
Finding	**M**	**F**	**M**	**F**	**M**	**F**
Injection site – Right (day 31 main necropsy) – 2 days post last injection						
Number examined / Number affected	**4 / 0**	**4 / 3**	**4 / 4**	**4 / 2**	**4 / 1**	**4 / 3**
Intermuscular connective tissue macrophages	0	0	1++	1++	0	0
Dermal hemorrhage	0	1++	0	0	1++	0
Panniculus muscle fiber degeneration	0	1+	2+ 1+++	1++	0	0
Deep epidermal acute inflammation	0	0	1+	0	0	1+ 1++
Deep dermal diffuse macrophage infiltration	0	0	1++	2++	0	3+
Panniculus focal macrophage infiltration	0	0	1++	0	0	0
Intermuscular acute inflammation	0	0	2++	0	0	0
Deep muscle focal degeneration	0	1++	0	1+	0	0
Deep muscle hemorrhage	0	1+	0	0	0	0
Deep muscle macrophage infiltration	0	1+	0	0	0	0
Injection site – Right (day 43 recovery necropsy) – 14 days post last injection						
Number examined / Number affected	**3 / 0**	**4 / 2**	**4 / 3**	**4 / 2**	**4 / 2**	**3 / 2**
Intermuscular connective tissue macrophages	0	0	0	0	1+	0
Panniculus muscle fiber degeneration	0	1+	1+ 1++ 1+++	1+	1++	2++
Follicular adnexa deficit	0	0	1++	0	0	0
Dermal hemorrhage	0	1+	0	1+	0	0
Superficial pustular dermatitis	0	1+	0	0	0	0

Findings are presented as number affected followed by severity.
Severity is scored as minimal (+), mild (++), or moderate (+++).

Partial to full reversibility of the findings in the right injection site was also indicated when the main and recovery necropsy animals were compared.

Based on the results of this study, three 0.5 mL intramuscular administrations of aCCD-H5N1-ivv (containing15 µg antigen and 0.25 mL MF59 adjuvant) in NZW rabbits was immunogenic, locally well tolerated, and was not associated with systemic toxicity.

Study No. 00-2673 A Multiple Dose Safety and Tolerability Study of Recombinant HCV Proteins and MF59 in Rabbits with a 14-Day Recovery Period

Although there was no separate saline control group included in this study, it provides relevant information on local and systemic effects of a 2× dose of MF59 administered six times in a 1.0 mL dose volume.

The purpose of this GLP study was to assess the local and systemic effects of multiple doses of HCV antigens (E1E2 and/or Core antigens) combined with MF59C.1 adjuvant. New Zealand White rabbits received six intramuscular doses of vaccine or individual components administered at 2-week intervals as shown below.

Study No. 00-2673 – Experimental Design

Group	Animals	Treatment	Dose of Antigen	Dose Volume
1	6M + 6F	Control[a]	0	1.0 mL
2	6M + 6F	HCV Core + MF59C.1[b]	300 µg	1.0 mL
3	6M + 6F	HCV E1E2 + MF59C.1[b]	100 µg	1.0 mL
4	6M + 6F	HCV Core + E1E2 + MF59C.1[b]	150 µg/100 µg	1.0 mL

[a] Control animals received 1.0 mL saline in right leg and 1.0 mL of 1:1 saline and MF59C.1 in the left leg
[b] Animals received 0.5 mL of MF59C.1 per injection

In-life evaluations included clinical signs, dermal injection site observations, body weights, food consumption, body temperatures, ophthalmoscopy, hematology, coagulation parameters, and clinical chemistry. Antibody titers were assessed 48 hours after each injection and at the end of the recovery period. Three animals per sex were necropsied 48 hours after the final dose administration (day 72); the remaining animals were necropsied 14 days after the final dose administration (day 84). Organ weights were obtained and macroscopic pathology examinations were performed. Histopathological evaluation of selected tissues, including injection sites, was conducted.

There were no test article-related clinical observations or ophthalmic findings. No treatment-related effects on body weight, food consumption, body temperature, or hematology parameters were observed. Fibrinogen levels were generally elevated (up to 2 times pretest levels) in all groups after treatment but returned to pretest levels after the recovery period. These increases have been observed previously with MF59C.1 administration and can be attributed to the immunomodulatory effects of this adjuvant. Consistent with the administration of immunogenic antigens, slight increases in globulin concentrations and concomitant decreases in albumin/globulin ratios were observed at many intervals throughout the study including at the end of the recovery period.

Compared to pretest values, creatine kinase values were increased sporadically across all groups. The increase in creatine kinase is likely due to animal restraint and/or administration of a relatively large volume by the intramuscular route.

Dermal observations of erythema, edema, and desquamation were seen sporadically in all groups including controls. Discoloration of the injection sites was observed at the day 72 necropsy in some of the animals receiving antigens. Minimal to moderate edema, inflammation (presence of mixed inflammatory infiltrate), and hemorrhage were observed microscopically in many of the animals at day 72 with the incidence and severity being slightly greater in the treated groups. Occasional muscle necrosis was also observed in the treated groups. Following the recovery period, there were no macroscopic effects and the microscopic effects were less frequent and severe with only minimal to slight edema and inflammation being observed at the injection sites.

There were no treatment-related effects on organ weights or any microscopic findings other than those at the injection sites.

Antibodies to Core were first observed following the second dose; titers increased throughout the dosing period and were maintained throughout the study including the recovery period in both groups 2 and 4. Similarly, antibodies to E1E2 were first observed following the second dose; titers increased throughout the dosing period (with some variability) and were maintained throughout the study including the recovery period in both groups 3 and 4.

The administration of six intramuscular doses of the vaccines, given at 2-week intervals, was well tolerated. Local effects at the injection sites, while slightly more frequent or severe in treated animals, were generally minimal to slight and were partially to fully reversible in the 2-week recovery period. Systemic findings of increased fibrinogen, globulin and creatine kinase levels were consistent with the intramuscular administration of an immunomodulatory agent.

Study No. 2670-100 8-Month Intramuscular Study in Rabbits

In this GLP study, New Zealand White rabbits received a 0.5 mL intramuscular injection of saline, or 0.5 mL MF59:saline (1:1) mixture, administered into alternate hind limbs approximately once every 3 weeks for 8 months (total of 12 administrations). Six animals/sex/group were dosed on days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, and 232. Necropsies were performed on days 233 and 247 (3/sex/group, respectively).

Parameters evaluated included survival, clinical signs, injection site observations, body weights, body temperatures, clinical pathology, ophthalmoscopy, macroscopic observations, organ weights, and histopathological examination of tissues. Urinalysis was performed at necropsy.

Study No. 2670-100 – Organs Weighed

Adrenal glands	Kidneys	Spleen
Brain including stem	Liver	Testes with epididymides
Heart	Ovaries	Thymus

Study No. 2670-100 – Tissues Evaluated

Adrenal glands	Ovaries
Bone marrow (femur)	Pancreas
Brain with brainstem (medulla/pons, cerebellar cortex, cerebral cortex)	Pituitary gland
	Prostate
Cecum	Rectum
Colon	Salivary glands (mandibular)
Duodenum	Skeletal muscle (psoas)
Epididymides	Skin
Eyes with optic nerve	Spinal cord - cervical, thoracic, lumbar
Femur (bone, including articular surface)	Spleen
Gall bladder	Stomach
Heart	Testes
Ileum	Thymus
Injection sites	Thyroid including parathyroid
Jejunum	Tongue
Kidneys	Trachea
Liver	Urinary bladder
Lungs (with bronchi)	Uterus
Mammary gland	Vagina
Mesenteric and cervical lymph nodes	All gross lesions

There were no deaths, no treatment-related clinical signs, and no effect on body weights, body temperatures, ophthalmoscopy, urinalysis, or organ weights. There were no treatment-related macroscopic observations.

Injection site observations consisted of slight dermal irritation at the injection sites of most animals, the incidence being slightly more frequent with MF59 than in the control group. Microscopically, treatment-related findings were limited to the injection sites at

the terminal necropsy on day 233, and consisted of generally minimal to mild inflammation. Incidence was reduced at the day 247 necropsy, indicating recovery.

A slight decrease in prothrombin times, slight increase in mean fibrinogen values, and an increase in creatine kinase values were consistent with inflammation at injection sites. In conjunction with antigen, there was also a slight increase in germinal centre activity within the white pulp of the spleen in a few animals (considered a normal immunological response).

Twelve intramuscular injections of MF59 over an 8-month period were well tolerated in rabbits.

Study No. 90-6081 14-Day Intramuscular Study in Rabbits

In this GLP study, New Zealand White rabbits received daily 0.5 mL intramuscular injections of saline or MF59 for 14 days. Half the animals were necropsied on day 15, the remainder on day 22, following a 7-day recovery period. The study design is shown below.

Study No. 90-6081 – Experimental Design

Group[a]	Number of Animals	Compound Administered	Dose Volume	Treatment Days	Necropsy Day	
					Day 15	Day 22
1	8M + 8F	0.45% saline	0.5 mL	1 - 14	4M + 4F	4M + 4F
5	8M + 8F	MF59 (water) [b]	0.5 mL	1 - 14	4M + 4F	4M + 4F

[a] Other groups received a second adjuvant + MF59, for clarity only the relevant treatment groups are shown
[b] MF59 was diluted 1:1 with saline prior to administration

Study parameters included clinical observations, body weight, food consumption, ophthalmology, clinical chemistry, hematology, urinalysis, and body temperatures. Comprehensive macroscopic examinations were performed and selected organs were weighed. A complete tissue list was collected and selected organs were weighed as shown below.

Study No. 90-6081 – Organs Evaluated

Organs weighed			
Adrenal glands*	Liver	Prostate	Thymus
Brain	Lung*	Seminal vesicles*	Thyroid with parathyroid glands
Epididymides*	Ovaries*	Spleen	
Heart	Pituitary gland	Submandibular glands	Uterus
Kidneys*	Popliteal lymph nodes*	Testes*	Vesicular gland

* Paired organs weighed together

Tissues were evaluated microscopically as shown below.

Study No. 90-6081 – Tissues Evaluated

Tissues examined histopathologically	
Heart with aorta	Thymus
Kidneys	Spleen
Lung	Eyes
Liver with gallbladder	Pituitary gland
Knee joint capsules	Bone marrow (of rib)
Iliac and para-aortic lymph nodes	All selected injection sites
Thymus	All gross lesions

There was no mortality and no clinical signs of toxicity. There was no effect on bodyweight, food consumption, body temperature, ophthalmology, clinical chemistry, or urinalysis. Fibrinogen levels were slightly elevated in MF59-treated females at the end of the dosing period but returned to normal during the recovery period.

There were no macroscopic observations consistent with systemic toxicity. Microscopically, slight, reversible thymic atrophy, slight increase in neutrophils and precursor cells in splenic red pulp (more marked in females) and slight bone marrow hypercellularity were observed in the MF59 group.

Macroscopic findings at the MF59 injection sites included hemorrhage, edema and discoloration of sub-fascial and subcutaneous tissues. These findings were observed more frequently after the last 4 injections, with incidence and severity reduced in recovery animals, indicating resolution.

Six of the 14 injection sites on each animal were evaluated microscopically. In MF59-treated animals, findings 1 day post-injection were generally minimal to mild and included neutrophil and macrophage infiltration, edema, hemorrhage, and muscle cell necrosis. Observations 7 days post-injection included inflammatory cell infiltrates,

macrophages and fibroblasts with occasional muscle cell necrosis, regeneration and calcification. By 12 days post-injection, observations consisted of minimal foci of macrophages and mononuclear cells accompanied by muscle cell regeneration.

Daily intramuscular administration of MF59 for 14 days was well tolerated in rabbits. There were no observations consistent with systemic toxicity, and local reactogenicity was of a low order of magnitude. Injection site findings were mild and reversible. The treatment schedule in this study was much more intense than that envisioned for any human vaccine usage.

2.6.6.3.2 Nonpivotal Studies

Study No. 940292 30-Day Subacute Toxicity Study in Rabbits by Intramuscular Route

This repeat-dose study was conducted in accordance with the OECD Principles of Good Laboratory Practice (GLP). The systemic toxicity and local tolerability of one to two intramuscular doses of vaccine formulations were studied in New Zealand White rabbits of both sexes. Treatment groups were Agrippal alone, MF59W.1 adjuvant alone (equivalent to MF59C.1 but without citrate buffer), or Agrippal+MF59W.1

Two intramuscular injections were administered 14 days apart into alternate hind limbs. The study design is shown below.

Study No. 940292 – Experimental Design

Group	Number of Animals	Compound Administered	Dose Volume	Treatment Days	Necropsy Day	
					Day 17	Day 31
1	6M + 6F	MF59W.1	0.5 mL	1, 15	3M + 3F	3M + 3F
2	6M + 6F	Agrippal (45 µg)	0.5 mL	1, 15	3M + 3F	3M + 3F
3	6M + 6F	Fluad equivalent (Agrippal (45 µg) + MF59W.1)	0.5 mL	1, 15	3M + 3F	3M + 3F

Each 0.5 mL injection contained the usual human dose of trivalent vaccine (15 µg HA from each of the three virus strains per 0.5 mL). On a body weight basis, each dose is equivalent to approximately 15 times the dose administered to adult humans (using 60 kg for man and 4 kg for rabbit).

The following parameters were evaluated at regular intervals for potential treatment-related effects: physical appearance, behavior, clinical signs, Draize scoring of injection

site reactions, rectal temperature, body weight gain, ophthalmology, hematology, clinical chemistry, macroscopic pathology and histopathology of selected organs and tissues, including the injection sites. Statistical analyses were performed on body weight gain, body temperature, hematology, clinical chemistry, and organ weights. In order to evaluate recovery, half the animals (3 per sex per group) were necropsied on day 17 and the remainder were necropsied on day 31.

Study No. 940292 – Parameters Evaluated

Organs weighed	Histopathology evaluation
Adrenal glands	Epididymides
Brain	Eye (with optic nerve)
Epididymides	Injection sites (left and right)
Heart	Liver
Kidneys	Lymph nodes (mesenteric and cervical)
Liver	Ovaries
Ovaries	Spleen
Spleen	Testes
Testes	Thymus
Thymus	Lesions identified macroscopically

Functional immunity was not directly assessed in this study, but the adjuvant effect of MF59 has been amply documented in several species, including rabbits. Systemic and local toxicity was evaluated, based on in-life parameters, following the first and second doses and observations for any additional effects following the second administration were performed on the same animals. The first and second doses were administered into the left (day 1) and right (day 15) hind limbs, respectively. Therefore, the left injection sites were evaluated 2 and 16 days post-dose (day 17 necropsy), and the right injection sites were evaluated 16 and 30 days post-dose (day 31 necropsy).

No animals died in this study. There were no clinical signs of systemic toxicity after a single dose or detectable reactions at the injection sites in any animal. Following two doses, there were no treatment-related effects on any study parameters indicative of local or systemic toxicity in any treatment group. There were some statistically significant differences in some hematology and clinical chemistry between the groups. However, the differences were of small magnitude, and within the expected normal ranges for untreated rabbits (historical controls), so were not considered to be treatment-related.

There were no relevant differences between groups in organ weights at either of the two scheduled necropsies, and individual values were all within the normal ranges. There were no macroscopic observations except for the injection sites. Macroscopic findings at the injection sites treated two days previously indicated an increased frequency of slight focal hemorrhage in the Agrippal+MF59W.1 group (equivalent to Fluad but without

citrate buffer) compared to the other two groups. There were no macroscopic findings at injection sites treated 16 or 30 days before necropsy.

Histological examination of the injection site 2 days post-injection revealed interstitial inflammation (mainly acute), interstitial hemorrhage, and/or muscle fiber degeneration in almost all animals. These observations were more notable in the Agrippal+MF59W.1 group, followed by the Agrippal group, then the MF59W.1 group. Sixteen days after injection, inflammatory and degenerative changes were still present, but to a lesser extent in most animals. Thirty days after injection, partial to full recovery was evident in most animals. At injection sites 16 and 30 days post-injection, there were no differences between the groups. There were no other tissues with treatment-related findings and any changes seen were comparable in frequency and severity to those commonly found in untreated New Zealand rabbits of similar age in the Test Facility.

In conclusion, no local or systemic adverse effects were seen in rabbits administered two intramuscular injections of MF59W.1 adjuvant, Agrippal, or the Fluad equivalent (Agrippal+MF59W.1).

Study No. 2777-102 Fourteen-Day Intramuscular Toxicity Study of Connaught Fluzone/MF59 Vaccine in Rabbits

This GLP study was designed to evaluate the local and systemic toxicity of Connaught Fluzone® Vaccine in combination with MF59 adjuvant when administered via intramuscular injection into alternating hind limbs of male and female adult rabbits. Injections were administered on days 1 and 15, and treatment was followed by a 14-day recovery period. Twenty-four Hra:(NZW)SPF/HRP rabbits were assigned to the study as shown below.

Study No. 2777-102 – Experimental Design

Group and treatment	Number of animals		Dose volume mL
	Male	Female	
1 (MF59 Control)	6	6	0.5
2 (Vaccine)	6	6	0.5

Control animals received 1:1 MF59:saline. Three animals/sex/group were necropsied approximately 48 hours after the dose on day 15. All remaining animals were necropsied on day 29 following a non-treatment recovery period.

Study parameters included survival, clinical signs, ophthalmology, injection-site irritation, body weight data, rectal temperatures, clinical pathology data, gross pathology observations, organ weight data, and microscopic examination of selected tissues from all animals.

All of the rabbits survived until their scheduled sacrifice. No treatment-related clinical signs were observed during the study, except for mild local irritation at the injection sites of some animals in both groups. Observations at the injection sites included very slight erythema on days 3, 4, 15, 16, 17, and 18 in a few animals of both sexes from each group; well-defined erythema in a Group 2 male on day 15; very slight edema in some Group 2 males on days 15, 16, 17, and 18, and in Group 1 females on days 15 and 17; slight edema in a Group 1 female on day 16; and moderate edema in a Group 1 female on day 15. The dermal findings appeared to resolve within a few days of dosing.

All animals gained weight during the study. There was no evidence of treatment-related effects in the ophthalmoscopic examinations, rectal temperature, hematology, serum chemistry, gross pathology, and organ weight data. Inflammation, hemorrhage, and focal skeletal muscle and collagen degeneration seen histologically at the intramuscular injection sites of some animals were similar between both groups. With the exception of focal skeletal muscle degeneration, these microscopic findings were reversible following the 14-day non-treatment recovery period.

Other microscopic findings in various tissues were generally inflammatory in nature, and were considered incidental to treatment with the Fluzone® Vaccine plus MF59 adjuvant. There were no histopathological findings suggestive of adverse systemic effects associated with the administration of the test material.

Based upon the results of this study, Fluzone® Vaccine in combination with MF59 was associated with localized tissue reactions when administered in two separate doses approximately 2 weeks apart via intramuscular injection to New Zealand White rabbits.

Study No. 433665 12-Week Vaccine Toxicity Study in Female New Zealand White Rabbits with Intranasal gp140 and LT-K63 Adjuvant Priming and Intramuscular gp140 and MF59 Adjuvant Boosting

The purpose of this GLP study was to assess the immunogenicity and potential toxicity of three intranasal priming doses of o-gp140subB + LT-K63 adjuvant with and without two intramuscular boosts with o-gp140subB + MF59 adjuvant to female rabbits. Six groups of 4 females per group were treated as shown below.

Study No. 433665 – Experimental Design

	Intranasal dosing			Intramuscular dosing		
Group and treatment	**o-gp140subB (mg) / LT-K63 (mg)**	**Dosing day**	**Necropsy day 31 animals**	**o-gp140subB (mg) / MF59 (mL)**	**Dosing day**	**Necropsy day 85 animals**
1 - Saline control	0 / 0	1, 15, 29	1-4	---	---	---
2 - Saline control	0 / 0	1, 15, 29	---	0 / 0	57, 71	5-8
3 - Adjuvant control	0 / 0.03	1, 15, 29	9-12	---	---	---
4 - Adjuvant control	0 / 0.03	1, 15, 29	---	0 / 0.25	57, 71	13-16
5 - Vaccine	0.1 / 0.03	1, 15, 29	17-20	---	---	---
6 - Vaccine	0.1 / 0.03	1, 15, 29	---	0.1 / 0.25	57, 71	21-24

Intranasal: Dose volume of 0.3 mL administered drop by drop, 150 µL to each nostril
Intramuscular: Dose volume of 0.5 mL to the hindlimb

Study parameters evaluated included: clinical signs (daily); body weights (weekly); food consumption (twice weekly); ophthalmoscopic examination (pretest and day 30); heart rate, respiratory rate and rectal temperature (pretest and on days 1, 15, 29, 57 and 71); and hematology and clinical chemistry (pretest and on days 14, 31, 43, 70 and 85). Blood was collected for serum antibody evaluations pretest and on days 14, 31, 43, 70 and 85. Vaginal washes (cervicovaginal secretions) were collected for antibody evaluations at necropsy on days 31 and 85.

Complete macroscopic examinations were performed on day 31 (groups 1, 3 and 5) and day 85 (groups 2, 4 and 6). A complete set of tissues was collected (based on the WHO Guideline on Nonclinical Evaluation of Vaccines, 2003). Organs (adrenals, brain, heart, kidneys, liver, ovaries, spleen and thymus) were weighed and a histopathological evaluation was performed on the tissues listed below.

Study No. 433665 – Histopathology tissue list

Brain (cerebellum, mid-brain, cortex, including brain stem and olfactory lobe)	Nasal cavity
Eyes	Spleen
Femur	Bone marrow
Kidneys	Thymus
Lung	Trachea
Lymph nodes (mesenteric, cervical)	All macroscopic lesions

There was no mortality. There were no treatment-related clinical signs, and no dermal irritation at the intramuscular injection sites. There was no effect on body weight, food consumption, ophthalmoscopy, heart rate, respiratory rate or body temperature, and no notable macroscopic findings.

There was no effect on hematology or clinical chemistry. There were occasional statistically significant changes. However, these changes were sporadic and small in magnitude and were, therefore, not considered to be toxicologically relevant.

There was a slight increase in absolute and relative adrenal weights at day 31 in animals that received vaccine or LT-K63 alone. This was considered unrelated to treatment because there were no macroscopic or microscopic correlates, and at day 85 there was no difference between groups.

There were no treatment-related microscopic findings. Any observations noted were within the range of background pathology seen in this strain and age of rabbit, and occurred at similar incidence and severity across all groups including controls.

In this study the clinical dose, regimen, and route of administration of o-gp140subB + LT-K63 adjuvant (intranasal prime) and o-gp140subB + MF59 adjuvant (intramuscular boost) were evaluated. Under the conditions of the study, 3 intranasal administrations of o-gp140subB + LT-K63 with or without 2 intramuscular administrations of o-gp140subB + MF59 were well tolerated in female NZW rabbits. There was no evidence of local or systemic toxicity. The intranasal prime was immunogenic in rabbits, and antibody titers increased following administration of the intramuscular boost.

Study No. 759-002 Subchronic Intramuscular Toxicity Study of Biocine® HPV-6 Vaccines in Rabbits (HPV-6 E7, HPV-6 L1 Antigens and MF59C.1 Adjuvant)

This GLP study was conducted to evaluate the toxicity of vaccines administered intramuscularly in rabbits. Six New Zealand White (Hra:(NZW)SPF) rabbits per sex received injections of 1:1 saline:MF59. There was no saline control for comparison. Doses were administered by intramuscular injection into the hind limb. Animals were injected on study days 1, 15 and 29 into alternating hind limbs. The injection volume was 0.5 mL. Three animals per sex per group were necropsied on day 31 and the remaining animals were necropsied on day 43.

Parameters evaluated included mortality, clinical signs, injection-site scores, body weights, body temperatures, ophthalmoscopic examinations, clinical pathology laboratory tests, macroscopic observations, organ weights, and histopathological examinations.

All animals survived to terminal or recovery necropsy. There were no significant clinical findings noted. There were no test article-related effects on body weights, body temperatures or ophthalmic findings. Bruising was noted at the injection site for 1 female

24 hours post-second injection and also on study days 17 and 18; the injection sites of all other animals at all time points had no evidence of erythema or edema.

Macroscopic pathology findings at the terminal necropsy consisted of red discoloration of the third injection site for 1 out of 3 males. In females, red discoloration of the third injection site was seen in 2 of 3 animals. This red discoloration correlated with the microscopic findings of hemorrhage, subacute inflammation, and/or muscle fiber degeneration. Other macroscopic findings noted at the terminal or recovery necropsies were considered incidental and not related to test article administration.

A decrease in incidence and severity of these lesions was noted in the first (day 1) and second (day 15) injection sites compared to the third injection site (day 29). No other MF59-related changes were evident at either the terminal or recovery necropsy in the organs evaluated microscopically. Injection-site findings present at the terminal necropsy had resolved by the end of the recovery period.

Study No. 2670-101 28-Day Intramuscular Study in Rabbits

In this GLP study, New Zealand White rabbits received 0.5 mL intramuscular injections of MF59 (1:1 MF59:saline equivalent to 1× human dose) on days 1, 15 and 29 followed by a 14-day recovery period.

There was no evidence of systemic toxicity or dermal irritation at the injection sites. There was focal hemorrhage and/or residual inflammation of minimal severity at injection sites, which mostly resolved after the recovery period.

Study No. 89-6192 Intramuscular Study in Chinchilla Rabbits

In this GLP study, Chinchilla rabbits received three courses of two separate intramuscular injections of 0.25 mL of saline or MF59, administered at 2 week intervals, with dosing on days 1, 15 and 29. There were no deaths and clinical/laboratory investigations indicated no adverse reactions. Postmortem examination showed no target-organ toxicity, however, a slight increase in fibrinogen and body temperature was noted in some MF59-treated animals. Injection sites indicated a slight inflammatory response that was also noted for the saline animals. MF59 emulsion was well tolerated under the conditions of the study.

Study No. 950031 Hepa Bio Vax B 6-Week Subacute Toxicity Study in New Zealand White Rabbits by Intramuscular Route

The purpose of this GLP study was to evaluate the toxicity and local tolerability of Hepa Bio Vax B with MF59 adjuvant administered by the intramuscular route to rabbits (6/sex/group) once every two weeks for 6 weeks. The control group was MF59 (0.5 mL of a 1:1 dilution with Tris buffer). Half the animals (3/sex/group) were necropsied 48 hours post-last dose. The remaining 3/sex/group were necropsied 2 weeks after the last dose.

Toxicity was evaluated based on clinical signs and injection site observations, body weights and temperatures, clinical pathology, ophthalmoscopy, macroscopic postmortem observations, selected organ weights, and histopathology of the injection sites.

There were no deaths and no systemic toxicity. The only treatment-related effects were mildly elevated lactic dehydrogenase and creatine kinase, both of which are related to intramuscular injection. Findings at the injection site consisted of reversible slight inflammatory and degenerative changes in the muscle.

Study No. 2670-102 Twenty-Eight Day Intramuscular Toxicity Study of BIOCINE® HSV Vaccine (30/30) in Rabbits (HSV gD2/gB2dTM Vaccine)

This GLP study was conducted in New Zealand White Rabbits. Two groups of rabbits, 6 per sex per group, were given three intramuscular injections two weeks apart. Each 0.5 mL dose was equivalent to one human dose, and contained 30 µg gD2 / 30 µg gB2 in MF59 adjuvant. The study design is shown below.

Study No. 2670-102 – Experimental Design

Group	# Animals	Placebo Vaccine	HSV Vaccine	Dose Days	Necropsy Day 30	Necropsy Day 42
1	6M + 6F	0.5 ml	0	1, 15, 29	3M + 3F	3M + 3F
2	6M + 6F	0	0.5 ml	1, 15, 29	3M + 3F	3M + 3F

M=male, F=female

The animals were observed daily for signs of toxicity. Local injection-site irritation was scored using the Draize scale, and was monitored daily, or until symptoms resolved. Body temperatures were measured pre-study, before dosing and at 48 hours post-dosing, as well as weekly on non-dosing weeks, and at sacrifice. The animals were weighed pre-study, before dosing, once weekly on non-dosing weeks, and at sacrifice. Blood was collected for chemistry after each dose and again before sacrifice. Ophthalmic exams were conducted pre study and at sacrifice.

Forty-eight hours after the third immunization, half of the animals were necropsied; the remaining animals were necropsied following a two-week recovery period.

No animals died on study, and there were no clinical observations, clinical pathology findings, ophthalmic observations, or histopathology findings of test article-related systemic effects. Histopathology findings at the injection site consisted of minimal to moderate chronic inflammation and minimal to slight hemorrhage and were similar between the vaccine and MF59 groups.

Study No. 656583 Subchronic Intramuscular Toxicity Study of Biocine HIVp24 Vaccine in Rabbits

The purpose of this GLP study was to evaluate local and systemic effects of the recombinant antigen combined with MF59C.1.

New Zealand White rabbits received 4 doses (0.5 ml on days 1, 15, 29 and 43) of 1:1 saline:MF59 via intramuscular injection into alternate hind limbs. In-life toxicological evaluations included clinical signs, injection-site scoring (Draize), body weights, body temperatures, ophthalmoscopy, hematology, and clinical chemistry. Three animals per sex were necropsied 48 hours after the fourth dose; the remaining animals were necropsied after a 14-day treatment-free period. Organ weights were obtained and macroscopic examinations performed.

There was no mortality. There were no treatment-related effects on body weights, ophthalmoscopy, or organ weights. Local reactions at injection sites were minimal in severity and of low incidence. Body temperatures increased slightly over time; 48 hours after the fourth dose the mean increase compared to pre-trial and pre-dose values was 0.69ºC for males and 0.14ºC for females. Enlarged popliteal lymph nodes were observed macroscopically on day 45 in 1 male and 2 females. On day 57 reddened lymph nodes were noted in 1 male and 1 female.

Microscopic evaluation of injection sites at day 45 showed inflammatory cell infiltrates; the severity was graded ‘very mild’ or ‘mild’. On day 57 the incidence and severity of findings was decreased, indicating reversibility.

Study No. 6549-166 Multiple-dose Intramuscular Injection Toxicity Study with HIV DNA/PLG Vaccine Formulation in New Zealand White Rabbits

The objective of this GLP study was to assess the local and systemic toxicity of the HIV Vaccine formulation in New Zealand white rabbits after repeated administration and to determine the reversibility of findings. The study consisted of two groups of eight animals/sex/group. Treated rabbits received four doses of the HIV DNA vaccine formulation (*env* and *gag* DNA/PLG) given every other week followed by four doses of the HIV Protein vaccine formulation, also given every other week. The last HIV DNA vaccine dose and the first o-gp 140 vaccine dose were administered on the same day (day 43). Doses were administered via intramuscular injections into the quadriceps leg muscle and legs were alternated except on day 43 when both legs were injected. Control animals received four intramuscular injections of saline solution followed by four intramuscular injections of MF59. Four animals/sex/group were necropsied 3 days (day 88, main necropsy) or 2 weeks post-dosing (day 99, recovery necropsy).

Study No. 6549-166 – Experimental Design

Group 1, 8/sex[a] – Control (mL)							
Treatment	**Day of Study**						
	1	**15**	**29**	**43**	**57**	**71**	**85**
Saline Control	1.0	1.0	1.0	1.0	–	–	–
MF59 Control[b]	–	–	–	0.5	0.5	0.5	0.5
Group 2, 8/sex[a] – DNA vaccine (mL)							
env & *gag* DNA /PLG[c]	1.0	1.0	1.0	1.0	–	–	–
Env (o-gp 140) protein[d]	–	–	–	0.5	0.5	0.5	0.5

[a] 4/sex/group necropsied on Day 88 (main necropsy) and on Day 99 (recovery necropsy)
[b] 0.25 mL of MF59 plus 0.25 mL saline
[c] 0.5 mL *env* DNA/PLG (2 mg DNA, 50 mg PLG/mL) plus 0.5 mL *gag* DNA/PLG (2 mg DNA, 50 mg PLG/mL)
[d] 0.25 mL of Env protein (0.4 mg/mL) plus 0.25 mL MF59

The animals were observed twice daily for mortality and morbidity and once daily for signs of toxicity. In addition, detailed observations were made before, and 4 hours after dose on each dosing day, weekly, and at each necropsy. Injection sites were assessed for signs of irritation and graded based on a modified Draize score before dosing and 24 and 48 hours after each injection. Body temperatures were taken before treatment, before each dose, and 24 hours after each dose. Body weight was recorded before treatment, weekly thereafter, and at necropsy. Food consumption was assessed weekly. The ophthalmology evaluation was performed before treatment and prior to each necropsy. Blood samples for hematology, serum chemistry, and coagulation (including fibrinogen) analysis were collected before treatment, before dose on days 29 and 57, and after dose on days 87 and 99. Additional blood samples were taken before treatment, before dose on days 15, 43, 71, and on days 87 and 99 for antibody (antinuclear and Env and Gag antibodies) analysis. At each necropsy, a complete macroscopic examination and microscopic evaluation of a full list of tissues were performed. Organ weight data on 11 selected organs were also collected. In addition, nine selected tissues were collected for possible assessment of distribution of the DNA vaccine into host tissues by PCR analysis.

There were no treatment-related effects on survival, clinical signs, body weight, food consumption, body temperature, and ophthalmic observations. Dermal scoring of the injection sites revealed occasional instances of edema or erythema in a few animals from both the control and treated group. Although the incidence of these dermal irritation reactions was slightly higher in Group 2 (HIV Vaccine treatment), the findings were mild

in severity (very slight to slight) and completely resolved by the next observation period. Findings in clinical pathology parameters were limited to slightly increased white blood cell counts (17% – 23%) and absolute lymphocyte counts (30%) in males. These minor effects were most apparent at day 87. There were no test article-related changes in absolute or relative organ weights and no macroscopic or microscopic findings except at the injection sites. Injection-site reactions were similar for the control and test article treated animals. However, myofiber degeneration and chronic-active inflammation were slightly increased for vaccine-treated animals of both sexes at the right injection site and males at the left injection site compared to the control animals that only received saline/MF59 (no DNA, PLG, or protein antigen). Analysis of serum for antinuclear antibodies was negative. Results of the analysis of anti-Env and anti-Gag antibodies are presented below.

Study No. 6549-166 – Geometric Mean Titers of HIV Env and Gag Antibodies in Immunized Rabbits

	Day 43			Day 71			Day 87		
	GM	**LCL**	**UCL**	**GM**	**LCL**	**UCL**	**GM**	**LCL**	**UCL**
HIV Gag Titers	127	65	251	794	480	1314	624	346	1126
HIV Env Titers	20	13	32	24,330	21,771	27,191	42,805	39,273	46,655

GM = Geometric Mean
LCL = Lower Confidence Limit
UCL = Upper Confidence Limit
n = 16 (Days 43 and 71)
n = 15 (Day 87)

Study No. 6549-166 – Percentage of Responders* to HIV Env or Gag Antigens in Immunized Rabbits

	Day 43	Day 71	Day 87
% Responders to HIV Gag in immunized rabbits	70	95	95
% Responders to HIV Env in immunized rabbits	44	100	100

* An animal was considered a responder if the antibody titer was ≥ 25.
n = 16 (Days 43 and 71)
n= 15 (Day 87)

No anti-Gag and anti-Env antibodies were detected in control animals. By day 43, 70% and 44% of the treated animals had positive titers against Gag and Env, respectively. By day 71, almost all animals (95%–100%) had positive responses.

In conclusion, under the conditions of this study, the HIV vaccine formulation was immunogenic and no systemic adverse effects related to the administration of the vaccine formulation were identified. Local effects consisted of occasional instances of very slight to slight erythema or edema at the injection sites, which appeared fully resolved by the next observation period. There were slight microscopic findings at the injection sites consistent with the intramuscular administration of immunogenic materials., Therefore,

four IM injections of the HIV DNA vaccine given every other week, followed by four IM injections of the o-gp 140 vaccine, also given every other week, were well tolerated by New Zealand white rabbits.

Study No. 501438 A Multiple Dose Intramuscular Toxicity and Local Tolerability Study of Rabies Vaccine Formulations in New Zealand White Rabbits

The objective of this GLP study was to evaluate the systemic toxicity and local tolerability of multiple (5) doses of rabies vaccine formulations administered once every one to two weeks by intramuscular injection to New Zealand White rabbits.

The study consisted of five groups of 6/sex/group. The MF59 dose volume was 0.5 mL injected alternately into the quadriceps or posterior thigh muscle on days 1, 8, 15, 29, and 43 of the study. A comprehensive macroscopic examination and tissue collection was performed on 3 animals/sex/group on days 45 and 57.

Potential toxicity was evaluated based on the following parameters: clinical signs, dermal injection site observations (0, 24, and 48 hours after each administration), body weights, ophthalmic examinations, food consumption, body temperatures, clinical pathology (hematology, coagulation, and serum chemistry parameters), terminal organ weights, full macroscopic postmortem examination, and microscopic evaluation of selected tissues.

There were no deaths or treatment-related adverse effects on any antemortem study parameters. There were no adverse clinical signs and no edema or erythema at the injection site at any of the observation time points. Globulin levels were slightly elevated on days 17 and 45 in males given MF59. By the end of the recovery period (day 57), levels were comparable to control values.

Macroscopic postmortem findings at the injection site consisted of reddening. The incidence of injection site findings was lower on day 57 than on day 45, indicating partial to full resolution. Microscopic findings at the injection consisted of inflammatory cell infiltrate in muscle and subcutaneous layer.

Under the conditions of the study, administration of five 0.5 mL intramuscular injections of MF59 once every one to two weeks was well tolerated.

2.6.6.4 Genotoxicity

Because MF59 contains a natural product with inherent contaminants (squalene), mutagenicity testing of the adjuvant was considered to be judicious. MF59 adjuvant, both water and citrate formulations, was investigated using the mouse micronucleus cytogenetic assay and the bacterial reverse mutation assay (Ames test) using standard study designs. The program of genotoxicity studies is shown below.

Genotoxicity Studies with MF59

Study type	Study number	MF59 formulation
Bacterial Reverse Mutation Assay	G96AQ62.502	Water
Bacterial Reverse Mutation Assay	G96AQ61.502	Citrate
Micronucleus Cytogenetic Assay in Mice	G96AQ62.122	Water
Micronucleus Cytogenetic Assay in Mice	G96AQ61.122	Citrate

Study Nos. G96AQ61.502 and G96AQ62.502 Bacterial Reverse Mutation Assay

The purpose of these GLP studies (Ames Test) was to evaluate the mutagenic potential of MF59 (both the water and citrate formulations) and/or its metabolites by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and one strain of *E. coli* in the presence and absence of S9 activation. The assay was conducted according to standard, published protocols.

MF59C.1 and MF59W.1 were tested using *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537 as well as *E. coli* tester strain WP2 uvrA both in the presence and absence of Aroclor 1254-induced rat liver, S9 (as the metabolic activation system). The assay was performed using the plate incorporation method.

Doses of 100, 333, 1000, 3333, and 5000 µg MF59/plate were used. Saline was used as the negative control and positive controls were included for each bacterial strain. The positive controls used were as follows: with S9 activation, 2-aminoanthracene; without S9 activation, TA98 = 2-nitrofluorene; TA100 and TA1535 = sodium azide; TA1537 = 9-aminoacridine; and WP2 uvrA = methyl methanesulfonate. No positive response was observed, and neither precipitate nor appreciable toxicity was observed. Under the conditions of this study, both MF59C.1 and MF59W.1 formulations were negative in the Bacterial Reverse Mutation Assay.

Study Nos. G96AQ61.122 and G96AQ62.122 Micronucleus Cytogenetic Assay in Mice

The purpose of these GLP studies was to evaluate the clastogenic potential of MF59C.1 and MF59W.1 formulations, as measured by their ability to induce micronucleated, polychromatic erythrocytes in the bone marrow of male and female mice. The assay was conducted according to standard, published protocols.

In the micronucleus assay, male and female ICR mice were dosed via intraperitoneal injection with vehicle or 1250, 2500, or 5000 mg/kg of MF59 (MF59C.1 or MF59W.1) in a constant volume of 20 mL/kg. Saline was used as the negative control and cyclophosphamide as the positive control. Animals (5/sex/group) were sacrificed at 24,

48, and 72 hours after dose administration except for positive controls where five animals per sex were sacrificed 24 hours after dose administration.

Study Nos. G96AQ61.122 and G96AQ62.122 – Experimental Design

Test materials	Number per sex used for bone marrow collection after dose administration		
	24 hours	48 hours	72 hours
Vehicle Control	5	5	5
Test Article			
Low Dose 1250 mg/kg	5	5	5
Mid Dose 2500 mg/kg	5	5	5
High Dose 5000 mg/kg	5	5	5
Positive Control	5	none	none

There was no mortality. Clinical signs following dose administration included lethargy in male and female mice at 5000 mg/kg. Slides of bone marrow cells, collected at 24, 48, and 72 hours after treatment were prepared and stained with May-Grunwald-Giemsa stain and read microscopically for micronucleated polychromatic erythrocytes. Slight reductions (up to 11% for MF59 citrate formulation, and up to 13% for the water formulation) in the ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the test article-treated groups relative to the respective vehicle controls.

There was no significant increase in the number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in test article treated groups, relative to the vehicle control group at any time point. Cyclophosphamide induced a significant increase in micronucleated polychromatic erythrocytes in both female and male mice. In this study both formulations of MF59 were negative in the mouse micronucleus assay.

2.6.6.5 Carcinogenicity

Not applicable.

2.6.6.6 Reproductive and Developmental Toxicity

Study No. 1303-002 Developmental Toxicity (Embryo-Fetal and Teratogenic Potential) Study of a Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to Crl:CD®BR VAF/Plus® Female Rats

The purpose of this GLP study was to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of a vaccine. The focus of this section is MF59; therefore only data pertaining to the saline and MF59-alone groups will be presented here.

Forty-five Crl:CD BR VAF/Plus (Sprague-Dawley) female rats were randomly assigned to each group. Rats were injected intramuscularly with either saline or MF59 on day 1 of the study (three weeks before cohabitation) and on days 0, 6, 8 and 10 of presumed gestation (rats assigned to Caesarean-sectioning) or on days 0, 6, 8, 10 and 20 of presumed gestation (rats assigned to natural delivery).

The test and control articles administered are shown in the table below:

Study No. 1303-002 – Test and control articles

Group	Number of females	Intramuscular injection
I	45	1.0 mL of saline (control) (0.5 mL in each of two separate sites)
II	45	0.5 mL of MF590 (2× clinical dose)[a]

[a] The clinical dose of MF59 is 0.25 mL

Body weight and feed consumption values were recorded weekly before cohabitation, on day 0 of presumed gestation and daily until necropsy on day 20 of presumed gestation (rats assigned to Caesarean-sectioning), day 25 of presumed gestation (rats assigned to natural delivery that did not deliver a litter) or day 21 postpartum (rats that delivered litters). Day 0 of presumed gestation was defined as the day spermatozoa were observed in a smear of vaginal contents or the presence of a copulatory plug *in situ* was noted.

Mating performance was evaluated daily during the cohabitation period and confirmed by observation of pregnancy (implantation sites present *in utero* on either day 20 or 25 of presumed gestation or natural delivery of a litter).

Twenty dams from each group underwent Caesarean sectioning on day 20 of presumed gestation. Parameters evaluated included number of corpora lutea, number and placement of implantation sites, early and late resorptions, and number of live and dead fetuses. Fetuses were weighed and examined to identify sex and gross external alterations. Approximately half the fetuses in each litter were evaluated for soft tissue alterations using a variation of Wilson's sectioning technique. The remaining fetuses were eviscerated, cleared, stained using alizarin red S, and examined for skeletal alterations and ossification sites.

The remaining pregnant dams were allowed to deliver naturally. Dams were evaluated for delivery complications, litter size and pup viability. Maternal behavior was recorded on days 1, 4, 7, 14 and 21 postpartum and observed on all days postpartum. Pup sex, bodyweight, gross external anomalies and mortality were evaluated. The pups in each litter were counted once each day and clinical signs recorded. Two pups per litter were euthanized and blood collected for antibody analysis on days 1, 4, 7, 14 and 21. Litters were culled to approximately 10 pups per litter on day 7. On day 21 all dams and

surviving pups were euthanized; all dams were examined for gross lesions and number and placement of implantation sites. Pups were necropsied and examined for macroscopic lesions.

Two animals in the MF59-treated group died. One animal, number 10951, had a swollen hind limb (associated with intramuscular injection) on day 1 of presumed gestation and was found dead on day 4 of presumed gestation. Feed consumption was reduced on days 1-2 and 3-4 of presumed gestation, and bodyweight was decreased on day 4. Necropsy observations included a fluid-filled bladder, green substance in the stomach, and large kidneys with tan spots and slight dilation of the pelvis. Pregnancy status could not be determined, as implantation had not yet occurred. This death was not considered to be related to innate toxicity of MF59, but rather to an individual susceptibility to the multiple administrations of a large dose volume (2× the clinical dosage) or a 'peculiarity' of MF59 administration in this animal (see Consultant's Report - Study 1303-002).

A second animal, number 10984, had a swollen hind limb on days 1, 11 and 21 of presumed gestation, and was found dead on day 11 of lactation. Body weight and feed consumption were unremarkable. Necropsy findings included a large liver and spleen, and red fluid in the abdominal cavity. This animal had delivered a litter of 14 live pups that appeared normal at the time of maternal death. This death was not considered to be related to innate toxicity of MF59 (see Consultant's Report - Study 1303-002).

One control group rat delivered on day 20 of presumed gestation (the mating date was incorrectly identified) and was euthanized on day 6 of lactation. No other deaths occurred, and there were no abortions or premature deliveries.

Clinical observations related to treatment consisted of hind limb swelling. Swelling was associated with intramuscular injection, and occurred during gestation in all animals receiving MF59 alone or with antigens. Injection of 0.9% saline was not associated with swelling in the hind limb.

Maternal body weights and body weight gains were unaffected throughout the pre-mating, gestation and lactation periods. Significant reductions ($p<0.05$ to $p<0.01$) in absolute and relative feed consumption values on days 8 to 10 of gestation were possibly caused by MF59; all other time points were comparable to the saline control.

There were no treatment-related macroscopic observations in dams caesarean-sectioned on gestation day 20. Litter averages for corpora lutea, implantations, litter sizes, early resorptions, percent live male fetuses, percent resorbed conceptuses, and number of dams with any resorptions was not affected by treatment.

There were no soft tissue or external alterations in fetuses. There was an increase in the litter and fetal incidences of incompletely ossified sternebrae, pubes and/or ischia in fetuses from MF59-treated dams. This finding was possibly related to treatment as the incidence exceeded historical ranges at the test facility.

Natural delivery was unaffected by treatment. There was no effect on litter parameters, pup sex ratios, pup body weights or clinical signs. Maternal behavior was unaffected, and there were no noteworthy necropsy findings for dams or pups.

In this study, MF59 was administered either 5 (Caesarean section group) or six (natural delivery group) times. Two maternal deaths (not considered related to innate toxicity) and a brief reduction in feed consumption were probably related to treatment. In fetuses an increased incidence of incomplete ossification of the sternebrae, pubes and/or ischia was seen. The MF59 dose used in this study was equivalent to twice the usual clinical dose of 0.25 mL. On a bodyweight basis, a 2× clinical dose in a 0.3 kg rat is approximately 200 times higher than the same dose in a 60 kg human. MF59 was not considered to be teratogenic or fetotoxic in this study.

Study No. 1303-001P Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to New Zealand White Rabbits

In this pilot GLP study, developmental toxicity (embryo-fetal toxicity and teratogenic potential) in female New Zealand White (NZW) rabbits was evaluated following intramuscular administration of MF59 alone or in combination with antigens. This study was used to select doses for a subsequent definitive study. The definitive study (Study No. 1303-001) did not contain an MF59-alone group, so is not summarized here, but is provided in Module 4.

Five artificially inseminated and presumed pregnant New Zealand White [Hra:(NZW)SPF] rabbits were assigned to each dose group. Saline (control) or MF59 was injected intramuscularly on days 6 through 28 of presumed gestation. Dose levels are shown below.

Study No. 1303-001P – Test and control articles

Group [a]	Number of females	Clinical dose equivalent	Test Material
I	5	—	0.5 mL of saline (control)
III	5	0.25 ×	MF59-0 diluted with saline
IV	5	0.50 ×	MF59-0 diluted with saline

[a] Groups II and V received antigen and are therefore not presented here

Body weights were recorded weekly before study assignment and days 0 and 6 through 29 of presumed gestation. Feed consumption values were recorded daily during the acclimation and study periods. Rabbits were observed on days 0 and 6 through 29 of presumed gestation for clinical signs, abortions and premature deliveries; viability was recorded at least twice daily. Ophthalmological evaluations were conducted during the acclimation period and on day 29 of presumed gestation.

On day 29 of presumed gestation all surviving rabbits were euthanized. A gross necropsy of the thoracic and abdominal viscera was performed. Uteri of apparently nonpregnant does were stained with 10% ammonium sulfide to confirm the absence of implantation sites. Lymphoid tissues from three sites (cervical, mesenteric and popliteal lymph nodes) and tissues with gross lesions were preserved in neutral buffered 10% formalin for possible histological evaluation. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses, and early and late resorptions. The number of corpora lutes present in each ovary was recorded. Each fetus was weighed and examined for sex and macroscopic external alterations.

No deaths occurred during the conduct of this study. One group IV (0.5 × MF59) doe prematurely delivered on day 29 of gestation. This was considered unrelated to the test article because it was a single occurrence and common in this strain of rabbit.

There were no treatment-related effects on clinical observations, body weight, feed consumption or necropsy observations. No Caesarean-sectioning or litter parameters were affected. The litter averages for corpora lutea, implantations, litter sizes, resorptions, percent male fetuses and percent resorbed conceptuses were comparable among groups. There were no dead fetuses, and no litter consisted of only resorbed conceptuses. One late resorption occurred in a litter in group III (0.25 × MF59). There were no macroscopic external alterations in fetuses.

This study was performed to select doses for a definitive study (Study No. 1303-001). The definitive study did not have an MF59-alone group, therefore the data is not presented here, however the same dosing schedule with 0.5× and 1.0× MF59 combined with antigens had no effect on litter parameters and was not teratogenic in rabbits.

2.6.6.7 Local Tolerance

Study No. 89-6280 Comparative Intramuscular Toxicity in Rabbits

Groups of 5 per sex New Zealand White rabbits received intramuscular injections of saline or MF59 on days 1 and 8 in this GLP study. The 0.5 mL dose volume per dosing occasion was split into two 0.25 mL injections (one per leg). Animals were necropsied 7-8 days after the second injection.

There was no mortality and no evidence of systemic toxicity. Injection site reactions were limited to small areas of erythema or scabbing, consistent with the physical introduction of a needle and reactions, where they occurred, had generally regressed within 72 hours of dosing.

Study No. 90-6230 Comparative Intramuscular Tolerability in Rabbits

In this GLP study, New Zealand White rabbits (5 per sex) received two 0.25 mL intramuscular injections of MF59 (1:1 MF59:vehicle equivalent to 1× human dose) on days 1, 15 and 29. At least 7 days after the third dose the animals were necropsied.

There was no evidence of systemic toxicity or dermal irritation at the injection sites. Injection site findings were generally minor in severity, and included chronic inflammation, degeneration of muscle fibers, multinucleate giant cell aggregation, and mineralization and aggregation of vacuolated macrophages.

Study No. 89-6281 Comparative Intramuscular Tolerability Study in Beagle Dogs

Beagle dogs were dosed with saline or MF59 on days 1 and 8 in this GLP study. One animal from each sex per group was euthanized on day 15 of the study, with remaining animals euthanized on either day 19 or 20.

Treatment-related findings were limited to local reactions of erythema or scabbing consistent with needle trauma.

Study No. 89-6193 Intramuscular Tolerability Study in Dogs

In this GLP study, beagle dogs received intramuscular injections of 0.5 mL of saline or MF59 on days 1, 16 and 29. Some animals showed a transient, non-recurring pain reaction after the first dose of MF59. There was no evidence of systemic toxicity.

Safety pharmacology (cardiovascular and neurological) parameters were also assessed in this study (section 2.6.2.4). There were no effects on either organ system.

Study No. 90-6231 Comparative Intramuscular Tolerability in Beagle Dogs

Beagle dogs (2 per sex) received 0.5 mL intramuscular injections of MF59 (1:1 MF59:vehicle, equivalent to 1× human dose) on days 1, 15 and 29 in this GLP study. Seven days after the third dose the animals were necropsied.

There was no evidence of systemic toxicity. Injection site reactions were acute and minor in severity, and consisted of a needle mark with erythema 1-3 mm in diameter that regressed 1-2 days following dosing. There were no notable histological changes related to treatment.

Safety pharmacology (cardiovascular and neurological) parameters were also assessed in this study (section 2.6.2.4). There were no effects on either organ system.

2.6.6.8 Other Toxicity Studies

2.6.6.8.1 Antigenicity

Study No. 564278 Biocine® MF59C.1 and Biocine® MF59W.1 Magnusson-Kligman Maximization Test in Guinea Pigs

The potential for MF59C.1 and MF59W.1 to cause delayed contact hypersensitivity was investigated using a Magnusson-Kligman Maximization Test.

Female Dunkin-Hartley Guinea pigs were used in this GLP study. Each MF59 formulation was tested in a group of 20 animals; 10 control animals received saline. MF59 concentrations for use in each phase of the study were determined by range finding; for the intradermal induction phase, 2% MF59 was used, and for the topical induction and challenge phases, 100% MF59 was used. Skin reactions were graded after each procedure by a standardized scoring system.

For intradermal induction in test groups, duplicate 0.1 mL injections of the following solutions were administered into scapular skin: 50% aqueous Freund's Complete Adjuvant (FCA), a 1:1 (v/v) 2% MF59 and 0.9% saline, and a 1:1 (v/v) mixture of 2% MF59 and 50% aqueous FCA. Control animals received duplicate 0.1 mL intradermal injections of the following solutions: 50% aqueous FCA, 0.9% saline, and 1:1 (v/v) mixture of 0.9% saline and 50% aqueous FCA.

Six days later, the scapular region was clipped free of hair and the injection sites observed for signs of irritation. On the following day (day 7) a second induction was performed via the topical route in the same animals. The test animals were treated with 0.5 mL of 100% MF59, and controls were treated with 0.5 mL of 0.9% saline. The site was covered with an occlusive dressing for 48 hours, and then examined for signs of irritation.

Thirteen days after the topical application, the scapular region was again clipped free of hair, and the following day (day 21), both test and control animals were treated with 0.5 mL of 0.9% saline, and 0.5 mL of 100% MF59 applied topically (challenge). The test site was covered with an occlusive dressing for 24 hours, after which it was again clipped, and observed for signs of irritation. All animals were examined for clinical signs twice daily, and body weights were recorded at the start and end of the study.

During intradermal induction, slight reactions were noted in all test animals (both MF59C.1 and MF59W.1). There was no reaction in any of the control animals.

During the topical induction phase, slight reactions were seen in 4/20 MF59C.1 and 10/20 MF59W.1 animals. In the control group, 2/10 animals had slight reactions.

On challenge, 3/20 MF59C.1 animals had a positive reaction at 24 hours; in 1 animal the reaction was still present at 48 hours. There was no positive reaction in any MF59W.1 or control animal.

Apart from injection site reactions, no other clinical signs were noted, and body weight gain was normal. Under the conditions of the assay, MF59C.1 and MF59W.1 were not considered to be sensitizers in guinea pigs.

2.6.6.8.2 Other (Other) Toxicity Studies

The safety of MF59 can also be inferred from efficacy studies. These studies were conducted with vaccine formulations composed of antigens combined with MF59 (no MF59-alone group). In these studies, the antigen/MF59 formulations were generally well tolerated. Based on the parameters evaluated, no treatment-related safety issues were identified. Adverse events were limited to inflammatory responses at the injection site. These were of a low grade of severity and partially to fully resolved by the end of the recovery period. Consistent systemic treatment-related findings consisted of increases in fibrinogen levels and slight increases in globulin. These findings are consistent with administration of adjuvanted vaccine formulations.

A list of completed and ongoing supportive studies follows. For completed studies, links to the study reports in Module 4 are provided.

Supportive Studies – MF59 with various antigens

Study Title	Study Number
Repeat Dose Toxicity	
Potential of Intratracheally-Administered MF59 or LTK63 to Induce Hypersensitivity Pneumonitis or Lipoid Pneumonia in the Lungs of Sprague-Dawley Rats	L08682
4-Week Vaccine Toxicity Study With Fluad® + IC31® Vaccine by 2 Intramuscular Injections in New Zealand White Rabbits Including a 2-Week Recovery Period	486688 (report in preparation)
2-Dose Intramuscular Injection Toxicity Study with MF59-Adjuvanted Influenza Vaccine with and without CpG 7909 in Rabbits	6560-106
4-week toxicity study with Fluad, Fluad High B, and Fluad High H3+IC31 influenza vaccine formulations by three intramuscular injections in New Zealand White rabbits followed by a 2-week recovery period	488182 (report in preparation)

Study Title	Study Number
A 10 Week Intramuscular Vaccine Safety Study in New Zealand White Rabbits With HCV E1E2 Antigen, CpG and MF59 Adjuvant	500757
Immunogenicity Study of a Vaccine (Antigen and Adjuvant Components) in Rabbits	1303-003
Intramuscular Toxicity Study of HCV (E2+Core) Vaccine in Rabbits	3-K84
Intramuscular Toxicity Study in Rabbits with HCV E2 Vaccine	N002833A
Rabbit / Intranasal Adjuvant-HA Toxicology	93-05-025R
Biocine HIV Vaccine GCP 52 121 gp120 Antigen Combined with Biocine MF59 Emulsion Containing MTP-PE Intramuscular Tolerability Study in Rabbits	91-6009
Biocine HSV Vaccine CGP 52 120 HSVgD2/gB2 Antigen Combined with BIOCINE MF59 Emulsion Containing MTP-PE Intramuscular Tolerability Study in Rabbits	90-6306
Subchronic Intramuscular Toxicity Study of BIOCINE HIV Thai E gp120/SF2 gp120 Vaccine in Rabbits	759-001
Administration of Woodchuck Hepatitis Virus Surface Antigen (WHsAg) to Woodchucks with and without Chronic Woodchuck Hepatitis Virus (WHV) Infection	98-07-263
HCV E2 and E2/Core Vaccines Adjuvanted with MF59-0 and Iscomatrix in Baboons	ACR 381-PC-0
Safety and Efficacy of Chiron HBV PreS2+S/MF59C.1 Vaccine in an Adult Chimpanzee with Chronic Hepatitis B Infection	420-PT-0
Reproductive Toxicity	
Intramuscular Reproductive and Developmental Toxicity Study of Fluad H5N1 Vaccine in Rabbits, Including a Postnatal Evaluation	UBA00021
Intramuscular Dosage-Range Developmental Toxicity Study of Biocine Vaccine (HSV gD2 and gB2dTM Antigens) in Rabbits	1303-004P
Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to New Zealand White Rabbits	1303-001
Other Toxicity	
Biocine FLU/MF59C.1 Magnusson-Kligman Maximization Test in Guinea Pigs	564110

2.6.6.9 Discussion and Conclusion

The nonclinical safety program, using several animal species, provides a complete and accurate assessment of the safety of MF59 for use as an adjuvant. Its use is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In

repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen and minor inflammatory and degenerative changes at the injection site are consistent with the effects of intramuscular injections of an immunological adjuvant. These findings are readily reversible within days to 1 to 2 weeks. MF59 is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat).

Findings in studies with antigens combined with MF59 or MF59 alone were attributable to the adjuvant, with no notable adverse effects seen with the antigen-adjuvant combination. In general, although immunogenicity is enhanced, toxicological findings with MF59-adjuvanted vaccines are comparable to findings with MF59 alone.

2.6.6.10 Tables and Figures

Not applicable.

2.6.7 毒性試験の概要表

目　次

表 一 覧

略号一覧

略号	省略していない表現（英）	省略していない表現（日）
MDCK	Madin-Darby Canine Kidney	イヌ腎由来 MDCK 細胞

1 緒言

FCC H1N1sw ワクチンの承認申請に際して提出する CTD2.6.7 毒性試験概要表の構成について説明する。

本資料は，本剤のドイツにおける承認申請に際して提出した CTD（以下，ドイツ CTD）を主な申請資料として構成している。しかしながら，ウイルスの培養細胞として使用しているイヌ腎臓由来 MDCK 細胞（Madin-Darby Canine Kidney，MDCK）の腫瘍原性及びがん原性に関しては，海外では細胞培養由来ワクチンである Optaful の承認申請時に既に評価されているため，ドイツ CTD には含まれていない。したがって，当該資料として Optaful の CTD2.6.7 Toxicology Tabulated Summary を添付した。また，本剤にアジュバントとして配合している MF59 アジュバントに関しては，海外では広く使用されているため，一部の試験報告書（重要な試験に関する資料）を除いてドイツ CTD には添付されていない。したがって，試験の記載に重複が生じるが，MF59 アジュバントに関する補足資料として，MF59 アジュバントの Drug Master File（DMF）から 2.6.7 Toxicology Tabulated Summary を添付した。

MDCK 細胞関連資料の記載箇所をTable 1-1に，MF59 アジュバント関連資料の記載箇所をTable 1-2にそれぞれ記載した。

Table 1-1 MDCK 細胞関連資料の CTD 記載箇所

試験の種類	試験系	試験番号	CTD 記載箇所
腫瘍原性 (細胞)	ヌードマウス	48329	OP: 2.6.7 Toxicology Tabulated Summary, p.15
がん原性 (溶解液)	ヌードマウス (幼若)	48330	OP: 2.6.7 Toxicology Tabulated Summary, p.16
	ラット (幼若)	48332	OP: 2.6.7 Toxicology Tabulated Summary, p.17
	ハムスター (幼若)	48331	OP: 2.6.7 Toxicology Tabulated Summary, p.18
がん原性 (DNA)	ヌードマウス (幼若)	48333	OP: 2.6.7 Toxicology Tabulated Summary, p.19
	ラット (幼若)	48335	OP: 2.6.7 Toxicology Tabulated Summary, p.20
	ハムスター (幼若)	48334	OP: 2.6.7 Toxicology Tabulated Summary, p.21
腫瘍原性 (細胞/溶解液)	ラット (幼若)	B012888/02	OP: 2.6.7 Toxicology Tabulated Summary, p.22
腫瘍原性 (細胞)	ヌードマウス	B96YG21.001	OP: 2.6.7 Toxicology Tabulated Summary, p.22

OP: OPTAFLU®の EMEA への申請資料（2006 年 5 月作成）

Table 1-2 MF59 アジュバント関連資料の CTD 記載箇所

試験の種類	試験系	試験番号	記載箇所
Pivotal MF59 studies			
反復投与毒性			
	ウサギ	90-6081	CTD2.6.7 Toxicology Tabulated Summary, p.50-54
遺伝毒性			
Ames test	*In vitro*	G96AQ62.502	CTD2.6.7 Toxicology Tabulated Summary, p.55-56
		G96AQ61.502	CTD2.6.7 Toxicology Tabulated Summary, p.57-58
小核	マウス	G96AQ62.122	CTD2.6.7 Toxicology Tabulated Summary, p.59
		G96AQ61.122	CTD2.6.7 Toxicology Tabulated Summary, p.60
皮膚感作性	モルモット	564278	CTD2.6.7 Toxicology Tabulated Summary, p.61
生殖発生毒性			
胚・胎児発生	ラット	1303-002	CTD2.6.7 Toxicology Tabulated Summary, p.62-65
	ウサギ	1303-001P	CTD2.6.7 Toxicology Tabulated Summary, p.66
Nonpivotal MF59 studies			
単回投与毒性	ウサギ	501464	CTD2.6.7 Toxicology Tabulated Summary, p.67
		00-2672	CTD2.6.7 Toxicology Tabulated Summary, p.68
反復投与毒性	ウサギ	89-6280	CTD2.6.7 Toxicology Tabulated Summary, p.68
		2777-102	CTD2.6.7 Toxicology Tabulated Summary, p.69
		89-6192	CTD2.6.7 Toxicology Tabulated Summary, p.69
		90-6230	CTD2.6.7 Toxicology Tabulated Summary, p.69
		2670-101	CTD2.6.7 Toxicology Tabulated Summary, p.70
		759-002	CTD2.6.7 Toxicology Tabulated Summary, p.70
		950031	CTD2.6.7 Toxicology Tabulated Summary, p.71
		656583	CTD2.6.7 Toxicology Tabulated Summary, p.71
		501438	CTD2.6.7 Toxicology Tabulated Summary, p.72
		00-2673	CTD2.6.7 Toxicology Tabulated Summary, p.72
		2670-100	MF59: 2.6.7 Toxicology Tabulated Summary, p.26-29
		466122	CTD2.6.7 Toxicology Tabulated Summary, p.12-17

試験の種類	試験系	試験番号	記載箇所
反復投与毒性 (続き)	イヌ	89-6281	CTD2.6.7 Toxicology Tabulated Summary, p.73
安全性薬理	イヌ	89-6193	CTD2.6.7 Toxicology Tabulated Summary, p.73
		90-6231	CTD2.6.7 Toxicology Tabulated Summary, p.74

CTD: 本ワクチンのドイツへの申請資料（CTD）
MF59: MF59 Drug Master File

2.6.7 Toxicology Tabulated Summary

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2.6.7 Toxicology Tabulated Summary

2.6.7.1 Toxicology Overview

Studies providing supportive data for the FCC H1N1sw vaccine

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.	Location
Single-Dose Toxicity								
	Rabbits	Intramuscular	Days 1 & 15	0.5 mL FCC/MF59-H5N1	Yes	[redacted] NL	466122	4.2.3.2
Repeat-Dose Toxicity - Pivotal								
	Rabbits	Intramuscular	Days 1 & 15	0.5 mL FCC/MF59-H5N1	Yes	[redacted] NL	466122	4.2.3.2
Repeat-Dose Toxicity - Supportive								
	Rabbits	Intramuscular	Days 1 & 8	0.5 mL Optaflu 0.5 mL Agrippal	Yes	[redacted]	191-44	4.2.3.2
	Rabbits	Intramuscular	Days 1, 15, & 29	0.5 mL Fluad	Yes	[redacted] NL	488182	4.2.3.7
	Rabbits	Intramuscular	Days 1 & 15	0.5 mL Fluad	Yes	[redacted] Italy	940292	4.2.3.7
Genotoxicity								
	Not applicable		—	—	—	—	—	—
Carcinogenicity								
	Not applicable		—	—	—	—	—	—
Reproductive and Developmental Toxicity - Pivotal								
	Not applicable		—	—	—	—	—	—

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.	Location
Reproductive and Developmental Toxicity - Supportive								
	Rabbits	Intramuscular	Days 1, 15 & 29 pre-mating, Days 7 & 20 gestation	0.5 mL Aflunov	Yes	[redacted] US	UBA00021	4.2.3.5
	Rabbits	Intramuscular	Days 1, 15 & 29 pre-mating, Days 7 & 20 gestation	0.5 mL Optaflu	Yes	[redacted] US	UBA00037	4.2.3.5
Local Tolerance								
	Rabbits	Intramuscular	Days 1 & 15	0.5 mL FCC/MF59-H5N1	Yes	[redacted] NL	466122	4.2.3.2
Other Toxicity Studies - Supportive								
Antigenicity Magnusson-Kligman	Guinea pig / D-Hartley	Intradermal & topical	Days 1, 7 & 21	0.1 & 0.5 mL Fluad	Yes	[redacted]	564110 / 14430	4.2.3.7
Other (Other) Toxicity Studies								
Pivotal Studies with MF59 water (MF59W) or citrate (MF59C.1) formulations								
Repeat dose Toxicity	Rabbit / NZW	Intramuscular	14 days	0.5 mL 1:1 MF59W:saline	Yes	Ciba-Geigy Ltd.	90-6081	4.2.3.7.7
Ames Test	*In vitro*	N/A	N/A	MF59W Up to 5000 µg per plate	Yes	[redacted]	G96AQ62.502	4.2.3.7.7
Ames Test	*In vitro*	N/A	N/A	MF59C.1 Up to 5000 µg per plate	Yes	[redacted]	G96AQ61.502	4.2.3.7.7

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.	Location
Mouse Micronucleus	Mice / ICR	Intraperitoneal	Single dose	MF59W 1250, 2500 & 5000 mg/kg	Yes	[redacted]	G96AQ62.122	4.2.3.7.7
Mouse Micronucleus	Mice / ICR	Intraperitoneal	Single dose	MF59C.1 1250, 2500 & 5000 mg/kg	Yes	[redacted]	G96AQ61.122	4.2.3.7.7
Magnusson-Kligman	Guinea pig / D- Hartley	Intradermal & topical		0.1 & 0.5 mL MF59C.1 or MF59W	Yes	[redacted]	564278 / 14465	4.2.3.7.7
Embryofetal Development	Rat / CD	Intramuscular	5 or 6 doses	0.5 mL MF59W	Yes	[redacted]	1303-002	4.2.3.7.7
Embryofetal Development	Rabbit / NZW	Intramuscular	Days 6-28	0.5 mL 1:7 MF59W:saline	Yes	[redacted]	1303-001P	4.2.3.7.7
Nonpivotal Studies with MF59 water (MF59W) or citrate (MF59C.1) formulations								
Single Dose Toxicity	Rabbit / NZW	Intramuscular	Single dose	0.5 mL MF59C.1	Yes	[redacted]	501464 / 20717	Available on request
Single Dose Toxicity	Rabbit / NZW	Intramuscular	Single dose	1.0 mL 1:1 MF59C.1:saline	Yes	[redacted]	00-2672	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1 & 8	0.25 mL×2 MF59W	Yes	[redacted]	89-6280	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1 & 15	0.5 mL 1:1 MF59W:saline	Yes	[redacted]	2777-102	Available on request
Tolerability	Rabbit / Chinchilla	Intramuscular	Days 1, 15 & 29	0.25 mL×2 MF59W	Yes	Ciba-Geigy Ltd.	89-6192	Available on request
Tolerability	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.25 mL×2 1:1 MF59:vehicle	Yes	[redacted]	90-6230	Available on request

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.	Location
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59W:saline	Yes	[redacted]	2670-101	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59C.1:saline	Yes	[redacted]	759-002	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59C.1:tris	Yes	[redacted] Italy	950031	Available on request
Repeat dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15, 29 & 43	0.5 mL 1:1 MF59C.1:saline	Yes	[redacted]	656583 / 14160	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 8, 15, 29 & 43	0.5 mL MF59C.1	Yes	[redacted]	501438 / 20611	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 0, 14, 28, 42, 56 & 70	1.0 mL 1:1 MF59C.1:saline	Yes	[redacted]	00-2673	Available on request
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	12 doses over 232 days	0.5 mL 1:1 MF59W:saline	Yes	[redacted]	2670-100	Available on request
Tolerability	Dog / Beagle	Intramuscular	Days 1 & 8	0.5 mL MF59W	Yes	[redacted]	89-6281	Available on request
Tolerability	Dog / Beagle	Intramuscular	Days 1, 16 & 29	0.5 mL MF59W	Yes	Ciba-Geigy Ltd.	89-6193	4.2.1.3
Tolerability	Dog / Beagle	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59W:vehicle	Yes	[redacted]	90-6231	4.2.1.3

2.6.7.2 Toxicokinetics: Overview of Studies

Not applicable.

2.6.7.3 Toxicokinetics: Overview of Data

Not applicable.

2.6.7.4 Toxicology: Drug Substances

Test Material	Batch No.	Study Dates	Study No.	Type of Study
Cell culture-derived influenza vaccines				
FCC/H5N1 MF59+Thiomersal Saline+Thiomersal	5[redacted]OX2 A[redacted]011 C[redacted]011	[redacted]/20[redacted] to [redacted]/20[redacted]	466122	Repeat Dose Toxicity in Rabbits
Optaflu	F[redacted]_01	[redacted]/20[redacted] to [redacted]/20[redacted]	191-44	Repeat Dose Toxicity in Rabbits
Optaflu	0[redacted]011	[redacted]/20[redacted] to [redacted]/20[redacted]	UBA00037	Embryofetal/Developmental Toxicity in Rabbits
Egg-derived influenza vaccines				
Agrippal	3[redacted]5	[redacted]/20[redacted] to [redacted]/20[redacted]	191-44	Repeat Dose Toxicity in Rabbits
Fluad Fluad High B Fluad High H3 IC31	0[redacted]703 F[redacted]B08 F[redacted]A08 I[redacted]/08	[redacted]/20[redacted] to [redacted]/20[redacted]	488182	Repeat Dose Toxicity in Rabbits
Agrippal Agrippal + MF59 (water) MF59 (water)	7[redacted] L[redacted]8H1 M[redacted]838	[redacted]/19[redacted] to [redacted]/19[redacted]	940292	Repeat Dose Toxicity in Rabbits
Aflunov (Fluad H5N1)	W[redacted]4H1	[redacted]/20[redacted] to [redacted]/20[redacted]	UBA00021	Embryofetal/Developmental Toxicity in Rabbits
Agrippal MF59C.1 (citrate)	L[redacted]18B K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	14430 / 564110	Guinea Pig Sensitization
Pivotal MF59 Studies				
MF59 (water)	1[redacted]0/2	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6081	14-Day Intramuscular Study in Rabbits
MF59-0 (water)	M[redacted]906	19[redacted]	1303-001P	Developmental Toxicity in Rabbits
MF59 (water)	M[redacted]489	[redacted]/19[redacted] to [redacted]/19[redacted]	1303-002	Developmental Toxicity in Rats
MF59W.1 (water)	K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ62.502	Bacterial Reverse Mutation Assay (Water Formulation)
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ61.502	Bacterial Reverse Mutation Assay (Citrate Formulation)

Test Material	Batch No.	Study Dates	Study No.	Type of Study
MF59W.1 (water)	[redacted]W[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ62.122	Micronucleus Cytogenetic Assay (Water Formulation)
MF59C.1 (citrate)	[redacted]C[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ61.122	Micronucleus Cytogenetic Assay (Citrate Formulation)
MF59W.1 (water) MF59C.1 (citrate)	[redacted]W[redacted]003 [redacted]C[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	14465 / 564278	Guinea Pig Sensitization
Non-pivotal MF59 Studies				
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6192	Intramuscular Study in Chinchilla Rabbits
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6280	Comparative Intramuscular Tolerability Study in Rabbits
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6281	Comparative Intramuscular Tolerability Study in Dogs
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6193	Intramuscular Tolerability Study in Dogs
MF59 (water / thiomersal)	1[redacted]1/1	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6231	Comparative Intramuscular Tolerability Study in Dogs
MF59 (water / thiomersal)	1[redacted]1/1	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6230	Intramuscular Tolerability Study in Rabbits
MF59-0 (water)	M[redacted] 849	[redacted]/19[redacted] to [redacted]/19[redacted]	HWA 2670-100	8-Month Intramuscular Toxicity Study in Rabbits
MF59-0 (water)	M[redacted] 147	[redacted]/19[redacted] to [redacted]/19[redacted]	HWA 2670-101	28-Day Intramuscular Toxicity Study in Rabbits
MF59C.1 (citrate)	K[redacted]8F1	[redacted]/19[redacted] to [redacted]/19[redacted]	950031	6-Week Subacute Toxicity Study in Rabbits
MF59 (water)	K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	CHV 2777-102	14-Day Toxicity Study in Rabbits
MF59C.1 (citrate)	M[redacted]02B	[redacted]/19[redacted] to [redacted]/19[redacted]	759-002	Subchronic Toxicity in Rabbits
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	14160 / 656583	Subchronic Toxicity in Rabbits
MF59C.1 (citrate)	M[redacted]01B	[redacted]/20[redacted] to [redacted]/20[redacted]	00-2673	A Multiple Dose Safety and Tolerability in Rabbits
MF59C.1 (citrate)	2[redacted]1	[redacted]/20[redacted] to [redacted]/20[redacted]	20611 / 501438	A Multiple Dose Intramuscular Toxicity in Rabbits
MF59C.1 (citrate)	2[redacted]1	[redacted]/20[redacted] to [redacted]/20[redacted]	20717 / 501464	A Single Dose Toxicity in Rabbits
MF59C.1 (citrate)	M[redacted]01B	[redacted]/20[redacted] to [redacted]/20[redacted]	00-2672	A Single Dose Safety and Tolerability in Rabbits

2.6.7.5 Single-Dose Toxicity

Test Article: FCC/H5N1-MF59

Species / Strain	Method of Administration (Vehicle/ Formulation)	Doses	Gender and No. per Group	Observed Maximum Nonlethal Dose	Approx. Lethal Dose (mL)	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular Saline MF59 FCC/H5N1-MF59	0.5 mL	8 per sex	0.5 mL	N/A	This study is described in detail in Table 2.6.7.7. Animals were not necropsied after a single dose but in-life parameters were evaluated. There was no mortality and no adverse effects. FCC/H5N1-MF59 was locally well tolerated.	466122

2.6.7.6 Repeat-Dose Toxicity: Nonpivotal

Not applicable.

2.6.7.7 Repeat Dose Toxicity: Pivotal

Study No. 466122

Report Title: 6-week vaccine toxicity study with H5N1 FCC + MF59 + thiomersal vaccine by three intramuscular injections in New Zealand White rabbits

Test Article: FCC/H5N1-MF59

Species/Strain: Rabbit / New Zealand White

Duration of Dosing: Days 1, 15 and 29

Study No.: 466122

Initial Age: Approximately 13 weeks

Duration of Postdose: 2 days (main groups) or 14 days (recovery groups)

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Intramuscular

GLP Compliance: Yes

Vehicle/Formulation: Control article: phosphate buffered saline (PBS).
Adjuvant control: 1:1 PBS:MF59.
Test article: 15µg antigens from Indonesia/5/2005(H5N1)/PR8-IBCDC-RG02 strain with 0.25mL MF59
All test materials contained 100 µg/mL thiomersal

Location: 4.2.3.2

Special Features: Modified Draize scoring of injection sites.
Immunogenicity evaluated pretest, on days 15 and 29 (all animals), day 31 (main groups), and day 43 (recovery groups).

Study No. 466122						
	Group 1		Group 2		Group 3	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (FCC/H5N1-MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Noteworthy Findings [a]						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight	–	–	–	–	–	–
Food Consumption	–	–	–	–	–	–

[a] – No noteworthy findings

Study No. 466122						
	Group 1		Group 2		Group 3	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (FCC/H5N1-MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Clinical Observations	–	–	–	–	–	–
Heart Rate	–	–	–	–	–	–
Respiratory Rate	–	–	–	–	–	–
Body Temperatures (ºC) [a]						
Day 29 (pre-dose)	39.10 ± 0.10	39.30 ± 0.16	39.00 ± 0.28	39.08 ± 0.19*	38.85 ± 0.25	39.19 ± 0.13
Day 29 (2h post-dose)	38.81 ± 0.44	39.32 ± 0.20	38.98 ± 0.23	39.04 ± 0.19*	39.18 ± 0.19*	39.26 ± 0.15‡
Ophthalmoscopy	–	–	–	–	–	–
Serum Chemistry						
Total globulin (g/l)						
Day 17	16.6 ± 0.8	15.9 ± 1.1	17.7 ± 1.2	17.0 ± 1.2	20.3 ± 1.2††, □□	19.6 ± 1.3††, □□
Day 31	15.4 ± 0.8	15.2 ± 0.9	17.2 ± 1.1††	16.5 ± 0.9†	18.1 ± 1.1††	18.4 ± 1.8††
Albumin / globulin ratio						
Day 17	2.4 ± 0.1	2.5 ± 0.2	2.2 ± 0.1††	2.4 ± 0.1	1.9 ± 0.1††, □□	2.0 ± 0.1††, □□
Day 31	2.7 ± 0.1	2.8 ± 0.2	2.3 ± 0.1††	2.6 ± 0.2	2.3 ± 0.1††	2.3 ± 0.2††, □

[a] Mean ± standard deviation. N= 7 or 8/sex/group
* Dunnett-test based on pooled variance; groups 2 and 3 significantly different from PBS control group 1 at 5% (*) or 1% (**) level
‡ Dunnett-test based on pooled variance; group 3 significantly different from group 2 at 5% (‡) or 1% (‡‡) level
□ Steel-test; group 3 significantly different from group 2 at 5% (□) or 1% (□□) level
† Steel-test; groups 2 and 3 significantly different from PBS control group 1 at 5% (†) or 1% (††) level

Study No. 466122						
	Group 1		**Group 2**		**Group 3**	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (FCC/H5N1-MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Hematology						
Prothrombin time (seconds)						
Day 17	7.6 ± 0.3	7.7 ± 0.3	7.2 ± 0.3**	7.5 ± 0.2	7.1 ± 0.2**	7.1 ± 0.3**, ‡‡
Day 31	7.7 ± 0.3	7.7 ± 0.2	7.2 ± 0.3*	7.3 ± 0.1**	7.2 ± 0.1**	7.1 ± 0.1**, ‡
Fibrinogen level (g/l)						
Day 17	2.67 ± 0.30	2.04 ± 0.16	3.95 ± 0.86*	2.38 ± 0.33	5.44 ± 1.23**, ‡	4.12 ± 0.75**, ‡‡
Day 31	2.62 ± 0.44	2.18 ± 0.17	4.81 ± 0.71**	2.94 ± 0.56**	4.49 ± 0.75**	3.64 ± 0.54**, ‡
Organ Weights	–	–	–	–	–	–
Macroscopic Pathology	–	–	–	–	–	–
Histopathology [a]						
Main Necropsy	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Injection sites						
Right hindlimb (2 days post last dose)						
Evaluated / Affected	4 / 0	4 / 3	4 / 4	4 / 2	4 / 1	4 / 3
Intermuscular connective tissue macrophages	0	0	1++	1++	0	0

* Dunnett-test based on pooled variance; groups 2 and 3 significantly different from PBS control group 1 at 5% (*) or 1% (**) level
‡ Dunnett-test based on pooled variance; group 3 significantly different from group 2 at 5% (‡) or 1% (‡‡) level
[a] Severity score + = minimal, ++ = mild, +++ = moderate

Study No. 466122						
	Group 1		**Group 2**		**Group 3**	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (FCC/H5N1-MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Dermal hemorrhage	0	1++	0	0	1++	0
Panniculus muscle fiber degeneration	0	1+	2+, 1+++	1++	0	0
Deep epidermal acute inflammation	0	0	1+	0	0	1+, 1++
Deep dermal diffuse macrophage infiltration	0	0	1++	2++	0	3+
Panniculus focal macrophage infiltration	0	0	1++	0	0	0
Intermuscular acute inflammation	0	0	2++	0	0	0
Deep muscle focal degeneration	0	1++	0	1+	0	0
Deep muscle hemorrhage	0	1+	0	0	0	0
Deep muscle macrophage infiltration	0	1+	0	0	0	0
Left hindlimb (16 days post last dose)						
Evaluated / Affected	4 / 0	4 / 1	4 / 4	4 / 3	4 / 3	4 / 1
Intermuscular connective tissue macrophages	0	0	0	0	2+	0
Dermal hemorrhage	0	0	1+	0	0	1++
Panniculus muscle fiber degeneration	0	1+	2++	3+	2++	0

Study No. 466122						
	Group 1		**Group 2**		**Group 3**	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (FCC/H5N1-MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Deep muscle focal degeneration	0	0	1++, 1+++	0	0	0
Deep muscle hemorrhage	0	0	1++	0	0	0
Recovery Necropsy	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Injection sites						
Right hindlimb (14 days post last dose)						
Evaluated / Affected	3 / 0	4 / 2	4 / 3	4 / 2	4 / 2	3 / 2
Intermuscular connective tissue macrophages	0	0	0	0	1+	0
Panniculus muscle fiber degeneration	0	1+	1+, 1++, 1+++	1+	1++	2++
Follicular adnexa deficit	0	0	1++	0	0	0
Dermal hemorrhage	0	1+	0	1+	0	0
Superficial pustular dermatitis	0	1+	0	0	0	0
Left hindlimb (28 days post last dose)						
Evaluated / Affected	3 / 0	4 / 1	4 / 0	4 / 0	4 / 1	3 / 1
Muscle fiber degeneration	0	1+	0	0	1++	1++

Study No. 466122						
	Group 1		Group 2		Group 3	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (FCC/H5N1-MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Antibody Titers (HI)[a]						
Pretest	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)
Day 15	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	10.9 (<10 – 20)
Day 29	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – 20)	207.5 (160 – 320)	246.8 (160 – 640)
Day 31	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	269.1 (160 – 320)	190.3 (160 – 320)
Day 43	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	380.5 (320 – 640)	320.0 (160 – 640)

[a] Titers are presented as geometric mean (range). N = 8/sex/group pretest and on days 15 and 29, N = 4/sex/group on days 31 and 43. Titers shown are heterologous (HI assay using Vietnam H5N1 strain; animals were vaccinated with antigens from the Indonesian H5N1 strain). Assay against the homologous virus strain will be performed when virus is available.

2.6.7.7.1 Repeat Dose Toxicity: Supportive

Repeat-Dose Toxicity

Report Title: Two Dose Intramuscular Toxicity Study of Influenza Vaccine Formulations in New Zealand White Rabbits

Test Article: Optaflu

Species/Strain: Rabbit / New Zealand White

Duration of Dosing: Days 1 and 8

Study No.: 191-44

Initial Age: Approximately 13 weeks

Duration of Postdose: 2 or 3 days (main necropsy), 14 or 15 days (recovery necropsy)

Location in CTD: 4.2.3.2-2

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Intramuscular

Vehicle/Formulation: Phosphate buffered saline (PBS). Comparator vaccine (Agrippal) is an egg-based subunit influenza vaccine.

GLP: Yes

Special Features: Modified Draize scoring of injection sites. Immunogenicity evaluated on days 1 and 8 (all animals), days 10, 15 and 22 (males), and 11, 15 and 23 (females).

No Observed Adverse Effect Level: Not applicable

Study No. 191-44						
Dose (µg antigen per dose)	**0 (PBS Control)**		**45 (Optaflu)**		**45 (Agrippal)**	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Noteworthy Findings						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight	–[a]	–	–	–	–	–
Food Consumption	–	–	–	–	–	–
Clinical Observations	–	–	–	–	–	–
Injection sites	–	–	–	–	–	–
Ophthalmoscopy	–	–	–	–	–	–

[a] – signifies no noteworthy findings

Study No. 191-44						
Dose (µg antigen per dose)	**0 (PBS Control)**		**45 (Optaflu)**		**45 (Agrippal)**	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Hematology	**–**	**–**	**–**	**–**	**–**	**–**
Serum Chemistry	**–**	**–**	**–**	**–**	**–**	**–**
Coagulation	–	–	–	–	–	–
Organ Weights						
Thymus[a] - day 10/11	3.53 ± 0.905	3.60 ± 0.933	2.64 ± 0.173	2.92 ± 0.475	2.64 ± 0.215	2.64 ± 0.724
Thymus – day 22/23	2.91 ± 0.232	3.52 ± 1.43	3.20 ± 0.738	4.28 ± 0.294	4.33 ± 1.26	4.30 ± 0.670
Macroscopic Pathology	–	–	–	–	–	–
Histopathology – day 10/11[b]						
Injection sites						
Left (injected day 1)						
Necrosis	–	1 (2.0)	–	1 (2.0)	1 (2.0)	2 (1.5)
Right (injected day 8)						
Hemorrhage	–	–	1 (2.0)	–	–	–
Postdose Evaluation:[c]						
Number Evaluated	**3**	**3**	**3**	**3**	**3**	**3**
Histopathology – day 22/23						
Injection sites						
Left (injected day 1)						
Necrosis	–	–	–	1 (1.0)	1 (2.0)	–

[a] Mean weight ± standard deviation. Statistical analysis was not performed because N=3/sex/group at each necropsy.
[b] Histopathology findings are presented as number of animals affected (mean severity). Severity score 1.0 = minimal, 2.0 = slight, 3.0 = moderate, 4.0 = marked.
[c] Parameters were evaluated as for terminal necropsy; there were no noteworthy findings.

Study No. 191-44						
Dose (µg antigen per dose)	**0 (PBS Control)**		**45 (Optaflu)**		**45 (Agrippal)**	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Right (injected day 8)						
Hemorrhage	1 (2.0)	–	–	–	–	–
Additional Examinations[a]						
HAI Titers – B/Guangdong						
Day 1	0 ± 0	0 ± 0	3 ± 8	3 ± 8	3 ± 8	3 ± 8
Day 8	0 ± 0	7 ± 10	5 ± 12	17 ± 15	33 ± 10	37 ± 8
Day 10/11 or 15	0 ± 0	0 ± 0	123 ± 138	318 ± 266	123 ± 115	160 ± 104
Day 22/23	0 ± 0	0 ± 0	320 ± 0	427 ± 185	227 ± 101	640 ± 320
HAI Titers – A/New Caledonia						
Day 1	0 ± 0	0 ± 0	0 ± 0	3 ± 8	0 ± 0	3 ± 8
Day 8	0 ± 0	18 ± 20	13 ± 16	27 ± 33	47 ± 30	50 ± 40
Day 10/11 or 15	10 ± 17	0 ± 0	47 ± 39	127 ± 109	37 ± 27	70 ± 47
Day 22/23	0 ± 0	0 ± 0	267 ± 92	360 ± 262	120 ± 40	426 ± 185
HAI Titers – A/Panama						
Day 1	67 ± 20	60 ± 22	67 ± 16	57 ± 27	53 ± 16	47 ± 16
Day 8	93 ± 55	80 ± 44	60 ± 22	93 ± 55	126 ± 53	120 ± 36
Day 10/11 or 15	67 ± 48	87 ± 39	73 ± 16	140 ± 33	107 ± 41	107 ± 41
Day 22/23	33 ± 12	40 ± 0	133 ± 46	213 ± 93	160 ± 0	160 ± 0

[a] Titers are presented as mean ± standard deviation

Species / Strain	Method of Administration (Formulation)	Duration of Dosing	Doses	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular Group 1: Saline Group 2: Fluad Group 3: Fluad High B Group 4: Fluad High H3+IC31	3 doses administered 2 weeks apart main necropsy: 2 days post last dose recovery necropsy: 14 days post last dose	**Fluad** 45 µg HA + 0.25 mL MF59 **Fluad High B** 60 µg HA + 0.25 mL MF59 **Fluad High H3+IC31** 60 µg HA + 0.25 mL MF59 + 0.5 mL IC31	8 per sex per group	There was no mortality, and no treatment-related effects on body weights, body weight gain, food consumption, or clinical signs in any group. There were no in-life dermal irritation or ophthalmoscopic findings, and no treatment-related effects on body temperatures, heart rates, and respiratory rates based on evaluable data. Treatment-related effects on hematology parameters in groups 2, 3 and 4 included increased fibrinogen levels, and slight decreases in prothrombin (PT) times. At the main necropsy (day 31), enlarged iliac lymph nodes in all treated males and in 3 group 2, 3 group 3, and 1 group 4 female(s) were noted, and 2 group 2 females had enlarged popliteal lymph nodes. At the recovery necropsy (day 43), one group 3 male and one group 4 female had enlarged iliac lymph nodes. Histopathology of the injection sites showed that administration of the vaccine formulations was associated with a low degree of inflammation, with no significant differences in the incidence or severity of findings between the three vaccine formulations.	488182 GLP

Species / Strain	Method of Administration (Formulation)	Duration of Dosing	Doses	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59 Agrippal Agrippal + MF59	2 doses administered 2 weeks apart main necropsy: 2 days post last dose recovery necropsy: 14 days post last dose	**MF59** 0.25 mL saline + 0.25 mL MF59 **Agrippal** 45 µg HA **Agrippal+MF59 (Fluad)** 45 µg HA + 0.25 mL MF59	6 per sex per group	There was no mortality, and no treatment related effects on physical appearance, behavior, clinical signs, in-life injection site reactions, body temperatures, body weight gain, ophthalmology, hematology, or clinical chemistry. There were no relevant differences between groups in organ weights at either necropsy, and no macroscopic observations except for the injection sites. Macroscopic findings at the injection sites treated two days previously indicated an increased frequency of slight focal hemorrhage in the Agrippal+MF59 group compared to the other two groups. There were no macroscopic findings at injection sites treated 16 or 30 days before necropsy. Histological examination of the injection site 2 days post-injection revealed interstitial inflammation (mainly acute), interstitial hemorrhage, and / or muscle fiber degeneration in almost all animals. These observations were more notable in the Agrippal+MF59 group, followed by the Agrippal group, then the MF59 group. Sixteen days after injection, inflammatory and degenerative changes were still present, but to a lesser extent in most animals. Thirty days after injection, partial to full recovery was evident in most animals.	940292 GLP

2.6.7.8 Genotoxicity: in vitro

Not applicable.

2.6.7.9 Genotoxicity: in vivo

Not applicable.

2.6.7.10 Carcinogenicity

Not applicable.

2.6.7.11 Reproductive and Developmental Toxicity: Nonpivotal

Not applicable.

2.6.7.12 Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development

Not applicable.

2.6.7.13 Reproductive and Developmental Toxicity: Effects on Embryofetal Development

Not applicable.

2.6.7.13.1 Reproductive and Developmental Toxicity: Effects on Embryofetal Development - Supportive

Study No. UBA00037 (Caesarean Section Evaluations)

Report Title: Intramuscular reproductive and developmental toxicity study of FCC vaccine in rabbits, including a postnatal evaluation

Test Article: Optaflu

Design similar to ICH 4.1.3? Yes

Study No.: UBA00037

Species/Strain: Rabbit/Hra: (NZW)SPF

Duration of Dosing: 5 doses over 55 days (days 1, 15 & 29 prior to mating, days 7 & 20 of gestation)

Location: 4.2.3.5

Initial Age: Approximately 7.5 months

GLP Compliance: Yes

Special Features: Single study performed to evaluate toxic effects of vaccine administration before cohabitation, through mating, gestation and lactation. This table describes C-sectioned groups; see table in section 2.6.7.14 for details regarding natural delivery groups.

No Observed Adverse Effect Level: Not determined

F_0Males: 0

F_0Females: 96

F_1Litters: 87/96 matings

Date of First Dose: [redacted] 20[redacted] – [redacted] 20[redacted]

Day of Mating: [redacted] 20[redacted] – [redacted] 20[redacted] (SD 36 = DG 0)

Day of C-Section: [redacted] 20[redacted] – [redacted] 20[redacted] (DG 29)

Method of Administration: Intramuscular

Formulation: Test article: 45 µg trivalent inactivated influenza surface antigen [15 µg each A/New Caledonia/20/99, A/New York 55/2004x-157, and B/Jiangsu/10/2003] in 0.5 mL phosphate buffered saline, lot number 008011A. Control article: 0.9% sodium chloride injection USP.

Study Number UBA00037 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Males: not evaluated; males were used for breeding only and were not treated	Not Applicable	Not Applicable
Does:		
No. Evaluated	24	24
No. Pregnant	23	24

Study Number UBA00037 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
No. Died or Sacrificed Moribund	0	0
No. Aborted	2 (8.7%)	4 (16.7%)
No. with Total Resorption of Litter	0	1
Clinical Observations	—	—
Necropsy Observations	—	—
Body Weight	—	—
Food Consumption	—	—
Number Pregnant and C-sectioned on Day 29 of Gestation	21	20
Mean No. Corpora Lutea (Mean ± SD)	9.2 ± 1.9	8.9 ± 2.5
Mean No. Implantations (Mean ± SD)	7.5 ± 2.9	7.0 ± 2.8
Litter Sizes (Mean ± SD)	7.0 ± 2.6	6.6 ± 2.8
Live fetuses (Mean ± SD)	7.0 ± 2.6	6.6 ± 2.8
Dead fetuses	0	0
Resorptions (Mean ± SD)	0.4 ± 0.7	0.4 ± 0.7
Early resorptions (Mean ± SD)	0.4 ± 0.7	0.4 ± 0.7
Late Resorptions	0	0
Does with any resorptions	7	5
% Resorbed Conceptuses per Litter (Mean ± SD)	4.7 ± 8.0	3.5 ± 7.8

Study Number UBA00037 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Litters:		
No. Litters with One or More Live Fetuses	21	19
No. Live Fetuses (total)	148	132
Live Fetal Body Weight (Grams) per Litter (Mean ± SD)	39.76 ± 9.08	39.75 ± 9.58
% Live Male Fetuses per Litter (Mean ± SD)	57.5 ± 19.6	50.1 ± 24.6
Fetal Anomalies:		
Litters with fetuses with any alterations observed N (%)	15 (71.4)	13 (68.4)
Fetuses with any alterations observed N (%)	38 (25.7)	32 (24.2)
Fetuses with any alteration / Litter (Mean ± SD)	24.0 ± 18.6	22.2 ± 21.8
Gross External Anomalies		
Tail: Thread-like Litter incidence N (%) Fetal incidence N (%)	 1 (4.8) 1 (0.7)	 0 (0.0) 0 (0.0)
Fore and/or hind limbs flexed Litter incidence N (%) Fetal incidence N (%)	 0 (0.0) 0 (0.0)	 1 (5.3) 1 (0.8)
Soft Tissue Alterations		
Vessels: Right subclavian arises to the left of the left subclavian Litter incidence N (%) Fetal incidence N (%) Affected fetus (9531-5) had other soft tissue alterations	 0 (0.0) 0 (0.0)	 1 (5.3) 1 (0.8)
Vessels: Right subclavian passes dorsal to the trachea and esophagus Litter incidence N (%) Fetal incidence N (%) Affected fetus (9531-5) had other soft tissue alterations	 0 (0.0) 0 (0.0)	 1 (5.3) 1 (0.8)

Study Number UBA00037 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Vessels: Innominate artery absent Litter incidence N (%) Fetal incidence N (%) Affected fetus (9531-5) had other soft tissue alterations	 0 (0.0) 0 (0.0)	 1 (5.3) 1 (0.8)
Lungs: Intermediate lobe absent Litter incidence N (%) Fetal incidence N (%)	 1 (4.8) 3 (2.0)	 0 (0.0) 0 (0.0)
Skeletal Anomalies		
Skull: Irregular ossification (sum of irregular ossification of the skull subcategories listed below) Litter incidence N (%) Fetal incidence N (%)	 11 (52.4) 21 (14.2)	 9 (47.4) 13 (9.8)
Skull: Nasals, Contain an internasal Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9509-4 and 9538-4) had other skeletal alterations	 5 (23.8) 6 (4.0)	 1 (5.3) 1 (0.8)
Skull: Nasals, Midline suture displaced Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9522-2 and 9522-4) had other skeletal alterations	 10 (47.6) 15 (10.1)	 9 (47.4) 12 (9.1)
Hyoid: Body small Litter incidence N (%) Fetal incidence N (%)	 0 (0.0) 0 (0.0)	 2 (10.5) 3 (2.3)
Hyoid: Body not ossified Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9511-5, 9519-1, 9519-5, 9519-8, 9521-3, 9522-2, 9538-4, and 9540-7) had other skeletal alterations	 5 (23.8) 8 (5.4)	 4 (21.0) 4 (3.0)

Study Number UBA00037 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Hyoid: Ala angulated Litter incidence N (%) Fetal incidence N (%)	 1 (4.8) 1 (0.7)	 2 (10.5) 3 (2.3)
Thoracic vertebrae: Hemivertebra Litter incidence N (%) Fetal incidence N (%) Affected fetus (9521-1) had other skeletal alterations	 1 (4.8) 1 (0.7)	 0 (0.0) 0 (0.0)
Thoracic vertebrae: centrum bifid Litter incidence N (%) Fetal incidence N (%) Affected fetus (9521-1) had other skeletal alterations	 1 (4.8) 1 (0.7)	 0 (0.0) 0 (0.0)
Caudal vertebrae: Bifid Litter incidence N (%) Fetal incidence N (%)	 0 (0.0) 0 (0.0)	 1 (5.3) 1 (0.8)
Caudal vertebrae: Misaligned Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9522-4 and 9535-6) had other skeletal alterations	 1 (4.8) 1 (0.7)	 2 (10.5) 2 (1.5)
Caudal vertebrae: 4 present Litter incidence N (%) Fetal incidence N (%) Affected fetus (9509-4) had other skeletal alterations	 1 (4.8) 1 (0.7)	 0 (0.0) 0 (0.0)
Ribs: Fused proximally Litter incidence N (%) Fetal incidence N (%) Affected fetus (9521-1) had other skeletal alterations	 1 (4.8) 1 (0.7)	 1 (5.3) 1 (0.8)
Manubrium: Small Litter incidence N (%) Fetal incidence N (%) Affected fetus (9538-4) had other skeletal alterations	 1 (4.8) 1 (0.7)	 1 (5.3) 1 (0.8)

Study Number UBA00037 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Sternal centra: incompletely ossified Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9511-5, 9519-1, 9519-5, 9519-8, 9521-3, 9526-7, 9535-6 and 9540-7) had other skeletal alterations	 6 (28.6) 11 (7.4)	 6 (31.6) 8 (6.1)
Sternal centra: fused Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9522-2 and 9538-4) had other skeletal alterations	 1 (4.8) 1 (0.7)	 1 (5.3) 1 (0.8)
Total Affected Fetuses (Litters)	38 (15)	32 (13)
Immunogenicity: Hemagglutination Inhibition [a] titers		
Maternal sera [b] geometric mean (range)		
Pre-study	Not tested	<10 (<10 – <10)
Study day 15	Not tested	<10 (<10 – 40)
Study day 29	Not tested	320.0 (160 – 1280)
Gestation day 7	Not tested	269.1 (80 – 1280)
Gestation day 20	Not tested	519.2 (160 – 1280)
Gestation day 29	<10 (<10 – <10)	226.3 (40 – 640)
Pooled fetal sera [c] geometric mean (range)		
Gestation day 29	Not tested	293.4 (80 – 1280)

[a] A representative subset of samples was tested against the A/New Caledonia strain only. For calculations a value of 5 was assigned where titer was <10.
[b] Mean of titers from N=8 does, and where applicable, their fetuses.
[c] Mean of titers from N=8 pools, each pool made up of sera from fetuses from a single doe.

Study No. UBA00021 (Caesarean Section Evaluations)

Report Title: Intramuscular reproductive and developmental toxicity study of Fluad H5N1 in rabbits, including a postnatal evaluation

Test Article: Aflunov

Design similar to ICH 4.1.3? Yes

Study Number: UBA00021

Species/Strain: Rabbit / Hra:(NZW)SPF

Duration of Dosing: 5 doses over 55 days (days 1, 15 & 29 prior to mating, days 7 & 20 of gestation

Location: 4.2.3.5

Initial Age: Approximately 7 months

GLP Compliance: Yes

Special Features: Single study performed to evaluate effects of vaccine administration before cohabitation, through mating, gestation and lactation. This table describes C-sectioned groups; see table in section 2.6.7.14 for details regarding natural delivery groups.

No Observed Adverse Effect Level: Not determined

Date of First Dose: [redacted] 20[redacted] – [redacted] 20[redacted]

F_0Males: 0

Day of Mating: [redacted] 20[redacted] – [redacted] 20[redacted] (SD 36 = DG 0)

F_0Females: 80

Day of C-Section: [redacted] 20[redacted] – [redacted] 20[redacted] (DG 29)

F_1Litters: 69/80 matings

Method of Administration: Intramuscular

Formulation: Test article: 0.5 mL containing 15 µg monovalent influenza surface antigen [NIBRG-14; A/Vietnam/1194/2004-like strain] inactivated and adjuvanted 0.25 mL MF59C.1. Control article: 0.5 mL saline.

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Males: Not evaluated; males were used for breeding only and were not treated	Not Applicable	Not Applicable
Dams/Does:		
No. Evaluated	20	20
No. Pregnant	18	16
No. Died or Sacrificed Moribund	0	0
No. Aborted or with Total Resorption of Litter	0	0
Clinical Observations Related to Test Article Administration	none	none

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Mean No. Corpora Lutea (Mean ± SD)	9.0 ± 2.0	8.6 ± 2.0
Mean No. Implantations (Mean ± SD)	7.9 ± 2.4	7.6 ±2.5
Litter sizes (Mean ± SD)	6.9 ± 2.3	6.4 ±3.0
Live fetuses (Mean ± SD)	6.9 ± 2.3	6.4 ±3.0
Dead fetuses (Mean ± SD)	0	0
Resorptions (Mean ± SD)	0.9 ± 0.9	1.2 ± 2.1
Early resorptions (Mean ± SD)	0.5 ± 0.8	1.2 ± 2.1
Late Resorptions (Mean ± SD)	0.4 ± 0.6	0.0 ± 0.0*
Does with any resorptions	11	8
% Resorbed Conceptuses per Litter (Mean ± SD)	11.7 ± 12.3	16.8 ± 25.7
Body Weights and Food Consumption[a]		
Body Weight (%) at end of premating period	3.96 ± 0.46 kg	102
Body Weight (%) at end of gestation period	4.12 ± 0.44 kg	104
Premating Absolute Food Consumption (%)	127.0 ± 27.2 g/day	105
Premating Relative Food Consumption (%)	32.6 ± 5.0 g/kg/day	104
Gestation Absolute Food Consumption (%)	118.2 ± 22.2 g/day	110
Gestation Relative Food Consumption (%)	29.0 ± 5.0 g/kg/day	108

* $p \leq 0.05$
[a] For controls, group means are shown. For treated groups, percent differences from controls are shown.

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Doe necropsy observations	None	None
Litters:		
No. Litters Evaluated	18	16
No. Live Fetuses (total)	125	102
Fetal Body Weight in Grams (Mean ± SD)	43.71 ± 6.35	47.58 ± 5.88
% Live Male Fetuses per Litter (Mean ± SD)	59.9 ± 27.4	53.3 ± 27.5
Fetal Anomalies:		
Litters with fetuses with any alterations observed N (%)	6 (33.3)	5 (31.2)
Fetuses with any alterations observed N (%)	11 (8.8)	10 (9.8)
Fetuses with any alteration / Litter (Mean ± SD)	8.7 ± 14.5	10.9 ± 19.0
Gross External Anomalies		
Fore and/or hind limbs flexed Litter incidence N (%) Fetal incidence N (%)	 1 (5.6) 1 (0.8)	 0 (0.0) 0 (0.0)
Abdominal distention Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other gross external alterations.	 1 (5.6) 1 (0.8)	 0 (0.0) 0 (0.0)
Edema Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other gross external alterations.	 1 (5.6) 1 (0.8)	 0 (0.0) 0 (0.0)
Soft Tissue Alterations		

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Vessels: Aorta distended Litter incidence N (%) Fetal incidence N (%) Affected fetus (9821-3) had other soft tissue alterations.	0 (0.0) 0 (0.0)	1 (6.2) 1 (1.0)
Lungs: intermediate lobe absent Litter incidence N (%) Fetal incidence N (%) Group II affected fetus (9821-3) had other soft tissue alterations.	1 (5.6) 1 (0.8)	1 (6.2) 2 (2.0)
Lungs: small Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other gross external alterations.	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Kidneys: Low set Litter incidence N (%) Fetal incidence N (%)Affected fetus (9821-3) had other soft tissue alterations.	0 (0.0) 0 (0.0)	1 (6.2) 1 (1.0)
Thoracic cavity: Contained clear pink fluid Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other gross external alterations.	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Skeletal Anomalies		
Skull: Irregular ossification (summarization of all skull ossification findings listed below) Litter incidence N (%) Fetal incidence N (%)	3 (16.7) 6 (4.8)	3 (18.8) 5 (4.9)
Skull: Nasal(s), Irregular ossification (summarization of findings below) Litter incidence N (%) Fetal incidence N (%)	3 (16.7) 5 (4.0)	2 (12.5) 4 (3.9)
Skull: Nasals, Contain an internasal Litter incidence N (%) Fetal incidence N (%)	2 (11.1) 2 (1.6)	2 (12.5) 3 (2.9)

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Skull: Nasals, Midline suture displaced Litter incidence N (%) Fetal incidence N (%) Group I fetus (9815-5) had other skeletal alterations.	1 (5.6) 2 (1.6)	1 (6.2) 1 (1.0)
Skull: Nasal, frontal suture irregular Litter incidence N (%) Fetal incidence N (%)	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Skull: Frontals fused Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other skeletal alterations.	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Skull: Interparietals, incompletely ossified Litter incidence N (%) Fetal incidence N (%) Affected fetus (9821-3) had other skeletal alterations.	0 (0.0) 0 (0.0)	1 (6.2) 1 (1.0)
Hyoid: Ala, angulated Litter incidence N (%) Fetal incidence N (%) Group I affected fetus (9818-7) had other skeletal alterations.	2 (11.1) 3 (2.4)	2 (12.5) 3 (2.9)
Thoracic vertebrae: Centrum, unilateral ossification Litter incidence N (%) Fetal incidence N (%) Affected fetuses (9818-7, 9821-3) had other skeletal alterations.	1 (5.6) 1 (0.8)	1 (6.2) 1 (1.0)
Sacral vertebrae: centrum, unilateral ossification Litter incidence N (%) Fetal incidence N (%) Affected fetus (9821-3) had other skeletal alterations.	0 (0.0) 0 (0.0)	1 (6.2) 1 (1.0)
Manubrium: Irregularly shaped Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other skeletal alterations.	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Sternal centra: incompletely ossified Litter incidence N (%) Fetal incidence N (%) Affected fetus (9821-3) had other skeletal alterations.	0 (0.0) 0 (0.0)	1 (6.2) 1 (1.0)
Sternal centra: fused Litter incidence N (%) Fetal incidence N (%) Group I affected fetuses (9815-5, 9818-7) had other skeletal alterations.	2 (11.1) 2 (1.6)	1 (6.2) 1 (1.0)
Sternal centra: Large Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other skeletal alterations.	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Sternal centra: Asymmetric Litter incidence N (%) Fetal incidence N (%)	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Scapulae: Ala, Irregularly shaped Litter incidence N (%) Fetal incidence N (%) Affected fetus (9818-7) had other skeletal alterations.	1 (5.6) 1 (0.8)	0 (0.0) 0 (0.0)
Total Affected Fetuses (Litters)	11 (6)	10 (5)

Study Number UBA00021 – Caesarean Sectioned Groups		
Group Number	**I**	**II**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Immunogenicity: Titer (hemagglutination inhibition) summary		
Maternal sera, geometric mean (range) of N=8 does		
Pre-study	Not tested	<10 (<10 – <10)
Observation day 15	Not tested	87.2 (40 – 160)
Observation day 29	Not tested	2152.7 (1280 – 5120)
Gestation day 7	Not tested	1974.0 (1280 – 5120)
Gestation day 20	Not tested	1660.0 (640 – 2560)
Gestation day 29	<10 (<10 – <10)	905.1 (320 – 2560)
Pooled fetal sera, geometric mean (range) of N=8 litters		
Gestation day 29	Not tested	1299.0 (480-2560)

2.6.7.14 Reproductive and Developmental Toxicity: Effects on Pre-and Postnatal Development

Not applicable.

2.6.7.14.1 Reproductive and Developmental Toxicity: Effects on Pre-and Postnatal Development – Supportive

Study No. UBA00037 (Natural Delivery Evaluation)

Report Title: Intramuscular reproductive and developmental toxicity study of FCC vaccine in rabbits, including a postnatal evaluation

Test Article: Optaflu

Design similar to ICH 4.1.2? Yes

Study No.: UBA00037

Species/Strain: Rabbit/Hra: (NZW)SPF

Duration of Dosing: 5 doses over 55 days (days 1, 15 & 29 prior to mating, days 7 & 20 of gestation)

Location: 4.2.3.5

Initial Age: Approximately 7.5 months

GLP Compliance: Yes

Special Features: Single study performed to evaluate toxic effects of vaccine administration before cohabitation, through mating, gestation and lactation. This table describes natural delivery groups; see table in section 2.6.7.13 for details regarding C-sectioned groups.

No Observed Adverse Effect Level: Not determined

Date of First Dose: [redacted] 20[redacted] – [redacted] 20[redacted]

F_0Males: 0

Day of Mating: [redacted] 20[redacted] – [redacted] 20[redacted] (SD 36 = DG 0)

F_0Females: 96

Day of C-Section: [redacted] 20[redacted] – [redacted] 20[redacted] (DG 29)

F_1Litters: 87/96 matings

Method of Administration: Intramuscular

Formulation: Test article: 45 µg trivalent inactivated influenza surface antigen [15 µg each A/New Caledonia/20/99, A/New York 55/2004x-157, and B/Jiangsu/10/2003] in 0.5 mL phosphate buffered saline, lot number 008011A. Control article: 0.9% sodium chloride injection USP.

Study Number UBA00037 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Males: not evaluated; males were used for breeding only and were not treated	Not Applicable	Not Applicable
No. Evaluated	0	0
Females		
No. Evaluated	24	24
No. Died or Sacrificed Moribund	4	2

Study Number UBA00037 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
No. Aborted	0	2
No. of Pregnant/Mated Females	19/23	21/23
Live fetuses / pups delivered	102 (6.4 ± 2.6 per litter)	103 (6.9 ± 2.4 per litter)
Dead fetuses / stillborn pups	11 (0.7 ± 2.0 per litter)	3 (0.2 ± 0.6 per litter)
Clinical Observations	—	—
Necropsy Observations	—	—
Body Weight	—	—
Food Consumption	—	—
F_1 Litters:		
No. Litters Included in Analysis	14	15
Total number of pups delivered	113	106
No. Pups/Litter (Mean ± SD)	7.1 ± 2.6	7.1 ± 2.2
No. Liveborn Pups/Litter (Mean ± SD)	6.4 ± 2.6	6.9 ± 2.4
No. of Litters With No Liveborn Pups N (%)	0 (0.0)	1 (6.2)
No. of Litters with Stillborn Pups N (%)	3 (18.8)	3 (18.8)
Postnatal Survival to Day 5 N/N (%)	88/94 (93.6)	102/103 (99.0)
Litters with All Pups Dying Days 1-4 Postpartum N (%)	1 (6.2)	0 (0.0)
Postnatal Survival to weaning Day 29 N/N (%)	86/88 (97.7)	91/102 (89.2)
Pup Body Weights / Litter on Day 5 (grams)	91.8 ± 26.6	88.4 ± 23.5
Pup Body Weights / Litter on Day 29 (grams)	560.0 ± 156.6	506.3 ± 113.8
Pup Sex Ratio (% males on Day 5)	63.2 ± 25.6	53.9 ± 22.8

Study Number UBA00037 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Reflex and Physical Development [a]		
Hair growth (Day on which criterion met, mean ± SD)	5.0 ± 0.0	5.0 ± 0.0
Eye opening (Day on which criterion met, mean ± SD)	9.9 ± 1.1	10.6 ± 0.8 *
Air righting (Day on which criterion met, mean ± SD)	12.0 ± 1.8	12.5 ± 1.4
Acoustic startle (Day on which criterion met, mean ± SD)	14.6 ± 0.8	14.5 ± 0.9
Pupil constriction (Day on which criterion met, mean ± SD)	22.0 ± 0.0	22.0 ± 0.0
Pup Necropsy Observations		
Litters evaluated N	17	16
Total pups stillborn, found dead or euthanized [b]	21	14
Stillborn	11	2
Found dead	8	11
Euthanized	2	1
No milk in stomach [c] N (%)	8 (100.0)	10 (90.9)
Pups Sacrificed and Necropsied on Day 1 or day 29 of lactation		
Litters evaluated N	15	15
Pups evaluated N	89	91

[a] Criterion day = average day postpartum that at least 50% of all pups in a litter met the criterion
* Significantly different from Group III ($p \leq 0.05$)
[b] Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full evaluation.
[c] Analysis restricted to pups found dead and necropsied

Study Number UBA00037 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days 1, 15, and 29 and gestation days 7 and 20	**0 µg**	**45 µg**
Appeared normal – Litter incidence N (%)	14 (93.3)	14 (93.3)
Appeared normal – Pup incidence N (%)	88 (98.9)	90 (98.9)
Brain, ventricle, moderate dilation – Litter incidence N (%)	0 (0.0)	1 (6.7)
Brain, ventricle, moderate dilation – Pup incidence N (%)	0 (0.0)	1 (1.1)
Accessory spleen – Litter incidence N (%)	1 (6.7)	0 (0.0)
Accessory spleen – Pup incidence N (%)	1 (1.1)	0 (0.0)
Immunogenicity: Hemagglutination Inhibition [a] titers		
Maternal sera [b] geometric mean (range)		
Pre-study	Not tested	Not tested
Study day 15	Not tested	Not tested
Study day 29	Not tested	Not tested
Gestation day 7	Not tested	Not tested
Gestation day 20	<10 (<10 – <10)	367.1 (160 – 1280)
Gestation day 29	Not tested	Not tested
Lactation day 29	<10 (<10 – <10)	56.6 (40 – 160)
Pup sera [c] geometric mean (range)		
Gestation day 29	Not tested	17.6 (<10 – 80)

[a] A representative subset of samples was tested against the A/New Caledonia strain only. For calculations a value of 5 was assigned where titer was <10.
[b] Mean of titers from N=8 does, and where applicable, their pups.
[c] Mean of titers from N=59 individual pups.

Study No. UBA00021 (Natural Delivery Evaluations)

Report Title: Intramuscular reproductive and developmental toxicity study of Fluad H5N1 in rabbits, including a postnatal evaluation

Test Article: Aflunov

Design similar to ICH 4.1.2? Yes

Study Number: UBA00021

Species/Strain: Rabbit / Hra:(NZW)SPF

Duration of Dosing: 5 doses over 55 days (days 1, 15 & 29 prior to mating, days 7 & 20 of gestation

Location: 4.2.3.5

Initial Age: Approximately 7 months

GLP Compliance: Yes

Special Features: Single study performed to evaluate effects of vaccine administration before cohabitation, through mating, gestation and lactation. This table describes natural delivery groups; see table in section 2.6.7.13 for details regarding C-sectioned groups.

No Observed Adverse Effect Level: Not determined

F_0Males: 0

F_0Females: 80

F_1Litters: 69/80 matings

Date of First Dose: [redacted] 20[redacted] – [redacted] 20[redacted]

Day of Mating: [redacted] 20[redacted] – [redacted] 20[redacted] (SD 36)

Day of C-Section: [redacted] 20[redacted] – [redacted] 20[redacted] (DG 29)

Method of Administration: Intramuscular

Formulation: Test article: 0.5 mL containing 15 µg monovalent influenza surface antigen [NIBRG-14; A/Vietnam/1194/2004-like strain] adjuvanted with 0.25 mL MF59C.1. Control article: 0.5 mL saline.

Study Number UBA00021 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Males: Not evaluated; males were used for breeding only and were not treated	Not Applicable	Not Applicable
Females		
No. Evaluated	20	20
No. Died or Sacrificed Moribund	0	0
No. of Pregnant Females	17	18
Live fetuses / pups delivered	6.8 ± 2.6	6.2 ± 2.7
Dead fetuses / stillborn pups	0.5 ± 1.3	0.4 ± 1.1

Study Number UBA00021 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Clinical Observations	**–**	**–**
Necropsy Observations	**–**	**–**
Body weights and Food Consumption [a]		
Body Weight (%) at end of premating period	4.07 ± 0.38 kg	99
Body Weight (%) at end of gestation period	4.43 ± 0.39 kg	94
Premating Absolute Food Consumption (%)	136.0 ± 14.4 g/day	97
Premating Relative Food Consumption (%)	34.5 ± 2.0 g/kg/day	98
Gestation Absolute Food Consumption (%)	128.5 ± 28.0 g/day	96
Gestation Relative Food Consumption (%)	29.6 ± 5.2 g/kg/day	101
F_1 Litters		
No. Litters Evaluated	17	18
Total number of pups delivered	125	107
No. Pups/Litter (Mean ± SD)	7.4 ± 2.3	6.7 ± 2.8
No. Liveborn Pups/Litter (Mean ± SD)	6.8 ± 2.6	6.2 ± 2.7
No. of Litters With No Liveborn Pups N (%)	0 (0)	2 (11.1)
No. of Litters with Stillborn Pups N (%)	3 (17.6)	5 (27.8)
Postnatal Survival to Day 5 N/N (%)	94/116 (81)	89/100 (89)
Litters with All Pups Dying Days 1-4 Postpartum N (%)	3 (17.6)	1 (6.2)

[a] For controls, group means are shown. For treated groups, percent differences from controls are shown.

Study Number UBA00021 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Postnatal Survival to Day 29 N/N (%)	87/116 (75)	83/100 (83)
Change in Pup Body Weights (%) Average weight of pup on Day 29 as a percent of weight on Day 5.	591	629
Pup Sex Ratio (% males on Day 5)	46.4	56.4
Pup Clinical Signs[b]		
Both hindlimbs, both hindpaws, left forelimb, head and/or tip of tail: purple or black	6/3	16/2
Cold to touch	5/3	7/2
Decreased motor activity	1/1	4/2
Limited use of both hindlimbs or right forelimb	0/0	3/2
Hindlimbs, back and/or caudal dorsal area: Scab(s)	0/0	3/2
Not nursing	8/ 3	1/ 1
Not nesting	6/2	1/1
Perianal region: urine- and feces-stained	0/0	2/1
Right shoulder: nip injury	0/0	1/1
Red perianal substance	0/0	1/1
Ungroomed coat	0/0	1/1
Right forelimb: red substance present	0/0	1/1
Dehydrated	6/2	0/0
No milk band present	2/1	0/0
Yellow perinasal substance	1/1	0/0

[b] Total frequency of observation (Days × Pups) / Litters with observations**.**

Study Number UBA00021 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Persistent Clinical Observations[c]		
Tip of tail: missing	0/0	10/1
Left eye: enophthalmos	12/1	0/0
Reflex and Physical Development[d]		
Hair growth (Criterion day, mean ± SD)	5.0 ± 0.0	5.0 ± 0.0
Eye opening (Criterion day, mean ± SD)	10.8 ± 0.5	10.8 ± 1.1
Air righting (Criterion day, mean ± SD)	12.4 ± 1.4	12.0 ± 1.8
Acoustic startle (Criterion day, mean ± SD)	14.1 ± 0.4	14.1 ± 0.4
Pupil constriction (Criterion day, mean ± SD)	22.0 ± 0.0	22.0 ± 0.0
Pup Necropsy Observations[e]		
Total pups stillborn, found dead or euthanized due to adverse clinical observations	38	37
Stillborn	9	20**
Found dead	28	13**
Euthanized	1	4
No milk in stomach N (%) among pups found dead or euthanized due to adverse clinical observations and necropsies. Presence of milk in the stomach was not documented for 4 pups.	24 (96.0)	10 (76.9)
Stomach: contained black gelatinous material N (%)	1 (2.6)	0 (0.0)

[c] Total frequency of observation (Days × Pups) / Litters with observations**.** Tabulation restricted to adverse observations; all other pups appeared normal.
[d] Criterion day = average day postpartum that at least 50% of all pups in a litter met the criterion
[e] Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full observation.

** $p \leq 0.01$

Study Number UBA00021 – Natural Delivery Groups		
Group Number	**III**	**IV**
Dosing Days: study days (SD) 1, 15, and 29 and gestation days (GD) 7 and 20	**0 µg**	**15 µg**
Stomach: numerous dark brown areas N (%)	1 (2.6)	0 (0.0)
Abdominal wall: dark red N (%)	0 (0.0)	1 (2.7)
Right forelimb: humerus, complete fracture N (%)	0 (0.0)	1 (2.7)
Immunogenicity: Titer (hemagglutination inhibition assay) summary		
Maternal sera, geometric mean (range) of N=8 does		
Gestation day 20	Not tested	1660.0 (1280-2560)
Lactation day 29	<10 (<10 - <10)	336.6 (160-640)
Group IV, Lactation Day 29 Pup sera, geometric mean (range)		
Litter number 9862 (9 pups)	Not tested	148.1 (80-160)
Litter number 9864 (8 pups)	Not tested	123.4 (80-160)
Litter number 9865 (8 pups)	Not tested	95.1 (80-160)
Litter number 9871 (6 pups)	Not tested	71.3 (40-80)
Litter number 9873 (6 pups)	Not tested	89.8 (80-160)
Litter number 9875 (7 pups)	Not tested	160.0 (160-160)
Litter number 9878 (7 pups)	Not tested	40.0 (40-40)
Litter number 9879 (8 pups)	Not tested	146.7 (80-320)

2.6.7.15 Studies in Juvenile Animals

Not applicable.

2.6.7.16 Local Tolerance

Species/ Strain	Method of Administration	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular Saline MF59 FCC/H5N1-MF59	0.5 mL on days 1, 15 & 29 into alternate hindlimbs 4/sex necropsied days 31 & 43	8/sex	Local tolerance was evaluated during the repeat dose toxicity study (described in Table 2.6.7.7). There were no clinical signs (including Draize scoring) at any of the injection sites. Histopathological findings at the injection sites consisted of the expected inflammatory changes associated with intramuscular injections. The findings in all groups included inflammation, infiltration and hemorrhage. For details see 2.6.6.3 and 2.6.7.7.	466122 GLP

2.6.7.17 Other Toxicity

2.6.7.17.1 Sensitization Study with Fluad

Species/ Strain	Method of Administration	Duration of Dosing	Doses	Gender and No. per Group	Noteworthy Findings	Study No.
Guinea Pig / Dunkin-Hartley	Induction phase: Intradermal and topical Challenge phase: Topical	Day 1 Intradermal induction Day 7 Topical induction Day 21 Topical challenge	Dose ranging: 100, 75, 50, 25, 10, 5, 2 and 1% solutions of Fluad. Topical induction: 0.5 mL of saline, Fluad (undiluted, per dose ranging study) Challenge: topical administration of 0.5 mL saline, Fluad (undiluted, per dose ranging study) Main study: Intradermal induction phase – duplicate 0.1 mL injections of: 50% aqueous FCA, 1:1 (v/v) Fluad:0.9% saline, & 1:1 (v/v) Fluad:50% aqueous FCA Controls received 50% aqueous FCA, 0.9% saline, & 1:1 (v/v) 0.9% saline:50% aqueous FCA	Dose ranging: 4 females / group Main study: 10 females (control) 20 females (test)	**Induction phase** Intradermal: Slight reactions noted in 5 test group animals 1 hour after dosing, and in 3 different test group animals 24 hours after dosing. No reactions in any control group animal. Topical: Slight to intense reactions in 17/20 test group animals at 1 hour; and 7/20 at 24 hours after patch removal. Slight reaction in 5/10 control animals at 1 hour. **Challenge phase** Two positive reactions noted at the saline site of the test group animals. No reactions were noted at any test material site in the test or control groups. In this study, flu antigens adjuvanted with MF59C.1 did not cause sensitization in Guinea pigs.	Project No. 564110 Report No. 14430 GLP

FCA = Freund's Complete Adjuvant
The test article, Fluad (Agrippal + MF59), is called Biocine Flu/MF59C.1 in the study report

2.6.7.17.2 Pivotal MF59 Studies

Repeat Dose Toxicity in Rabbits (14 Days)

Report Title: 14-day Intramuscular Toxicity Study in Rabbits **Test Article:** MF59 (New Zealand White)

Species/Strain: Rabbit / New Zealand White **Duration of Dosing:** 14 days **Study No.:** 90-6081

Initial Age: Approximately 13 weeks **Duration of Postdose:** 7 days

Date of First Dose: [redacted] 19[redacted] **Method of Administration:** Intramuscular **Location** 4.2.3.7.7

Vehicle/Formulation: Saline: 0.9% diluted 1:1 with water for injection. MF59: diluted 1:1 with saline **GLP Compliance:** Yes

Special Features: This study evaluated other test articles; for clarity only the MF59 and saline groups are presented here.

No Observed Adverse Effect Level: > 0.5 mL 1:1 MF59:saline

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Noteworthy Findings				
Died or Sacrificed Moribund	0	0	0	0
Body Weight	—	—	—	—
Food Consumption	—	—	—	—
Body Temperature	—	—	—	—
Clinical Observations	—	—	—	—
Ophthalmoscopy	—	—	—	—

— No significant findings + Steel-test significant at 5% level

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Hematology				
Fibrinogen Pretest	278	199	270	234
Fibrinogen End of Treatment	320	219	477	498+
Fibrinogen Recovery	404	228	310	301
Serum Chemistry				
Urinalysis	—	—	—	—
Terminal Evaluation: Day 15				
Organ Weights	—	—	—	—
Macroscopic Pathology				
Injection Sites – 1 day post-last dose				
Subcutaneous Hemorrhage	1	0	4	4
Focal discoloration and/or reddening	2	2	4	4
Histopathology[a]				
Thymus				
Reduction of lymphatic tissue	1 (1.0)	0	3 (1.0)	2 (1.0)
Spleen				
Congestion	0 (1.0)	2 (1.5)	3 (1.0)	4 (1.0)
Haemosiderosis	2 (1.0)	2 (1.0)	2 (2.5)	3 (1.3)
Red pulp, neutrophils	4 (1.2)	4 (2.2)	4 (2.2)	4 (2.7)

[a] Results are displayed as number of animals affected (mean severity).
Severity scores: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Neutrophil precursors	2 (1.5)	3 (1.6)	4 (2.2)	2 (4.0)
Bone Marrow				
Hypercellularity	0	2 (1.0)	2 (1.0)	3 (2.6)
Liver				
Infiltration - mixed cells	0	1 (1.0)	1 (1.0)	1 (1.0)
Injection Sites – 1 day post-last dose				
Neutrophil infiltrates	3 (1.0)	2 (1.0)	1 (3.0)	2 (2.5)
Monocyte infiltrates	4 (1.2)	2 (1.0)	3 (1.6)	4 (1.7)
Macrophages	4 (1.5)	1 (1.0)	2 (2.0)	4 (2.0)
Muscle cell necrosis	3 (1.3)	2 (1.0)	1 (2.0)	3 (1.6)
Muscle cell regeneration	2 (1.5)	1 (1.0)	3 (1.0)	3 (1.0)
Edema	0	0	1 (1.0)	2 (2.0)
Hemorrhage	1 (1.0)	0	0	1 (1.0)
Postdose Evaluation: Day 22				
Number Evaluated	4	4	4	4
Macroscopic Pathology				
Injection Sites – 7 days post-last dose				
Subcutaneous Hemorrhage	0	0	2	2
Focal discoloration and/or reddening	2	3	2	1

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Histopathology[a]				
Thymus				
Reduction of lymphatic tissue	0	2 (2.0)	2 (1.5)	1 (1.0)
Histopathology[a]				
Spleen				
Congestion	1 (1.0)	1 (2.0)	1 (2.0)	4 (1.7)
Haemosiderosis	1 (1.0)	1 (2.0)	1 (4.0)	3 (1.0)
Red pulp, neutrophils	4 (2.0)	4 (1.5)	4 (3.5)	4 (3.0)
Neutrophil precursors	4 (1.7)	4 (1.0)	4 (2.7)	4 (3.0)
Bone Marrow				
Hypercellularity	0	3 (2.3)	4 (1.7)	4 (2.7)
Liver				
Infiltration - mixed cells	1 (1.0)	1 (1.0)	1 (1.0)	0
Injection Sites – 7 days post-last dose				
Neutrophil infiltrates	0	0	2 (1.0)	0
Monocyte infiltrates	2 (1.0)	2 (1.0)	4 (1.0)	4 (1.0)
Macrophages	0	0	4 (1.2)	0
Muscle cell necrosis	0	2 (1.0)	0	1 (1.0)
Muscle cell regeneration	2 (1.0)	1 (1.0)	4 (1.0)	4 (1.0)

[a] Results are displayed as number of animals affected (mean severity).
Severity scores: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Edema	0	0	0	0
Hemorrhage	0	0	0	0

Reverse Mutation MF59W.1

Report Title: Bacterial Reverse Mutation Assay with MF59W.1 (Water Formulation)

Test Article: MF59

Test for Induction of: Reverse Mutation in Bacterial Cells

Strains: S. typhimurium and E. coli

Metabolizing System: Aroclor-induced Rat Liver S9, 10%

Vehicle: Saline

Treatment: Plate incorporation for 48 - 72 hr

Cytotoxic Effects: None

Genotoxic Effects: None

No. of Independent Assays: 2

No. of Replicate Cultures: 3

No. of Cells Analysed /Culture: $\geq 0.3 \times 10^9$

Study No.: G96AQ62.502

Location 4.2.3.7.7

GLP Compliance: Yes

Date of Treatment: [redacted] 19[redacted]

Study No.: G96AQ62.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
Without Activation	MF59W.1	0	15 ± 3	110 ± 9	9 ± 2	6 ± 2	15 ± 3
		100	14 ± 4	97 ± 6	7 ± 1	3 ± 1	14 ± 3
		333	14 ± 3	109 ± 11	10 ± 5	3 ± 1	10 ± 1
		1000	13 ± 4	117 ± 16	7 ± 1	5 ± 3	17 ± 2
		3333	20 ± 2	122 ± 3	6 ± 3	6 ± 1	16 ± 4
		5000	15 ± 1	115 ± 17	10 ± 1	3 ± 4	20 ± 7
	2-nitrofluorene	1.0	124 ± 23				
	Sodium azide	1.0		534 ± 133	428 ± 51		
	9-aminoacridine	75				99 ± 24	
	Methyl methanesulfonate	1000					134 ± 19
With Activation		0	21± 6	140 ± 2	12 ± 2	7 ± 2	17 ± 7
		100	16 ± 4	133 ± 7	7 ± 1	5 ± 5	12 ± 1
		333	17 ± 5	139 ± 23	8 ± 1	8 ± 3	16 ± 1

Study No.: G96AQ62.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
		1000	11 ± 9	137 ± 8	10 ± 3	5 ± 2	12 ± 3
		3333	20 ± 8	117 ± 29	14 ± 4	5 ± 3	16 ± 5
		5000	18 ± 6	119 ± 12	12 ± 3	4 ± 1	16 ± 4
	2-aminoanthracene	1.0	774 ± 183	687 ± 45	66 ± 9	73 ± 19	87 ± 10

Reverse Mutation MF59C.1

Report Title: Bacterial Reverse Mutation Assay with MF59C.1 (Citrate Formulation) **Test Article:** MF59

Test for Induction of: Reverse Mutation in Bacterial Cells
Strains: S. typhimurium and E. coli
Metabolizing System: Aroclor-induced Rat Liver S9, 10%
Vehicle: Saline
Treatment: Plate incorporation for 48 - 72 hr
Cytotoxic Effects: None
Genotoxic Effects: None

No. of Independent Assays: 2
No. of Replicate Cultures: 3
No. of Cells Analysed /Culture: $\geq 0.3 \times 10^9$

Study No.: G96AQ61.502
Location: 4.2.3.7.7
GLP Compliance: Yes
Date of Treatment: [redacted] 19[redacted]

Study No.: G96AQ61.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
Without Activation	MF59C.1	0	13 ± 4	121 ± 8	11 ± 1	6 ± 1	22 ± 3
		100	20 ± 5	122 ± 0	9 ± 1	6 ± 0	23 ± 3
		333	18 ± 4	126 ± 10	8 ± 1	6 ± 3	15 ± 4
		1000	15 ± 3	129 ± 12	9 ± 2	7 ± 2	22 ± 4
		3333	11 ± 2	116 ± 11	11 ± 4	4 ± 3	26 ± 11
		5000	16 ± 1	131 ± 11	9 ± 3	3 ± 3	25 ± 1
	2-nitrofluorene	1.0	133 ± 40				
	Sodium azide	1.0		586 ± 87	346 ± 65		
	9-aminoacridine	75				274 ± 55	
	Methyl methanesulfonate	1000					171 ± 14
With Activation		0	24 ± 9	133 ± 9	16 ± 5	9 ± 4	17 ± 5
		100	19 ± 2	125 ± 18	10 ± 5	4 ± 2	19 ± 2
		333	20 ± 1	124 ± 7	12 ± 6	4 ± 2	19 ± 6

Study No.: G96AQ61.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
		1000	17 ± 3	128 ± 21	15 ± 3	6 ± 2	18 ± 7
		3333	27 ± 9	108 ± 4	10 ± 3	7 ± 6	16 ± 2
		5000	16 ± 1	119 ± 5	9 ± 2	9 ± 4	14 ± 5
	2-aminoanthracene	1.0	506 ± 86	572 ± 67	75 ± 17	43 ± 20	172 ± 51

Mouse Micronucleus MF59W.1

Report Title: Micronucleus Cytogenetic Assay in Mice With MF59W.1 (Water Formulation) **Test Article:** MF59

Test for Induction of: Bone marrow micronuclei **Treatment Schedule:** Single dose **Study No.:** G96AQ62.122

Species/Strain: Mice / ICR **Sampling Time:** 24, 48, and 72 hours post-dose

Age: 6-8 Weeks **Method of Administration:** Intraperitoneal injection, 20 mL/kg **Location:** 4.2.3.7.7

Cells Evaluated: Polychromatic erythrocytes **Vehicle Formulation:** Saline **GLP Compliance:** Yes

No. of Cells Analysed/Animal: 1000 **Special Features:** None **Date of Dosing:** [redacted] 19[redacted]

Toxic/Cytotoxic Effects: No mortality, lethargy in all animals dosed with 5000 mg/kg MF59W.1

Genotoxic Effects: None **Evidence of Exposure:** Lethargy at 5000 mg/kg

Test Article (sampling time)	Dose (mg/kg)	No. of Animals	PCE/Total erythrocytes (Mean ± SD)		Micronucleated PCE per 1000 PCEs (Mean ± SD)	
			Males	Females	Males	Females
20 mL/kg Saline (24hr)	0	5M/5F	0.61 ± 0.08	0.60 ± 0.09	1.0 ± 0.71	1.2 ± 0.84
MF59W.1 (24hr)	1250	5M/5F	0.59 ± 0.06	0.60 ± 0.06	0.8 ± 0.84	0.8 ± 0.84
	2500	5M/5F	0.56 ± 0.03	0.57 ± 0.01	0.2 ± 0.45	0.6 ± 0.89
	5000	5M/5F	0.60 ± 0.03	0.59 ± 0.10	0.8 ± 0.84	0.2 ± 0.45
Cyclophosphamide (24hr)	60	5M/5F	0.46 ± 0.07	0.47 ± 0.09	37.2 ± 15.42	32.0 ± 7.97
20 mL/kg Saline (48hr)	0	5M/5F	0.53 ± 0.05	0.53 ± 0.07	1.0 ± 0.71	0.6 ± 0.55
MF59W.1 (48hr)	1250	5M/5F	0.56 ± 0.05	0.52 ± 0.04	1.0 ± 1.00	1.2 ± 0.84
	2500	5M/5F	0.51 ± 0.05	0.52 ± 0.02	0.4 ± 0.55	1.0 ± 1.22
	5000	5M/5F	0.53 ± 0.06	0.46 ± 0.08	0.4 ± 0.55	1.2 ± 0.84
20 mL/kg Saline (72hr)	0	5M/5F	0.51 ± 0.08	0.55 ± 0.06	0.8 ± 0.84	0.2 ± 0.45
MF59W.1 (72hr)	1250	5M/5F	0.48 ± 0.10	0.61 ± 0.13	1.0 ± 0.00	0.6 ± 0.55
	2500	5M/5F	0.50 ± 0.07	0.59 ± 0.09	0.4 ± 0.55	0.4 ± 0.55
	5000	5M/5F	0.53 ± 0.6	0.53 ± 0.08	1.0 ± 1.00	0.6 ± 0.55

Mouse Micronucleus MF59C.1

Report Title: Micronucleus Cytogenetic Assay in Mice With MF59C.1 (Citrate Formulation) **Test Article:** MF59

Test for Induction of: Bone marrow micronuclei **Treatment Schedule:** Single dose **Study No.:** G96AQ61.122

Species/Strain: Mice / ICR **Sampling Time:** 24, 48, and 72 hours post-dose

Age: 6-8 Weeks **Method of Administration:** Intraperitoneal injection, 20 mL/kg **Location:** 4.2.3.7.7

Cells Evaluated: Polychromatic erythrocytes (PCE) **Vehicle Formulation:** Saline **GLP Compliance:** Yes

No. of Cells Analysed/Animal: 1000 **Special Features:** None **Date of Dosing:** [redacted] 19[redacted]

Toxic/Cytotoxic Effects: No mortality, lethargy in 9/20 males and 1/20 females dosed with 5000 mg/kg MF59C.1

Genotoxic Effects: None **Evidence of Exposure:** Lethargy at 5000 mg/kg

Test Article (sampling time)	Dose (mg/kg)	No. of Animals	PCE/Total erythrocytes (Mean ± SD)		Micronucleated PCE per 1000 PCEs (Mean ± SD)	
			Male	Female	Male	Female
20 mL/kg Saline (24hr)	0	5M/5F	0.61 ± 0.08	0.60 ± 0.09	1.0 ± 0.71	1.2 ± 0.84
MF59C.1 (24hr)	1250	5M/5F	0.54 ± 0.03	0.55 ± 0.05	1.0 ± 0.71	1.0 ± 0.71
	2500	5M/5F	0.56 ± 0.03	0.62 ± 0.05	1.4 ± 1.14	1.0 ± 1.00
	5000	5M/5F	0.54 ± 0.04	0.55 ± 0.04	1.0 ± 0.71	0.6 ± 0.55
Cyclophosphamide (24hr)	60	5M/5F	0.46 ± 0.07	0.47 ± 0.09	37.2 ± 15.42	32.0 ± 7.97
20 mL/kg Saline (48hr)	0	5M/5F	0.53 ± 0.05	0.53 ± 0.07	1.0 ± 0.71	0.6 ± 0.55
MF59C.1 (48hr)	1250	5M/5F	0.55 ± 0.04	0.54 ± 0.03	0.4 ± 0.55	1.0 ± 0.71
	2500	5M/5F	0.54 ± 0.05	0.55 ± 0.04	0.8 ± 0.84	1.0 ± 0.71
	5000	5M/5F	0.51 ± 0.06	0.50 ± 0.02	0.6 ± 0.55	0.4 ± 0.55
20 mL/kg Saline (72hr)	0	5M/5F	0.51 ± 0.08	0.55 ± 0.06	0.8 ± 0.84	0.2 ± 0.45
MF59C.1 (72hr)	1250	5M/5F	0.51 ± 0.07	0.55 ± 0.08	1.4 ± 0.55	0.8 ± 0.84
	2500	5M/5F	0.55 ± 0.08	0.52 ± 0.05	0.4 ± 0.55	0.8 ± 0.45
	5000	5M/5F	0.51 ± 0.10	0.56 ± 0.09	0.4 ± 0.55	0.2 ± 0.45

Guinea Pig Sensitization

Test Article: MF59

Species/ Strain	Method of Administration	Duration of Dosing	Doses	Gender and No. per Group	Noteworthy Findings	Study No.
Guinea Pig / Dunkin-Hartley	Induction phase: Intradermal and topical Challenge phase: Topical	Day 1 Intradermal induction Day 7 Topical induction Day 21 Topical challenge	Dose ranging: 100, 75, 50, 25, 10, 5, 2 and 1% solutions. Main study: Intradermal induction phase – duplicate 0.1 mL injections: Test group 1 (MF59C.1) 50% aqueous FCA, 1:1 (v/v) 2% MF59C.1:0.9% saline, 1:1 (v/v) 2% MF59C.1:50% aqueous FCA Test group 2 (MF59W.1) 50% aqueous FCA, 1:1 (v/v) 2% MF59W.1:0.9% saline, 1:1 (v/v) 2% MF59W.1:50% aqueous FCA Control group 50% aqueous FCA, 0.9% saline, 1:1 (v/v) 0.9% saline:50% aqueous FCA Topical induction phase: 0.5 mL administrations of saline, undiluted MF59W.1 and MF59C.1 Challenge phase – 05mL saline, undiluted MF59W.1 and MF59C.1	Dose ranging: 4 females / group Main study: 10 females (saline) 20 females (MF59W.1) 20 females (MF59C.1)	**Induction phase** Intradermal: MF59C.1– slight reactions noted in all animals. MF59W.1 – slight reactions noted in all animals. Control – no reactions. Topical: MF59C.1 – slight reactions in 4/20 animals. MF59W.1 – slight reactions in 10/20 animals. Control – slight reactions in 2/20 animals. **Challenge phase** MF59C.1 – 3/20 animals had a positive reaction at 24 hrs, in one animal the reaction was still present at 48 hrs. MF59W.1 – no reaction in any animal. Control – no reactions. In this study, MF59 citrate and water formulations were not considered to be sensitizers in Guinea pigs.	Project No. 564278 Report No. 14465 GLP

FCA = Freund's Complete Adjuvant

Developmental Toxicity in Rats

Report Title: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of a Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to Crl:CD BR VAF/Plus Female Rats

Test Article: MF59

Design See below

Species/Strain: Rat / Crl:CD BR VAF/Plus

Initial Age: Approximately 66 days

Date of First Dose: [redacted] 19[redacted]

Special Features: None

No Observed Adverse Effect Level:

Duration of Dosing: Days –21, 0, 6, 8 & 10 of PG[a] for C-section group, Days –21, 0, 6, 8, 10 & 20 of PG for natural delivery group

Study No.: 1303-002

Day of Mating: Day 0

Location: 4.2.3.7.7

Method of Administration: Intramuscular

GLP Compliance: Yes

Vehicle/Formulation: MF59 – water formulation. Saline – 0.9%

Litters Culled/Not Culled: Culled to 5/sex/litter where possible (naturally delivered only)

F_0Females: 0.5 mL MF59

F_1Litters: 0.5 mL MF59

20 dams per group underwent Caesarean sections on Day 20 of presumed gestation; the litters were evaluated for gross, skeletal, or soft tissue alterations. The remaining dams were allowed to deliver naturally; weight, sex and nursing behaviour of the litters were recorded. Pups were euthanized on Day 21 postpartum and examined macroscopically.

Note: Adjuvanted vaccine was also evaluated in this study, however for clarity only the MF59 and saline control data are presented. Saline was administered as two 0.5 mL injections to separate sites. MF59 (0.5 mL) is equivalent to 2 × the human dose.

Dose	1.0mL Saline (2 × 0.5 mL injections)	0.5 mL MF59
F_0 Females	45	45
No. Pregnant	30	33
No. Died or Sacrificed Moribund	1 (day 6 L[b])	2 (day 4 PG and day 11 L)
No. Aborted or with Total Res. of Litter	0	0
Clinical Observations		
Swollen Hindlimb (associated with IM injection)	0	40**
Localized Alopecia (limb)	0	4**

[a] PG = Presumed Gestation
[b] L = Lactation

** $P \leq 0.01$ * $P \leq 0.5$

Dose		1.0mL Saline (2 × 0.5 mL injections)	0.5 mL MF59
Necropsy Observations		—	—
Gestation Body Weight (%[a])		131.8 ± 21.5	2
Lactation Body Weight (%[a])		315.1 ± 24.5	4
Gestation Food Consumption (%[a])			
Days 1-20		22.4 ± 1.6	0
Days 8-10		22.5 ± 2.0	-6
Lactation Food Consumption (%[a])		41.1 ± 6.8	7
F_0 Females – Caesarean Section Day 20 PG			
Number evaluated:		20	20
Mean No. Corpora Lutea ± SD		16.8 ± 2.7	16.8 ± 3.2
Mean No. Implantations ± SD		14.5 ± 2.2	13.9 ± 3.5
Dams with any Resorptions N (%)		12 (60)	8 (40)
All Conceptuses Dead or Resorbed N (%)		0	0
Dams with Viable Fetuses N (%)		20 (100)	20 (100)
Litters (Caesarean Section Day 20 PG dams):			
No. Litters Evaluated		20	20
No. Live Fetuses		271	262
Litter Sizes	**Mean Live Fetuses ± SD (N)**	13.6 ± 2.4	13.1 ± 3.4
	Mean Dead Fetuses ± SD (N)	0	0
Resorptions	**Mean Early Resorptions ± SD (N)**	0.9 ± 1.0 (18)	0.8 ± 1.2 (16)
	Mean Late Resorptions ± SD (N)	0.0 ± 0.2 (1)	0.0 ± 0.0 (0)

— No significant findings ** $P \leq 0.01$ * $P \leq 0.5$

[a] For controls, group means (grams ± SD) are shown. For treated groups, percent differences from controls are shown.

Dose	1.0mL Saline (2 × 0.5 mL injections)	0.5 mL MF59
Mean Fetal Body Weight (g/litter)	3.35 ± 0.22	3.45 ± 0.27
% Live Male Fetuses per Litter	49.3 ± 17.2	53.6 ± 15.9
Fetal Anomalies (any alteration) N (%)	9 (3.3)	20 (7.6)
Sternebrae: Incomplete Ossification **Litter Incidence N (%)** **Fetal Incidence N (%)**	 0 0	 5 (25.0)** 5 (3.7)**
Pelvis: Incomplete Ossification of Pubes **Litter Incidence N (%)** **Fetal Incidence N (%)**	 1 (5.0) 1 (0.7)	 7 (35.0)** 12 (8.8)**
Pelvis: Incomplete Ossification of Ischia **Litter Incidence N (%)** **Fetal Incidence N (%)**	 0 0	 3 (15.0)** 4 (2.9)**
F_0 Females – Natural Delivery		
Number evaluated:	10	13
Mean Duration of Gestation (days)	22.7 ± 0.7	22.8 ± 0.4
F_1 Litters:		
No. Litters Evaluated	10	13
Mean No. of Implantation Sites per Litter	15.8 ± 1.8	15.2 ± 1.5
Mean No. Pups/Litter	13.4 ± 3.9	13.6 ± 2.6
No. Liveborn Pups/total no. pups	129/134	172/177
No. of Litters with Stillborn Pups N (%)	0	1 (7.7)
Postnatal Mortality to Day 4[a]	2	4
Postnatal Mortality to Day 21[a]	2	5

— No significant findings ** $P \leq 0.01$ * $P \leq 0.5$

[a] Pups were culled or euthanized for blood collection (for antibody analysis) between birth and weaning. Only unscheduled deaths are included here.

Dose	1.0mL Saline (2 × 0.5 mL injections)	0.5 mL MF59
No. of Total Litter Losses	0	0
Pup Sex Ratios – Male pups on Day 1	50.6 ± 15.0	49.9 ± 21.0
Pup Weight / Litter (grams)		
Day 1	6.0 ± 0.6	6.1 ± 1.1
Day 21	39.4 ± 5.0	42.4 ± 5.5
Pup Clinical Signs	—	—
Pup Necropsy Observations	—	—

— No significant findings ** P ≤ 0.01 *P ≤ 0.5

Developmental Toxicity in Rabbits

Test Article: MF59

Species/ Strain	Method of Administration (Vehicle/Formulation)	Dosing Period	Doses (0.5 mL)	No. per Group)	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59 concentrations equivalent to 0.25× and 0.5× the human dose were tested. MF59 was diluted with saline and water.	Day 6 – 28 of presumed gestation Caesarean sections performed day 29	Saline, 0.25× MF59 0.5× MF59	5 females	There were no deaths. One animal (MF59, 0.5×) prematurely delivered on day 29 of gestation. Body weights and food consumption were unaffected. There were no test article-related necropsy observations. No Caesarean-sectioning or litter parameters were affected. Litter averages for corpora lutea, implantations, litter sizes, resorptions, percent male fetuses, and percent resorbed conceptuses were comparable among groups. There were no dead fetuses, and no litter consisted of only resorbed conceptuses. One late resorption occurred in a litter from a dam treated with MF59 (0.25×). There were no macroscopic external fetal alterations in this study. This study was performed to select doses for a definitive study. The definitive study did not have an MF59-alone group, therefore the data is not presented here, however the same dosing schedule with 0.5× and 1.0× MF59 combined with antigens had no effect on litter parameters and was not teratogenic in rabbits.	1303-001P GLP

2.6.7.17.3 Nonpivotal MF59 Studies

Test Article: MF59

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59C.1	Single dose 2/sex necropsied on days 3 & 15	0.5	4/sex	No mortality. No effects on clinical signs, body weight, food consumption, body temperature, ophthalmoscopy, or hematology/clinical chemistry. No edema or erythema at the injection sites. Elevated fibrinogen levels on day 3 in males. Complete reversal by day 15. Histopathologic evaluation of injection sites on day 3 primarily showed inflammation, inflammatory cell infiltrate, and hemorrhage. By day 15, findings seen on day 3 had partially to fully resolved. On day 15, two animals exhibited signs of hepatic coccidiosis (parasitic granuloma around bile ducts and/or moderate cholangitis). These findings were not related to treatment but were consistent with a subclinical *Eimeria stiedae* infection.	Project No. 501464 Report No. 20717 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59C.1 Dose volume: 1.0 mL Saline (1 mL) in the right leg 1:1 MF59:saline (1 mL) in the left leg	Single dose (on day 0) 2/sex necropsied on days 2 & 15	1.0	4/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology parameters. No irritation or inflammation was observed at the injection site. Occasional slight desquamation/erythema at injection sites was observed 24 and 48 hrs post-dosing. Elevated fibrinogen levels were seen on day 2. Levels returned to baseline values by day 15 indicating complete reversal. Increased creatine kinase levels were observed on day 2. Levels returned to baseline values by day 15. This finding was considered consistent with an intramuscular injection of a 1 mL volume into rabbit muscle. On day 2, discoloration of injection sites was observed at both saline and MF59 injection sites. Minimal to moderate edema, inflammation, and/or hemorrhage was noted at injection sites of most animals. Local muscle necrosis with mineral deposition was occasionally seen. Lesions were partially to fully resolved by day 15.	00-2672 GLP
Rabbit / New Zealand White	Intramuscular MF59W	Dosed on days 1 & 8 3/sex necropsied on day 15/16, 2/sex on day 16/17	0.5 (0.25 per leg)	5/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, organ weights, clinical chemistry or hematology parameters. Injection site reactions were limited to slight erythema or scabbing, consistent with the physical introduction of a needle.	89-6280 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59W:saline	Dosed on days 1 & 15 3/sex necropsied on days 15 & 29	0.5	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or clinical chemistry parameters. Mild local irritation at the injection site was observed. Slight erythema was observed at injection sites in some animals for 2-4 days following injection. Inflammation, hemorrhage and focal muscle degeneration were seen microscopically at injection sites; findings were partially to fully resolved by the end of the recovery period.	CHV 2777-102 GLP
Rabbit / Chinchilla	Intramuscular MF59W	Dosed on days 1, 15 & 29 All animals necropsied on day 38	0.5 (0.25 per leg)	5/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or clinical chemistry parameters. Increased fibrinogen levels in males and females after the first injection. Microscopic changes at the injection sites indicated a slight inflammatory response.	89-6192 GLP
Rabbit / New Zealand White	Intramuscular 1:1 vehicle:MF59W Vehicle consisted of saline/buffer 50µg thiomersal per 0.5 mL MF59W	Dosed on days 1, 15 & 29 All animals necropsied on day 36	0.5 (0.25 per leg)	5/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology parameters. Injection site observations included dermal bruising and reddening of the quadriceps muscle. These minimal reactions were seen across all groups and are consistent with intramuscular dosing. Microscopically, minimal to moderate focal inflammation and muscle fiber degeneration were seen.	90-6230 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59W:saline	Dosed on days 1, 15 & 29 3/sex necropsied on days 29 & 43	0.5	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology or serum chemistry parameters. There was no evidence of systemic toxicity or dermal irritation at the injection sites. There was focal hemorrhage and/or residual inflammation at injection sites; severity was minimal after the recovery period.	HWA 2670-101 GLP
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline	Dosed on days 1, 15 & 29 3/sex necropsied on days 31 & 43	0.5	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, organ weights, clinical chemistry or hematology parameters. Macroscopic observations at the terminal necropsy consisted of red discoloration of the third injection site in two of three animals; this correlated with microscopic findings of hemorrhage, subacute inflammation, and/or muscle fiber degeneration. Incidence and severity of injection site findings were reduced at the recovery necropsy.	759-002 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:Tris buffer	Dosed on days 1, 15, & 29 3/sex necropsied on days 31 & 43	0.5	6/sex	No mortality. No treatment-related effects on clinical signs (including injection sites), body weight, body temperature, ophthalmoscopy, organ weights or hematology parameters. Mildly elevated serum LDH and creatine kinase levels were seen at day 31. By day 43 levels had returned to normal in most animals; 2 females still had slightly higher CK levels. Macroscopically, slight hemorrhagic areas were seen at the third injection site (48 hours post-injection). Microscopically, inflammatory and degenerative changes (subacute inflammation, histiocytosis, and muscle fiber degeneration and/or necrosis) at all injection sites were seen. By day 43 incidence and severity of macroscopic and microscopic findings were reduced, indicating resolution.	950031 GLP
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline	Dosed on days 1, 15, 29 & 43 3/sex necropsied on days 45 & 57	0.5	6/sex	There was no mortality. There were no treatment-related effects on body weights, ophthalmoscopy, or organ weights. Local reactions at injection sites were minimal and of low incidence. Body temperatures increased slightly over time. Enlarged popliteal lymph nodes were observed macroscopically on day 45; this finding was not seen on day 57. On day 57 two animals had reddened lymph nodes. Microscopic evaluation of injection sites at day 45 showed inflammatory cell infiltrates; the severity was graded ‘very mild’ or ‘mild’. On day 57 the incidence and severity of findings was decreased, indicating reversibility.	Project No. 656583 Report No. 14160 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline	Dosed on days 1, 8, 15, 29 & 43 3/sex necropsied on days 45 & 57	0.5	6/sex	No mortality. No treatment-related effects on clinical observations (including injection sites), body weight, food consumption, body temperature, organ weights or ophthalmoscopy. Globulin was elevated in some males on days 17 and 45. Redding or enlargement in the draining lymph nodes was noted in one male. Inflammatory cell infiltrate at injection sites was seen in all animals at day 45. On day 57 incidence and severity were reduced, indicating recovery. Approximately half the rabbits had a subclinical *Eimeria stiedae* infection; this did not prevent the evaluation of local or systemic toxicity.	Project No. 501438 Report No. 20611 GLP
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline Saline (1 mL) in the right leg 1:1 MF59C.1:saline (1 mL) in the left leg	Dosed on days 0, 14, 28, 42, 56, & 70 3/sex necropsied on days 72 & 84	1.0	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology parameters. Sporadic erythema, edema, and desquamation were seen at injection sites, severity was generally very slight. Fibrinogen was increased relative to pretest values and had returned to pre-treatment levels by the end of recovery (day 84). Sporadic and reversible increases in creatine kinase levels were seen. On day 72, edema, inflammation, and hemorrhage were noted at injection sites along with occasional muscle necrosis and mineral deposition. Findings were generally more severe and/or frequent on day 72 (minimal to moderate severity) as compared to day 84 (minimal to slight severity) indicating partial resolution.	00-2673 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59W:saline	Dosed on days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211 & 232 3/sex necropsied on days 233 & 247	0.5	6/sex	No mortality. No treatment-related effects on clinical observations with the exception of transient and mild dermal irritation at the injection sites of most animals. There was no effect on body weight, body temperature, urinalysis, organ weights or ophthalmoscopy. There were occasional incidences of decreased prothrombin times 2 days post-injection. Occasional increases in globulin and mild decreases in albumin were seen. Increases were noted in creatine kinase values at the 2-day post injection intervals; these change were statistically significant and are indicative of muscular damage associated with injections. Microscopically, acute to subacute inflammation was noted at injection sites.	HWA 2670-100
Dog / Beagle	Intramuscular MF59W	Dosed on days 1 & 8 1/sex necropsied on days 15 & 19/20	0.5	2/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, organ weights, hematology or serum chemistry parameters. Occasional macroscopic injection site reactions were limited to small (1-2 mm) areas of redness or scabbing consistent with the physical introduction of a needle. Microscopically, inflammation was present at injection sites.	89-6281 GLP
Dog / Beagle	Intramuscular MF59W	Dosed on days 1, 16 & 29 All animals necropsied on day 36	0.5	2/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, cardiography, ophthalmoscopy, organ weights, hematology or serum chemistry parameters. Some dogs displayed a pain reaction after the first injection, which subsided quickly (within a few seconds) and did not recur. Microscopic evaluation of injection sites showed a generally minimal inflammatory response.	89-6193 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Dog / Beagle	Intramuscular 1:1 MF59W:vehicle 50µg thiomersal per 0.5 mL MF59W	Dosed on days 1, 15 & 29 All animals necropsied on day 36	0.5	2/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, cardiography, urinalysis, organ weights, hematology or serum chemistry parameters. Neurological parameters were unaffected. Macroscopic injection site reactions were limited to acute areas of redness surrounding the entry point.	90-6231 GLP

2.6.7 Toxicology Tabulated Summary

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2.6.7 Toxicology Tabulated Summary

2.6.7.1 Toxicology: Overview

Test Article: FCC vaccine

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses	GLP	Testing Facility	Study No.	Location	
								Mod.	Section
Single-Dose Toxicity									
	Mouse and Guinea pig	Intraperitoneal	Single dose	45 µg antigens (0.5 mL)	No	Chiron	Not assigned (lot release)	3	3.2.P.5.1
	Rabbit / NZW	Refer to repeat-dose toxicity and local tolerability below – in-life parameters and injection site histopathology were evaluated after a single dose					191-44	4	4.2.3.2
Repeat-Dose Toxicity									
	Rabbit / NZW	Intramuscular	2 doses days 1 & 8	0 & 45 µg antigens (0.5 mL)	Yes	[redacted]	191-44	4	4.2.3.2
Genotoxicity									
	Not applicable		—	—	—	—	—	—	
Carcinogenicity									
	Not applicable		—	—	—	—	—	—	
Reproductive and Developmental Toxicity									
	Not applicable		—	—	—	—	—	—	
Local Tolerance									
	Rabbit / NZW	Intramuscular	2 doses days 1 & 8	0 & 45 µg antigens (0.5 mL)	Yes	[redacted]	191-44	4	4.2.3.2

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses	GLP	Testing Facility	Study No.	Location	
								Mod.	Section
Other Toxicity Studies									
Other (Other) Studies conducted using process intermediates (not vaccine product)									
Tumorigenicity of intact MDCK cells	Mouse / Nu/Nu	Subcutaneous	Single dose	10^1, 10^3, 10^5, or 10^7 MDCK cells	No	[redacted] UK	48329	4	4.2.3.7.7
Oncogenicity of MDCK cell lysates in neonatal animals	Mouse / Nu/Nu	Subcutaneous	Single dose	MDCK cell lysates equal to ~5×10^6 cells	No	[redacted] UK	48330	4	4.2.3.7.7
	Rat / Wistar	Subcutaneous	Single dose	MDCK cell lysates equal to ~10^7 cells	No	[redacted] UK	48332	4	4.2.3.7.7
	Hamster / Aura	Subcutaneous	Single dose	MDCK cell lysates equal to ~10^7 cells	No	[redacted] UK	48331	4	4.2.3.7.7
Oncogenicity of MDCK cell DNA in neonatal animals	Mouse / Nu/Nu	Subcutaneous	Single dose	~27 – 35µg DNA	No	[redacted] UK	48333	4	4.2.3.7.7
	Rat / Wistar	Subcutaneous	Single dose	~55 – 70µg DNA	No	[redacted] UK	48335	4	4.2.3.7.7
	Hamster / Aura	Subcutaneous	Single dose	~55 – 70µg DNA	No	[redacted] UK	48334	4	4.2.3.7.7
Other (Other) Supportive studies conducted with previous MDCK cells (serum-free but not protein-free adapted)									
Tumorigenicity of intact MDCK cells	Mouse / Nu/Nu	Subcutaneous	Single dose	10^7 MDCK cells	Yes	[redacted] USA	B96YG21.001	4	4.2.3.7.7
Tumorigenicity of intact MDCK cells or MDCK cell lysate	Rat anti-thymocyte model	Subcutaneous	Single dose	10^7 MDCK cells or MDCK cell lysates equal to ~10^7 cells	No	[redacted]	B012888/02	4	4.2.3.7.7

2.6.7.2 Toxicokinetics – Overview of Studies

Not applicable.

2.6.7.3 Toxicokinetics – Overview of Data

Not applicable.

2.6.7.4 Toxicology – Drug Substances

2.6.7.4.1 FCC Vaccine

Test Material	Batch No.	Study Dates	Study No.	Type of Study
FCC vaccine	5[redacted]012	[redacted]/20[redacted] – [redacted]/20[redacted]	Ph. Eur test	Abnormal toxicity
FCC vaccine	5[redacted]011	[redacted]/20[redacted]	Ph. Eur test	Abnormal toxicity
FCC vaccine	5[redacted]011	[redacted]/20[redacted]	Ph. Eur test	Abnormal toxicity
FCC vaccine	F[redacted]_01	[redacted]/20[redacted] – [redacted]/20[redacted]	191-44	Toxicology

2.6.7.4.2 Process Intermediates

Test Material	Batch No.	Study Dates	Study No.	Type of Study
MDCK cells (serum-free, not protein-free adapted)	3[redacted] 96	[redacted]/19[redacted] – [redacted]/19[redacted]	B96YG21.001	Tumorigenicity
MDCK cells (serum-free, not protein-free adapted)	3[redacted]97 I	[redacted]/20[redacted] – [redacted]/20[redacted]	B012888/02	Tumorigenicity
MDCK cells (serum-free and protein-free adapted)	3[redacted]517	[redacted]/20[redacted] – [redacted]/20[redacted]	48329	Tumorigenicity
MDCK cell-free lysate BPL-treated MDCK cells	0[redacted]0-b 0[redacted]0-c	[redacted]/20[redacted] – [redacted]/20[redacted]	48330	Oncogenicity
		[redacted]/20[redacted] – [redacted]/20[redacted]	48331	Oncogenicity
		[redacted]/20[redacted] – [redacted]/20[redacted]	48332	Oncogenicity
Untreated MDCK cell DNA Flu-infected MDCK cell DNA Flu-infected BPL-treated MDCK cell DNA	0[redacted]0-a 0[redacted]0-d 0[redacted]0-e	[redacted]/20[redacted] – [redacted]/20[redacted]	48333	Oncogenicity
		[redacted]/20[redacted] – [redacted]/20[redacted]	48334	Oncogenicity
		[redacted]/20[redacted] – [redacted]/20[redacted]	48335	Oncogenicity

2.6.7.5 Single-Dose Toxicity

Test Article: FCC vaccine

Species	Method of Administration	Doses	Gender & No. per Group	Observed Maximum Nonlethal Dose	Approx. Lethal Dose (mL)	Noteworthy Findings	Study No.
Mouse and Guinea pig	Intraperitoneal	0.5 mL (containing 45 µg antigens)	Mouse: 5 animals Guinea pigs: 2 animals	Not applicable (0.5 mL maximum tested)	Not applicable	The Ph. Eur. Test for Abnormal Toxicity is performed for each lot of FCC vaccine. Animals are injected and observed for 7 days. Mortality, body weights and clinical signs are recorded. Test results (see also 3.2.P.5.4) Batch 522 008 012 – pass Batch 522 009 011 – pass Batch 522 011 011 – pass	Not assigned
Rabbit / NZW	Intramuscular	0.5 mL (containing 45 µg antigens)	6/sex	Not applicable (0.5 mL maximum tested)	Not applicable	Single-dose toxicity was evaluated as part of the repeat-dose toxicity study (2.6.7.7). There were no in-life observations of irritation and no macroscopic injection site findings. Microscopic findings were seen in all groups, including controls. Findings consisted of minimal to slight necrosis and hemorrhage, and were partially to fully resolved by the end of the recovery period.	191-44

2.6.7.6 Repeat-Dose Toxicity: Nonpivotal

Not applicable.

2.6.7.7 Repeat-Dose Toxicity: Pivotal Studies

Report Title: Two Dose Intramuscular Toxicity Study of Influenza Vaccine Formulations in New Zealand White Rabbits

Test Article: FCC vaccine

Species/Strain: Rabbit / New Zealand White

Duration of Dosing: Days 1 and 8

Study No.: 191-44

Initial Age: Approximately 13 weeks

Duration of Postdose: 2 or 3 days (main necropsy), 14 or 15 days (recovery necropsy)

Location in CTD:

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Intramuscular

Mod. 4 **Section** 4.2.3.2

Vehicle/Formulation: Phosphate buffered saline (PBS)

GLP Compliance: Yes

Special Features: Modified Draize scoring of injection sites. Immunogenicity evaluated on days 1 and 8 (all animals), days 10, 15 and 22 (males), and 11, 15 and 23 (females).

No Observed Adverse Effect Level: Not applicable

Study No. 191-44						
Dose (µg antigen per dose)	**0 (PBS Control)**		**45 (FCC vaccine)**		**45 (Agrippal™ vaccine)**	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Noteworthy Findings						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight	–[a]	–	–	–	–	–
Food Consumption	–	–	–	–	–	–
Clinical Observations	–	–	–	–	–	–
Injection sites	–	–	–	–	–	–
Ophthalmoscopy	–	–	–	–	–	–

[a] – signifies no noteworthy findings

Study No. 191-44						
Dose (µg antigen per dose)	**0 (PBS Control)**		**45 (FCC vaccine)**		**45 (Agrippal™ vaccine)**	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Hematology	–	–	–	–	–	–
Serum Chemistry	–	–	–	–	–	–
Coagulation	–	–	–	–	–	–
Organ Weights						
Thymus[b] - day 10/11	3.53 ± 0.905	3.60 ± 0.933	2.64 ± 0.173	2.92 ± 0.475	2.64 ± 0.215	2.64 ± 0.724
Thymus – day 22/23	2.91 ± 0.232	3.52 ± 1.43	3.20 ± 0.738	4.28 ± 0.294	4.33 ± 1.26	4.30 ± 0.670
Macroscopic Pathology	–	–	–	–	–	–
Histopathology – day 10/11[c]						
Injection sites						
Left (injected day 1)						
Necrosis	–	1 (2.0)	–	1 (2.0)	1 (2.0)	2 (1.5)
Right (injected day 8)						
Hemorrhage	–	–	1 (2.0)	–	–	–
Postdose Evaluation:[d]						
Number Evaluated	**3**	**3**	**3**	**3**	**3**	**3**
Histopathology – day 22/23						
Injection sites						
Left (injected day 1)						
Necrosis	–	–	–	1 (1.0)	1 (2.0)	–

[b] Mean weight ± standard deviation. Statistical analysis was not performed because N=3/sex/group at each necropsy.
[c] Histopathology findings are presented as number of animals affected (mean severity). Severity score 1.0 = minimal, 2.0 = slight, 3.0 = moderate, 4.0 = marked.
[d] Parameters were evaluated as for terminal necropsy; there were no noteworthy findings.

Study No. 191-44						
Dose (µg antigen per dose)	**0 (PBS Control)**		**45 (FCC vaccine)**		**45 (Agrippal™ vaccine)**	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Right (injected day 8)						
Hemorrhage	1 (2.0)	–	–	–	–	–
Additional Examinations[e]						
HAI Titers – B/Guangdong						
Day 1	0 ± 0	0 ± 0	3 ± 8	3 ± 8	3 ± 8	3 ± 8
Day 8	0 ± 0	7 ± 10	5 ± 12	17 ± 15	33 ± 10	37 ± 8
Day 10/11 or 15	0 ± 0	0 ± 0	123 ± 138	318 ± 266	123 ± 115	160 ± 104
Day 22/23	0 ± 0	0 ± 0	320 ± 0	427 ± 185	227 ± 101	640 ± 320
HAI Titers – A/New Caledonia						
Day 1	0 ± 0	0 ± 0	0 ± 0	3 ± 8	0 ± 0	3 ± 8
Day 8	0 ± 0	18 ± 20	13 ± 16	27 ± 33	47 ± 30	50 ± 40
Day 10/11 or 15	10 ± 17	0 ± 0	47 ± 39	127 ± 109	37 ± 27	70 ± 47
Day 22/23	0 ± 0	0 ± 0	267 ± 92	360 ± 262	120 ± 40	426 ± 185
HAI Titers – A/Panama						
Day 1	67 ± 20	60 ± 22	67 ± 16	57 ± 27	53 ± 16	47 ± 16
Day 8	93 ± 55	80 ± 44	60 ± 22	93 ± 55	126 ± 53	120 ± 36
Day 10/11 or 15	67 ± 48	87 ± 39	73 ± 16	140 ± 33	107 ± 41	107 ± 41
Day 22/23	33 ± 12	40 ± 0	133 ± 46	213 ± 93	160 ± 0	160 ± 0

[e] Titers are presented as mean ± standard deviation

2.6.7.8 Genotoxicity: In Vitro

Not applicable.

2.6.7.9 Genotoxicity: In Vivo

Not applicable.

2.6.7.10 Carcinogenicity

Not applicable.

2.6.7.11 Reproductive and Developmental Toxicity: Nonpivotal

Not applicable.

2.6.7.12 Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development

Not applicable.

2.6.7.13 Reproductive and Developmental Toxicity: Effects on Embryofetal Development

Not applicable.

2.6.7.14 Reproductive and Developmental Toxicity: Effects on Pre- and Post-natal Development

Not applicable.

2.6.7.15 Studies in Juvenile Animals

Not applicable.

2.6.7.16 Local Tolerance

Test Article: Flu Cell Culture vaccine

Species/ Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study No.
Rabbit / NZW	Intramuscular	0.5 mL dose on day 1 (left hindlimb) & day 8 (right hindlimb) Group 1: saline control Group 2: FCC vaccine, 45µg antigen Group 3: Agrippal™ (comparator vaccine), 45µg antigen	6/sex 3/sex necropsied on day 10 (males) or 11 (females) 3/sex necropsied on day 22 (males) or 23 (females)	Local tolerability was evaluated as part of the repeat-dose toxicity study (2.6.7.7). There were no in-life observations of irritation and no macroscopic injection site findings. Microscopic findings were seen in all groups, including controls. Findings consisted of minimal to slight necrosis and hemorrhage, and were partially to fully resolved by the end of the recovery period.	191-44

2.6.7.17 Other Toxicity Studies

2.6.7.17.1 Antigenicity

Not applicable.

2.6.7.17.2 Immunotoxicity

Not applicable.

2.6.7.17.3 Mechanistic

Not applicable.

2.6.7.17.4 Dependence

Not applicable.

2.6.7.17.5 Metabolites

Not applicable.

2.6.7.17.6 Impurities

Not applicable.

2.6.7.17.7 Other (Other) Studies Conducted with Process Intermediates

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Mice / NuNu ~4 weeks old (adult)	Subcutaneous	Single 0.2 mL dose on day 0, necropsy on day 150 or 151 Type and number of intact cells administered to each group is shown below. Cells were suspended in fresh media. Group A (negative control): 10^7 MRC-5 Group B: 10^1 MDCK Group C: 10^3 MDCK Group D: 10^5 MDCK Group E: 10^7 MDCK Group F (positive control): 10^7 HeLa	13/sex	Number of mice surviving to necropsy: Group A: 12 females, 11 males Group B: 10 females, 10 males Group C: 12 females, 13 males Group D: 12 females, 11 males Group E: 10 females, 11 males Group F: 5 females, 7 males (culled due to tumor size) Assay was valid based on presence of tumors (>90%) in positive control group. No negative control animals had tumors. Mortality: a few animals in each group were either found dead or euthanized due to poor condition and/or weight loss. Positive control (HeLa) animals were culled as tumor size exceeded 10 mm in one dimension. Clinical observations: measurable/palpable nodules at injection sites were present at various times in some animals from groups C, D, E and F. Observations of distorted hindlimb (site of injection) were first noted on day 88 and affected 1 to 3 animals from each MDCK-treated group. Macroscopic observations: distorted hindlimb noted during life corresponded with observations of flat nodular masses at necropsy. Microscopic evaluation: neoplastic MDCK cells (subset confirmed by PCR) at site of injection in 3 (group B), 2 (group C), 10 (group D) and 11 (group E) animals. Metastasis in at least one animal per treatment group. Conclusion: Intact MDCK cells were tumorigenic in nude mice at all doses tested.	48329

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Mice / NuNu < 4 days old	Subcutaneous	Single 0.1 mL dose on day 0, necropsy on day 150. Group A (negative control): 1:2000 Beta-propiolactone (BPL) in tris buffer Group B: MDCK cell free lysate in cell culture media Group C: BPL-treated MDCK cell lysate in tris buffer	14-18/sex	Number of mice surviving to necropsy (re-sexed at weaning): Group A: 9 females, 5 males Group B: 4 females, 7 males Group C: 7 females, 5 males Mortality: high mortality was observed in animals from all groups predominately in the first 2 weeks of the study. Possible contributing factors are associated with disturbing the dams & pups during early stages of the study; excessive handling and/or injection trauma can contribute to increased cannibalism. Mortality was not considered to be due to any toxicity of the control and test articles. Clinical observations: no significant findings Macroscopic observation: no significant findings Microscopic evaluation of lung and injection sites: no tumors Conclusion: MDCK cell lysate and BPL-treated MDCK cell lysate were not oncogenic in infant nude mice.	48330

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Rat / Wistar < 4 days old	Subcutaneous	Single 0.2 mL dose on day 0, necropsy on day 151 or 152 Group A (negative control): 1:2000 Beta-propiolactone (BPL) in tris buffer Group B: MDCK cell free lysate in cell culture media Group C: BPL-treated MDCK cell lysate in tris buffer	15/sex (groups A and B) or 14/sex (group C)	Number of rats surviving to necropsy (re-sexed at weaning): Group A: 16 females, 13 males Group B: 14 females, 16 males Group C: 16 females, 12 males Mortality: 1 male (group A) cannibalized day 1 Clinical observations: no significant findings Macroscopic observation: no significant findings Microscopic evaluation of lung and injection sites: no tumors Conclusion: MDCK cell lysate and BPL-treated MDCK cell lysate were not oncogenic in infant rats.	48332

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Hamster / Aura < 4 days old	Subcutaneous	Single 0.2 mL dose on day 0, necropsy on day 150. Group A (negative control): 1:2000 Beta-propiolactone (BPL) in tris buffer Group B: MDCK cell free lysate in cell culture media Group C: BPL-treated MDCK cell lysate in tris buffer	15/sex (groups A and C) or 14/sex (group B)	Number of hamsters surviving to necropsy (re-sexed at weaning): Group A: 14 females, 14 males Group B: 15 females, 13 males Group C: 9 females, 21 males Mortality: 1 male (group A) cannibalized day 1, 1 female (group A) cannibalized day 29 Clinical observations: no significant findings Macroscopic observation: no significant findings Microscopic evaluation of lung and injection sites: no tumors Conclusion: MDCK cell lysate and BPL-treated MDCK cell lysate were not oncogenic in infant hamsters.	48331

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Mice / NuNu < 4 days old	Subcutaneous	Single 0.1 mL dose on day 0, necropsy on day 150. Negative control: ~35µg DNA from murine tissue in phosphate buffered saline (PBS) Group 1: ~35µg DNA from uninfected and untreated MDCK cells in PBS Group 2: ~35µg DNA from Influenza-infected, untreated MDCK cells in PBS Group 3: ~27µg DNA from Influenza-infected, Beta-propiolactone (BPL)-treated MDCK cells in PBS	16-24/sex	Number of mice surviving to necropsy (re-sexed at weaning): Negative control group: 3 females, 8 males Group 1: 4 females, 0 males Group 2: 11 females, 5 males Group 3: 15 females, 14 males Mortality: high mortality was observed in animals from all groups predominately in the first 2 weeks of the study. Possible contributing factors are associated with disturbing the dams & pups during early stages of the study; excessive handling and/or injection trauma can contribute to increased cannibalism. Mortality was not considered to be due to any toxicity of the control and test articles. Clinical observations: no significant findings Macroscopic observation: no significant findings Microscopic evaluation of lung and injection sites: no tumors Conclusion: DNA from MDCK cells, influenza-infected MDCK cells, and influenza-infected BPL-treated MDCK cells was not oncogenic in infant nude mice.	48333

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Rat / Wistar < 4 days old	Subcutaneous	Single 0.2 mL dose on day 0, necropsy on day 152. Negative control: ~70µg DNA from murine tissue in phosphate buffered saline (PBS) Group 1: ~70µg DNA from uninfected and untreated MDCK cells in PBS Group 2: ~70µg DNA from Influenza-infected, untreated MDCK cells in PBS Group 3: ~55µg DNA from Influenza-infected, Beta-propiolactone (BPL)-treated MDCK cells in PBS	15/sex	Number of rats surviving to necropsy (re-sexed at weaning): Negative control group: 16 females, 12 males Group 1: 14 females, 15 males Group 2: 14 females, 14 males Group 3: 16 females, 14 males Mortality: 2 females (control group) found dead day 2 with no prior signs of sickness. No cause of death could be assigned. 1 female (group 1) was euthanized day 98 due to a congenital jaw condition. 1 female (group 2) cannibalized day 27. 1 female (group 2) was euthanized day 63 due to a congenital jaw condition. Clinical observations: no significant findings Macroscopic observation: no significant findings Microscopic evaluation of lung and injection sites: no tumors Conclusion: DNA from MDCK cells, influenza-infected MDCK cells, and influenza-infected BPL-treated MDCK cells was not oncogenic in infant rats.	48335

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Hamster / Aura < 4 days old	Subcutaneous	Single 0.2 mL dose on day 0, necropsy on day 150. Negative control: ~70µg DNA from murine tissue in phosphate buffered saline (PBS) Group 1: ~70µg DNA from uninfected & untreated MDCK cells in PBS Group 2: ~70µg DNA from Influenza-infected, untreated MDCK cells in PBS Group 3: ~55µg DNA from Influenza-infected, Beta-propiolactone (BPL)-treated MDCK cells in PBS	15/sex	Number of hamsters surviving to necropsy (re-sexed at weaning): Negative control group: 16 females, 12 males Group 1: 14 females, 16 males Group 2: 15 females, 12 males Group 3: 13 females, 17 males Mortality: 2 females (control group) cannibalized on days 1 & 8. 1F (group 2) cannibalized day 15. 2 males (group 2) cannibalized on days 15 & 16. Clinical observations: no significant findings Macroscopic observation: no significant findings Microscopic evaluation of lung and injection sites: no tumors Conclusion: DNA from MDCK cells, influenza-infected MDCK cells, and influenza-infected BPL-treated MDCK cells was not oncogenic in infant hamsters.	48334

Species / Strain	Method of Administration	Doses	Gender & No. per Group	Noteworthy Findings	Study number
Rat anti-thymocyte model < 24 hours old	Subcutaneous	Single 0.2 mL dose 5/group and all HeLa-treated animals necropsied on day 21, remainder on day 84 Group 1: 10^7 MDCK cells Group 2: 10^7 MDCK cells lysed by freeze/thaw Group 3: 10^7 MDCK cells lysed by treatment with Beta-propiolactone (BPL) Group 4: 10^7 HeLa cells	10/group, sex not specified	Anti-thymocyte serum (0.1 mL) was administered subcutaneously on days 0, 2, 7 and 14. Injection site tumors with or without metastasis to lung/lymph node were seen in all group 1 and group 4 animals necropsied on or before day 21. There were no tumors in group 2 and 3 animals at day 21. At day 84, remaining group 1, 2 and 3 animals were necropsied, there were no tumors in any of these animals. Conclusion: In the rat anti-thymocyte model, 10^7 MDCK cells are tumorigenic. Lysed or BPL-treated MDCK cells are not tumorigenic.	B012888/02
Mouse / NuNu ~4 weeks old	Subcutaneous	Single 0.2 mL dose, necropsy on day 57 10^7 MDCK cells 10^7 18C1-10T cells (positive control) 10^7 SHE cells (negative control, produces non-progressing nodules)	10 females	Injection site tumors with or without metastasis in 8 of 10 MDCK-treated animals. Assay was valid based on positive and negative control results. Conclusion: In nude mice, 10^7 MDCK cells are tumorigenic.	B96YG21.001

2.6.7 Toxicology Tabulated Summary

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2.6.7.1 Toxicology Overview – Completed Studies

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.
Single-Dose Toxicity							
	Rabbit / NZW	Intramuscular	Single dose	0.5 mL MF59C.1	Yes	[redacted]	501464
	Rabbit / NZW	Intramuscular	Single dose	1.0 mL 1:1 MF59C.1:saline	Yes	[redacted]	00-2672
Repeat-Dose Toxicity							
	Rabbit / NZW	Intramuscular	Days 1 & 15	0.5 mL 1:1 MF59:saline	Yes	[redacted] Italy	940292
	Rabbit / NZW	Intramuscular	Days 1 & 15	0.5 mL 1:1 MF59W:saline	Yes	[redacted]	2777-102
	Rabbit / NZW	Intramuscular	Day 57 & 71	0.5 mL 1:1 MF59:saline	Yes	[redacted]	433665
	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59C.1:saline & thiomersal	Yes	[redacted]	466122
	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59C.1:saline	Yes	[redacted]	759-002
	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59W:saline	Yes	[redacted]	2670-101
	Rabbit / Chinchilla	Intramuscular	Days 1, 15 & 29	0.25 mL×2 MF59W	Yes	Ciba-Geigy Ltd.	89-6192
	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59C.1:tris	Yes	[redacted] Italy	950031
	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL Placebo vaccine	Yes	[redacted]	2670-102
	Rabbit / NZW	Intramuscular	Days 1, 15, 29 & 43	0.5 mL 1:1 MF59C.1:saline	Yes	[redacted]	656583

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.
	Rabbit / NZW	Intramuscular	Days 43, 57, 71 & 85	0.5 mL 1:1 MF59C.1:saline	Yes	[redacted]	6549-166
	Rabbit / NZW	Intramuscular	Days 1, 8, 15, 29 & 43	0.5 mL MF59 alone	Yes	[redacted]	501438
	Rabbit / NZW	Intramuscular	Days 0, 14, 28, 42, 56 & 70	1.0 mL 1:1 MF59C.1:saline	Yes	[redacted]	00-2673
	Rabbit / NZW	Intramuscular	12 doses over 232 days	0.5 mL 1:1 MF59W:saline	Yes	[redacted]	2670-100
	Rabbit / NZW	Intramuscular	14 days	0.5 mL 1:1 MF59W:saline	Yes	Ciba-Geigy Ltd.	90-6081
Genotoxicity							
Ames Test	*In vitro*	N/A	N/A	MF59W Up to 5000 µg per plate	Yes	[redacted]	G96AQ62.502
Ames Test	*In vitro*	N/A	N/A	MF59C.1 Up to 5000 µg per plate	Yes	[redacted]	G96AQ61.502
Mouse Micronucleus	Mice / ICR	Intraperitoneal	Single dose	MF59W 1250, 2500 & 5000 mg/kg	Yes	[redacted]	G96AQ62.122
Mouse Micronucleus	Mice / ICR	Intraperitoneal	Single dose	MF59C.1 1250, 2500 & 5000 mg/kg	Yes	[redacted]	G96AQ61.122
Carcinogenicity							
	Not applicable		—	—	—	—	—
Reproductive and Developmental Toxicity							
	Rat / CD	Intramuscular	5 or 6 doses	0.5 mL MF59W	Yes	[redacted]	1303-002
	Rabbit / NZW	Intramuscular	Days 6-28	0.5 mL 1:7 & 1:3 MF59W:saline	Yes	[redacted]	1303-001P

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.
Local Tolerance							
	Rabbit / NZW	Intramuscular	Days 1 & 8	0.25 mL×2 MF59W	Yes	[redacted]	89-6280
	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.25 mL×2 1:1 MF59:vehicle	Yes	[redacted]	90-6230
	Dog / Beagle	Intramuscular	Days 1 & 8	0.5 mL MF59W	Yes	[redacted]	89-6281
	Dog / Beagle	Intramuscular	Days 1, 16 & 29	0.5 mL MF59W	Yes	Ciba-Geigy Ltd.	89-6193
	Dog / Beagle	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 MF59W:vehicle	Yes	[redacted]	90-6231
Other Toxicity Studies							
Magnusson-Kligman	Guinea pig / D-Hartley	Intradermal & topical	Days 1, 7 & 21	0.1 & 0.5 mL MF59C.1 or MF59W	Yes	[redacted]	564278
Other (Other) Toxicity Studies							
Hypersensitivity	Rat / Sprague-Dawley	Intratracheal	Days 1 & 15	0.1 mL 1:1 MF59C.1+saline	Yes	[redacted]	L08682
Magnusson-Kligman	Guinea pig / D-Hartley	Intradermal & topical	Days 1, 7 & 21	0.1 & 0.5 mL Fluad	Yes	[redacted]	564110
Repeat dose Toxicity	Rabbit / NZW	Intramuscular	Days 1 & 15	0.5 mL Fluad +/- CpG7909	Yes	[redacted] USA	6560-106
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15 & 29	0.5 mL 1:1 HCV E2+MF59C.1	Yes	[redacted] Ohio	N002833A
Tolerability	Rabbit / NZW	Intramuscular	Days 1, 15 & 27	0.5mL 1:1 MF59/MTP-PE:0.9% NaCl gp120:MF59 gp120:MF59/ MTP-PE	Yes	CIBA Geigy Limited	91-6009

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Dose and Test Article	GLP	Testing Facility	Study No.
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15 & 30	0.5mL 1:1 MF59/MTP-PE:0.9% NaCl gD2/gB2:MF59 gD2/gB2:MF59/MTP-PE	Yes	[redacted]	90-6306
Safety & Efficacy	Rabbit / NZW	Intramuscular	Days 0, 21, & either 42 or 70	0.5 mL CMVgB+MF59	No	[redacted]	1303-003
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15, 29 & 43	0.5 mL 1:1 HCV core+MF59C.1 HCV E2/core+MF59C.1	Yes	[redacted]	3-K84
Repeat Dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15, 29 & 43	0.5 mL Thai E gp120:MF59C.1 Thai E gp120:SF 2 gp120:MF59C.1	Yes	[redacted] USA	759-001
Safety & Efficacy	Rabbit / NZW	Intranasal	Days 0, 7, 14, & 21	0.1 mL 1:1 MF59C.1+saline	No	[redacted]	93-05-025R
Embryofetal Development	Rabbit / NZW	Intramuscular	Days 1, 15 & 29 pre-mating, days 7 & 20 gestation	0.5 mL aH5N1-IVV	Yes	[redacted] US	UBA00021
Embryofetal Development	Rabbit / NZW	Intramuscular	Days 1, 21, 42 & 6, 12, 18 days of gestation	0.5 mL HSV gD2/gB2/MF59 1.0 mL HSV gD2/gB2/MF59	Yes	[redacted]	1303-004P
Repeat dose Toxicity	Rabbit / NZW	Intramuscular	Days 1, 15, 29, 43, 57 & 71	0.6 mL 1:1 $HCVE_1E_2$/CpG:MF59	Yes	[redacted] Canada	500757
Safety & Efficacy	Woodchuck	Intramuscular	Day 0, Week 3, 6 & 12	0.5 mL 1:1 WHsAg+MF59C.1	No	[redacted] NY	98-07-263
Safety & Efficacy	Chimpanzee	Intramuscular	Months 0, 1, 2 & 6	0.5 ml 1:1 HBV:MF59C.1	Yes	[redacted] Texas	420-PT-0

2.6.7.2 Toxicokinetics – Overview of Studies

Not applicable.

2.6.7.3 Toxicokinetics – Overview of Data

Not applicable.

2.6.7.4 Toxicology Drug Substance – Completed Studies

Test Material	MF59 Batch No.	Study Dates	Study No.	Study Title
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6192	Intramuscular Study in Chinchilla Rabbits
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6280	Comparative Intramuscular Tolerability Study in Rabbits
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6281	Comparative Intramuscular Tolerability Study in Dogs
MF59 (water)	1[redacted]9/2	[redacted]/19[redacted] to [redacted]/19[redacted]	89-6193	Intramuscular Tolerability Study in Dogs
MF59 (water / thiomersal)	1[redacted]1/1	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6231	Comparative Intramuscular Tolerability Study in Dogs
MF59 (water / thiomersal)	1[redacted]1/1	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6230	Intramuscular Tolerability Study in Rabbits
MF59 (water)	1[redacted]0/2	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6081	14-Day Intramuscular Study in Rabbits
MF59 (water)	1[redacted]0/2	[redacted]/19[redacted] to [redacted]/19[redacted]	90-6306	BIOCINE HSV Vaccine CGP 52 120 HSVgD2/gB2 Antigen Combined with BIOCINE MF59 Emulsion Containing MTP-PE Intramuscular Tolerability Study in Rabbits
MF59 (water)	1[redacted]0/2	[redacted]/19[redacted] to [redacted]/19[redacted]	91-6009	Biocine HIV Vaccine GCP 52 121 gp120 Antigen Combined with Biocine MF59 Emulsion Containing MTP-PE Intramuscular Tolerability Study in Rabbits
MF59-0 (water)	M[redacted] 906	19[redacted]	1303-001P	Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to New Zealand White Rabbits
MF59-0 (water)	M[redacted] 849	[redacted]/19[redacted] to [redacted]/19[redacted]	2670-100	8-Month Intramuscular Toxicity Study in Rabbits of BIOCINE® HIV gp120 Antigen and BIOCINE® HIV Env 2-3 Antigen in Rabbits
MF59 (water)	M[redacted] 489	[redacted]/19[redacted] to [redacted]/19[redacted]	1303-002	Developmental Toxicity (Embryo-Foetal and Teratogenic Potential) Study of a Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to Crl:CD®BR VAF/Plus® Female Rats
MF59-0 (water)	M[redacted] 147	[redacted]/19[redacted] to [redacted]/19[redacted]	2670-101	28-Day Intramuscular Toxicity Study in Rabbits
MF59 (water)	M[redacted] 838	[redacted]/19[redacted] to 10/19[redacted]	940292	30-Day Subacute Toxicity Study in Rabbits by Intramuscular Route

Test Material	MF59 Batch No.	Study Dates	Study No.	Study Title
MF59C.1 (citrate)	K[redacted]F1	[redacted]/19[redacted] to [redacted]/19[redacted]	950031	Hepa Bio Vax B 6-Week Subacute Toxicity Study in New Zealand White Rabbits by Intramuscular Route
MF59C.1 (citrate)	3[redacted]5B	[redacted]/19[redacted] to [redacted]/19[redacted]	95-0455, 461, 93-05-025R	Rabbit / Intranasal Adjuvant-HA Toxicology
MF59W.1 (water)	K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ62.502	Bacterial Reverse Mutation Assay (Water Formulation)
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ61.502	Bacterial Reverse Mutation Assay (Citrate Formulation)
MF59W.1 (water)	K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ62.122	Micronucleus Cytogenetic Assay (Water Formulation)
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	G96AQ61.122	Micronucleus Cytogenetic Assay (Citrate Formulation)
MF59W.1 (water) MF59C.1 (citrate)	K[redacted]003 K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	564278	Guinea Pig Sensitization
MF59C.1 (citrate)	K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	564110	Guinea Pig Sensitization
MF59 (water)	K[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	2777-102	Fourteen-Day Intramuscular Toxicity Study of Connaught Fluzone/MF59 Vaccine in Rabbits
MF59C.1 (citrate)	M[redacted]002B	[redacted]/19[redacted] to [redacted]/19[redacted]	759-002	Subchronic Intramuscular Toxicity Study of Biocine® HPV-6 Vaccines in Rabbits (HPV-6 E7, HPV-6 L1 Antigens and MF59C.1 Adjuvant)
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	656583	Subchronic Intramuscular Toxicity Study of Biocine HIVp24 Vaccine in Rabbits
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	656583	Subchronic Intramuscular Toxicity Study of BIOCINE HIVp24 Vaccine in Rabbits
MF59C.1 (citrate)	M[redacted]001A	[redacted]/19[redacted] to [redacted]/19[redacted]	759-001	Subchronic Intramuscular Toxicity Study of BIOCINE HIV Thai E Gp120/SF2 GP120 Vaccine in Rabbits
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	3-K84	Intramuscular Toxicity Study of HCV (E2+Core) Vaccine in Rabbits
MF59C.1 (citrate)	M[redacted]002C	[redacted]/19[redacted] to [redacted]/19[redacted]	N002833A	Intramuscular Toxicity Study in Rabbits with HCV E2 Vaccine
MF59C.1 (citrate)	M[redacted]001	[redacted]/19[redacted] to [redacted]/19[redacted]	L08682	Potential of Intratracheally-Administered MF59 or LTK63 to Induce Hypersensitivity Pneumonitis or Lipoid Pneumonia in the Lungs of Male Sprague-Dawley Rats

Test Material	MF59 Batch No.	Study Dates	Study No.	Study Title
MF59C.1 (citrate)	K[redacted]007 and M[redacted]001A	[redacted]/19[redacted] to [redacted]/20[redacted]	98-07-263	Administration of Woodchuck Hepatitis Virus Surface Antigen (WHsAg) to Woodchucks with and without Chronic Woodchuck Hepatitis Virus (WHV) Infection
MF59C.1 (citrate)	M[redacted]002	[redacted]/19[redacted] to [redacted]/19[redacted]	420-PT-0	Safety and Efficacy of Chiron HBV PreS2+S/MF59C.1 Vaccine in an Adult Chimpanzee with Chronic Hepatitis B Infection
MF59C.1 (citrate)	M[redacted]001B	[redacted]/20[redacted] to [redacted]/20[redacted]	00-2672	A Single Dose Safety and Tolerability Study of Recombinant HCV Proteins and MF59 in Rabbits with a 15-Day Recovery Period
MF59C.1 (citrate)	M[redacted]001B	[redacted]/20[redacted] to [redacted]/20[redacted]	00-2673	A Multiple Dose Safety and Tolerability Study of Recombinant HCV Proteins and MF59 in Rabbits with a 14-Day Recovery Period
MF59C.1 (citrate)	b[redacted]001	[redacted]/20[redacted] to [redacted]/20[redacted]	501464	A Single Dose Intramuscular Toxicity Study of Rabies Vaccine Formulations in New Zealand White Rabbits
MF59C.1 (citrate)	b[redacted]001	[redacted]/20[redacted] to [redacted]/20[redacted]	501438	Rabies Vaccine Formulations Intramuscular Toxicity and Local Tolerability Study of Rabies Vaccine Formulations in NZW Rabbits
MF59C.1 (citrate)	0[redacted]1	[redacted]/20[redacted] to [redacted]/20[redacted]	6549-166	Multiple-Dose Intramuscular Injection Toxicity Study with HIV DNA Vaccine Formulation in New Zealand White Rabbits
MF59C.1 (citrate)	l[redacted]01C	[redacted]/20[redacted] to [redacted]/20[redacted]	500757	A 10 Week Intramuscular Vaccine Safety Study in New Zealand White Rabbits With HCV E1E2 Antigen, CpG and MF59 Adjuvant
MF59C.1 (citrate)	0[redacted]1	[redacted]/20[redacted] to [redacted]/20[redacted]	433665	12-Week Vaccine Toxicity Study in Female NZW Rabbits with Intranasal gp140 and LT-K63 Adjuvant Priming and Intramuscular gp140 and MF59 Adjuvant Boosting
MF59C.1 (citrate)	0[redacted]8	[redacted]/20[redacted] to [redacted]/20[redacted]	UBA00021	Intramuscular Reproductive and Developmental Toxicity Study of Fluad H5N1 Vaccine in Rabbits, Including a Postnatal Evaluation
MF59C.1 (citrate)	0[redacted]01	[redacted]/20[redacted] to [redacted]/20[redacted]	CBI-PCS-008	A study to determine the efficacy of an H5N1 Influenza vaccine adjuvanted with MF59 in the ferret experimental challenge model
MF59C.1 (citrate)	A[redacted]011	[redacted]/20[redacted] to [redacted]/20[redacted]	466122	6-Week vaccine toxicity study with H5N1 FCC + MF59 + Thiomersal vaccine by 3 intramuscular injections in NZW Rabbits

2.6.7.5 Single-Dose Toxicity

Test Article: MF59

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59C.1	Single dose 2/sex necropsied on days 3 & 15	0.5	4/sex	No mortality. No effects on clinical signs, body weight, food consumption, body temperature, ophthalmoscopy, or hematology/clinical chemistry. No edema or erythema at the injection sites. Elevated fibrinogen levels on day 3 in males. Complete reversal by day 15. Histopathological evaluation of injection sites on day 3 primarily showed inflammation, inflammatory cell infiltrate, and hemorrhage. By day 15, findings seen on day 3 had partially to fully resolved. On day 15, two animals exhibited signs of hepatic coccidiosis (parasitic granuloma around bile ducts and/or moderate cholangitis). These findings were not related to treatment but were consistent with a subclinical *Eimeria stiedae* infection.	Project No. 501464 Report No. 20717 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59C.1 Dose volume: 1.0 mL Saline (1 mL) in the right leg 1:1 MF59:saline (1 mL) in the left leg	Single dose (on day 0) 2/sex necropsied on days 2 & 15	1.0	4/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology parameters. No irritation or inflammation was observed at the injection site. Occasional slight desquamation/erythema at injection sites was observed 24 and 48 hrs post-dosing. Elevated fibrinogen levels were seen on day 2. Levels returned to baseline values by day 15 indicating complete reversal. Increased creatine kinase levels were observed on day 2. Levels returned to baseline values by day 15. This finding was considered consistent with an intramuscular injection of a 1 mL volume into rabbit muscle. On day 2, discoloration of injection sites was observed at both saline and MF59 injection sites. Minimal to moderate edema, inflammation, and/or hemorrhage were noted at injection sites of most animals. Local muscle necrosis with mineral deposition was occasionally seen. Lesions were partially to fully resolved by day 15.	00-2672 GLP

2.6.7.6 Repeat Dose Toxicity – Nonpivotal Studies

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59:saline 45 µg Agrippal 45 µg Agrippal + MF59 (Fluad)	Days 1 & 15 3/sex necropsied on days 17 &31	0.5	6/sex	No mortality, no clinical signs or effects on study parameters indicative of local or systemic toxicity in any treatment group. There were no treatment-related effects on hematology and clinical chemistry between the groups. There were no relevant differences between groups in organ weights, and no macroscopic observations except for the injection sites. Macroscopic findings at the injection sites treated two days previously indicated an increased frequency of slight focal hemorrhage in the Agrippal+MF59W.1 group compared to the other two groups. There were no macroscopic findings at injection sites treated 16 or 30 days before necropsy. Histological examination of the injection site 2 days post-injection revealed interstitial inflammation (mainly acute), interstitial hemorrhage, and/or muscle fiber degeneration in almost all animals. These observations were more notable in the Agrippal+MF59W.1 group, followed by the Agrippal group, then the MF59W.1 group. Sixteen days after injection, inflammatory and degenerative changes were still present, but to a lesser extent in most animals. Thirty days after injection, partial to full recovery was evident in most animals. At injection sites 16 and 30 days post-injection, there were no differences between the groups. There were no other tissues with treatment-related findings.	940292 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59W:saline	Dosed on days 1 & 15 3/sex necropsied on days 15 & 29	0.5	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or clinical chemistry parameters. Mild local irritation at the injection site was observed. Slight erythema was observed at injection sites in some animals for 2-4 days following injection. Inflammation, hemorrhage and focal muscle degeneration were seen microscopically at injection sites; findings were partially to fully resolved by the end of the recovery period.	CHV 2777-102 GLP
Rabbit / Chinchilla	Intramuscular MF59W	Dosed on days 1, 15 & 29 All animals necropsied on day 38	0.5 (0.25 per leg)	5/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or clinical chemistry parameters. Increased fibrinogen levels in males and females after the first injection. Microscopic changes at the injection sites indicated a slight inflammatory response.	89-6192 GLP
Rabbit / New Zealand White	Intramuscular 1:1 MF59W:saline	Dosed on days 1, 15 & 29 3/sex necropsied on days 29 & 43	0.5	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology or serum chemistry parameters. There was no evidence of systemic toxicity or dermal irritation at the injection sites. There was focal hemorrhage and/or residual inflammation at injection sites; severity was minimal after the recovery period.	HWA 2670-101 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline	Dosed on days 1, 15 & 29 3/sex necropsied on days 31 & 43	0.5	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, organ weights, clinical chemistry or hematology parameters. Macroscopic observations at the terminal necropsy consisted of red discoloration of the third injection site in two of three animals; this correlated with microscopic findings of hemorrhage, subacute inflammation, and/or muscle fiber degeneration. Incidence and severity of injection site findings were reduced at the recovery necropsy.	759-002 GLP
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:Tris buffer	Dosed on days 1, 15, & 29 3/sex necropsied on days 31 & 43	0.5	6/sex	No mortality. No treatment-related effects on clinical signs (including injection sites), body weight, body temperature, ophthalmoscopy, organ weights or hematology parameters. Mildly elevated serum LDH and creatine kinase levels were seen at day 31. By day 43 levels had returned to normal in most animals; 2 females still had slightly higher CK levels. Macroscopically, slight hemorrhagic areas were seen at the third injection site (48 hours post-injection). Microscopically, inflammatory and degenerative changes (subacute inflammation, histiocytosis, and muscle fiber degeneration and/or necrosis) at all injection sites were seen. By day 43 incidence and severity of macroscopic and microscopic findings were reduced, indicating resolution.	950031 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline	Dosed on days 1, 15, 29 & 43 3/sex necropsied on days 45 & 57	0.5	6/sex	There was no mortality. There were no treatment-related effects on body weights, ophthalmoscopy, or organ weights. Local reactions at injection sites were minimal and of low incidence. Body temperatures increased slightly over time. Enlarged popliteal lymph nodes were observed macroscopically on day 45; this finding was not seen on day 57. On day 57 two animals had reddened lymph nodes. Microscopic evaluation of injection sites at day 45 showed inflammatory cell infiltrates; the severity was graded 'very mild' or 'mild'. On day 57 the incidence and severity of findings was decreased, indicating reversibility.	Project No. 656583 Report No. 14160 GLP
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline	Dosed on days 1, 8, 15, 29 & 43 3/sex necropsied on days 45 & 57	0.5	6/sex	No mortality. No treatment-related effects on clinical observations (including injection sites), body weight, food consumption, body temperature, organ weights or ophthalmoscopy. Globulin was elevated in some males on days 17 and 45. Redding or enlargement in the draining lymph nodes was noted in one male. Inflammatory cell infiltrate at injection sites was seen in all animals at day 45. On day 57 incidence and severity were reduced, indicating recovery. Approximately half the rabbits had a subclinical *Eimeria stiedae* infection; this did not prevent the evaluation of local or systemic toxicity.	Project No. 501438 Report No. 20611 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59C.1:saline Saline (1 mL) in the right leg 1:1 MF59C.1:saline (1 mL) in the left leg	Dosed on days 0, 14, 28, 42, 56, & 70 3/sex necropsied on days 72 & 84	1.0	6/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology parameters. Sporadic erythema, edema, and desquamation were seen at injection sites, severity was generally very slight. Fibrinogen was increased relative to pretest values and had returned to pre-treatment levels by the end of recovery (day 84). Sporadic and reversible increases in creatine kinase levels were seen. On day 72, edema, inflammation, and hemorrhage were noted at injection sites along with occasional muscle necrosis and mineral deposition. Findings were generally more severe and/or frequent on day 72 (minimal to moderate severity) as compared to day 84 (minimal to slight severity) indicating partial resolution. Note: although this study is considered pivotal (2× MF59 administered 6 times), there was no saline-alone control group and therefore the tabular format for pivotal studies was not used.	00-2673 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular 1:1 MF59W:saline	Dosed on days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211 & 232 3/sex necropsied on days 233 & 247	0.5	6/sex	No mortality. No treatment-related effects on clinical observations with the exception of transient and mild dermal irritation at the injection sites of most animals. There was no effect on body weight, body temperature, urinalysis, organ weights or ophthalmoscopy. There were occasional incidences of decreased prothrombin times 2 days post-injection. Occasional increases in globulin and mild decreases in albumin were seen. Increases were noted in creatine kinase values at the 2-day post injection intervals; these changes were statistically significant and are indicative of muscular damage associated with injections. Microscopically, acute to subacute inflammation was noted at injection sites.	2670-100 GLP
Woodchuck (*Marmota monax*)	Intramuscular Group 1: 20 µg WHsAg + MF59C.1 Group 2: 20 µg WHsAg + MF59C.1 Group 3: 20 µg WHsAg + Alum	Four injections on day 0, and weeks 3, 6, and 12.	0.5	Group 1: WHV-infected 4M;5F Group 2: Uninfected 2M;2F Group 3: Uninfected 2M;2F	No treatment-related clinical signs or changes in liver pathology parameters relative to pre-dose values. Slight decrease in body weights seen in Group 1 was possibly related to chronic infection with WHV. Treatment was well tolerated, but evidence of efficacy was not seen.	98-07-263 non-GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Chimpanzee (*Pan troglodytes*)	Intramuscular 20 µg HBsAg + MF59C.1	Part 1: Four injections on day 1 and months 1, 2, and 6. Part 2: Above regimen was repeated: months 9, 10, 11, and 15.	0.5	1 male animal	No signs of systemic or local toxicity based on: physical examinations, injection-site observations, ophthalmoscopy, serum chemistry, hematology, coagulation, or urinalysis. Liver histopathology pre- and post-treatment comparable. Due to the presence of HBsAg at the end of the treatment period, the animal was still considered to be chronically infected.	420-PT-0 GLP

2.6.7.7 Repeat Dose Toxicity – Pivotal Studies

2.6.7.7.1 Study No. 466122

Report Title: 6-week vaccine toxicity study with H5N1 FCC + MF59 + thiomersal vaccine by three intramuscular injections in New Zealand White rabbits

Test Article: aCCD-H5N1-ivv

Species/Strain: Rabbit / New Zealand White

Duration of Dosing: Days 1, 15 and 29

Study No.: 466122

Initial Age: Approximately 13 weeks

Duration of Postdose: 2 days (main groups) or 14 days (recovery groups)

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Intramuscular

GLP Compliance: Yes

Vehicle/Formulation: Control article: phosphate buffered saline (PBS).
Adjuvant control: 1:1 PBS:MF59.
Test article: 15µg antigens from Indonesia/5/2005(H5N1)/PR8-IBCDC-RG02 strain with 0.25mL MF59
All test materials contained 100 µg/mL thiomersal

Location: 4.2.3

Special Features: Modified Draize scoring of injection sites.
Immunogenicity evaluated pretest, on days 15 and 29 (all animals), day 31 (main groups), and day 43 (recovery groups).

Study No. 466122						
	Group 1		Group 2		Group 3	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (H5N1 FCC+ MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Noteworthy Findings [a]						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight	–	–	–	–	–	–
Food Consumption	–	–	–	–	–	–

[a] – No noteworthy findings

Study No. 466122						
	Group 1		Group 2		Group 3	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (H5N1 FCC+ MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Clinical Observations	–	–	–	–	–	–
Heart Rate	–	–	–	–	–	–
Respiratory Rate	–	–	–	–	–	–
Body Temperatures (ºC) [a]						
Day 29 (pre-dose)	39.10 ± 0.10	39.30 ± 0.16	39.00 ± 0.28	39.08 ± 0.19[*]	38.85 ± 0.25	39.19 ± 0.13
Day 29 (2h post-dose)	38.81 ± 0.44	39.32 ± 0.20	38.98 ± 0.23	39.04 ± 0.19[*]	39.18 ± 0.19[*]	39.26 ± 0.15[‡]
Ophthalmoscopy	–	–	–	–	–	–
Serum Chemistry						
Total globulin (g/l)						
Day 17	16.6 ± 0.8	15.9 ± 1.1	17.7 ± 1.2	17.0 ± 1.2	20.3 ± 1.2[††, □□]	19.6 ± 1.3[††, □□]
Day 31	15.4 ± 0.8	15.2 ± 0.9	17.2 ± 1.1[††]	16.5 ± 0.9[†]	18.1 ± 1.1[††]	18.4 ± 1.8[††]
Albumin / globulin ratio						
Day 17	2.4 ± 0.1	2.5 ± 0.2	2.2 ± 0.1[††]	2.4 ± 0.1	1.9 ± 0.1[††, □□]	2.0 ± 0.1[††, □□]
Day 31	2.7 ± 0.1	2.8 ± 0.2	2.3 ± 0.1[††]	2.6 ± 0.2	2.3 ± 0.1[††]	2.3 ± 0.2[††, □]

[a] Mean ± standard deviation. N= 7 or 8/sex/group
[*] Dunnett-test based on pooled variance; groups 2 and 3 significantly different from PBS control group 1 at 5% ([*]) or 1% ([**]) level
[‡] Dunnett-test based on pooled variance; group 3 significantly different from group 2 at 5% ([‡]) or 1% ([‡‡]) level
[□] Steel-test; group 3 significantly different from group 2 at 5% ([□]) or 1% ([□□]) level
[†] Steel-test; groups 2 and 3 significantly different from PBS control group 1 at 5% ([†]) or 1% ([††]) level

Study No. 466122						
	Group 1		**Group 2**		**Group 3**	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (H5N1 FCC+ MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Hematology						
Prothrombin time (seconds)						
Day 17	7.6 ± 0.3	7.7 ± 0.3	7.2 ± 0.3**	7.5 ± 0.2	7.1 ± 0.2**	7.1 ± 0.3**, ‡‡
Day 31	7.7 ± 0.3	7.7 ± 0.2	7.2 ± 0.3*	7.3 ± 0.1**	7.2 ± 0.1**	7.1 ± 0.1**, ‡
Fibrinogen level (g/l)						
Day 17	2.67 ± 0.30	2.04 ± 0.16	3.95 ± 0.86*	2.38 ± 0.33	5.44 ± 1.23**, ‡	4.12 ± 0.75**, ‡‡
Day 31	2.62 ± 0.44	2.18 ± 0.17	4.81 ± 0.71**	2.94 ± 0.56**	4.49 ± 0.75**	3.64 ± 0.54**, ‡
Organ Weights	–	–	–	–	–	–
Macroscopic Pathology	–	–	–	–	–	–
Histopathology [a]						
Main Necropsy	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Injection sites						
Right hindlimb (2 days post last dose)						
Evaluated / Affected	4 / 0	4 / 3	4 / 4	4 / 2	4 / 1	4 / 3
Intermuscular connective tissue macrophages	0	0	1++	1++	0	0

* Dunnett-test based on pooled variance; groups 2 and 3 significantly different from PBS control group 1 at 5% (*) or 1% (**) level
‡ Dunnett-test based on pooled variance; group 3 significantly different from group 2 at 5% (‡) or 1% (‡‡) level
[a] Severity score + = minimal, ++ = mild, +++ = moderate

Study No. 466122						
	Group 1		**Group 2**		**Group 3**	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (H5N1 FCC+ MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Dermal hemorrhage	0	1++	0	0	1++	0
Panniculus muscle fiber degeneration	0	1+	2+, 1+++	1++	0	0
Deep epidermal acute inflammation	0	0	1+	0	0	1+, 1++
Deep dermal diffuse macrophage infiltration	0	0	1++	2++	0	3+
Panniculus focal macrophage infiltration	0	0	1++	0	0	0
Intermuscular acute inflammation	0	0	2++	0	0	0
Deep muscle focal degeneration	0	1++	0	1+	0	0
Deep muscle hemorrhage	0	1+	0	0	0	0
Deep muscle macrophage infiltration	0	1+	0	0	0	0
Left hindlimb (16 days post last dose)						
Evaluated / Affected	4 / 0	4 / 1	4 / 4	4 / 3	4 / 3	4 / 1
Intermuscular connective tissue macrophages	0	0	0	0	2+	0
Dermal hemorrhage	0	0	1+	0	0	1++
Panniculus muscle fiber degeneration	0	1+	2++	3+	2++	0

Study No. 466122						
	Group 1		**Group 2**		**Group 3**	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (H5N1 FCC+ MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Deep muscle focal degeneration	0	0	1++, 1+++	0	0	0
Deep muscle hemorrhage	0	0	1++	0	0	0
Recovery Necropsy	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Injection sites						
Right hindlimb (14 days post last dose)						
Evaluated / Affected	3 / 0	4 / 2	4 / 3	4 / 2	4 / 2	3 / 2
Intermuscular connective tissue macrophages	0	0	0	0	1+	0
Panniculus muscle fiber degeneration	0	1+	1+, 1++, 1+++	1+	1++	2++
Follicular adnexa deficit	0	0	1++	0	0	0
Dermal hemorrhage	0	1+	0	1+	0	0
Superficial pustular dermatitis	0	1+	0	0	0	0
Left hindlimb (28 days post last dose)						
Evaluated / Affected	3 / 0	4 / 1	4 / 0	4 / 0	4 / 1	3 / 1
Muscle fiber degeneration	0	1+	0	0	1++	1++

Study No. 466122						
	Group 1		Group 2		Group 3	
Dose (µg antigen per dose)	**0 – Control Article (PBS)**		**0 – Adjuvant Control (MF59)**		**15 – Vaccine (H5N1 FCC+ MF59)**	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Antibody Titers (HI)[a]						
Pretest	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)
Day 15	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	10.9 (<10 – 20)
Day 29	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – 20)	207.5 (160 – 320)	246.8 (160 – 640)
Day 31	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	269.1 (160 – 320)	190.3 (160 – 320)
Day 43	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	<10 (<10 – <10)	380.5 (320 – 640)	320.0 (160 – 640)

[a] Titers are presented as geometric mean (range). N = 8/sex/group pretest and on days 15 and 29, N = 4/sex/group on days 31 and 43. Titers shown are heterologous (HI assay using Vietnam H5N1 strain; animals were vaccinated with antigens from the Indonesian H5N1 strain). Assay against the homologous virus strain will be performed when virus is available.

2.6.7.7.2 Study No. 2670-100

Report Title: 8-Month Intramuscular Toxicity Study in Rabbits of BIOCINE® HIV gp120 Antigen and BIOCINE® HIV Env 2-3 Antigen in Rabbits

Test Article: MF59

Species/Strain: Rabbit / New Zealand White

Duration of Dosing: Days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211 & 232

Study No.: 2670-100

Initial Age: Approximately 4 months

Duration of Postdose: 1 and 15 days

Date of First Dose: [redacted] 19[redacted]

Method of Administration: Intramuscular

Location 4.2.3

Vehicle/Formulation: MF59 diluted 1:1 with saline

GLP Compliance: Yes

Special Features: This study evaluated other test articles; for clarity only the MF59 and saline groups are presented here.

No Observed Adverse Effect Level: > 0.5 mL 1:1 MF59:saline

Daily Dose	Study Number 2670-100			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Noteworthy Findings				
Died or Sacrificed Moribund	0	0	0	0
Body Weight	—	—	—	—
Body Temperature	—	—	—	—
Clinical Observations	—	—	—	—
Injection Site (Draize) Observations	—	—	—	—
Ophthalmoscopy	—	—	—	—
Urinalysis	—	—	—	—

— No significant findings

Daily Dose	Study Number 2670-100			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Hematology[a]				
Fibrinogen				
Day 24	253	—	309	—
Day 42	—	196	—	221
Day 45	—	210	—	276
Day 66	—	185	—	307
Day 87	—	192	—	278
Day 108	230	—	352	—
Day 129	229	175	384	320
Day 150	224	172	413	302
Day 171	219	168	337	327
Day 213	213	175	328	287
Day 233	193	165	290	266
Prothrombin Time (seconds)				
Day 66	—	6.2	—	6.1
Day 108	—	6.0	—	5.9
Day 129	—	6.0	—	5.9
Day 150	—	6.0	—	5.9
Day 168	6.0	—	6.1	—

[a] Only significantly different ($p \leq 0.05$) values are shown

Daily Dose	Study Number 2670-100			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Day 171	6.0	—	5.9	—
Serum Chemistry				
Creatine Kinase				
Day 129	281 (SD64.1)	427 (SD 207.4)	1098 (SD 732.8)	2177 (SD 944.5)
Day 150	251 (SD 68.1)	261 (SD 88.5)	3301 (SD 2617.0)	3416 (SD 2631.6)
Day 171	292 (SD 113.1)	254 (SD 74.2)	2085 (SD 1229.3)	1525 (SD 574.3)
Day 192	290 (SD 62.3)	340 (SD 244.2)	1490 (SD 1059.1)	2425 (SD 1810.4)
Day 213	258 (SD63.7)	275 (SD 87.3)	1762 (SD 992.0)	3060 (SD 2000.6)
Day 233	305 (84.8)	443 (SD 230.1	6751 (SD 4694.9)	8770 (SD 4314.5)
Terminal Necropsy: Day 233	**M: 3**	**F: 3**	**M: 3**	**F: 3**
Organ Weights	—	—	—	—
Macroscopic Pathology	—	—	—	—
Histopathology[a]				
Injection Sites				
Muscle necrosis	0	0	1	0
Inflammation/muscle, acute/subacute	0	0	1	0
Inflammation/SC, acute/subacute	0	0	0	2
Inflammation/muscle, chronic	1	0	0	0
Hemorrhage/muscle	0	0	1	1
Hemorrhage/SC	0	0	0	1

[a] Results are displayed as number of animals affected; severity was generally slight to minimal

Daily Dose	Study Number 2670-100			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Recovery Necropsy: Day 247	**M: 3**	**F: 3**	**M: 3**	**F: 3**
Macroscopic Pathology	—	—	—	—
Injection Sites				
Inflammation/muscle, acute/subacute	0	0	1	0

2.6.7.7.3 Study No. 90-6081

Report Title: 14-day Intramuscular Toxicity Study in Rabbits (New Zealand White)

Test Article: MF59

Species/Strain: Rabbit / New Zealand White

Duration of Dosing: 14 days

Study No.: 90-6081

Initial Age: Approximately 13 weeks

Duration of Postdose: 7 days

Date of First Dose: [redacted] 19[redacted]

Method of Administration: Intramuscular

Location 4.2.3

Vehicle/Formulation: Saline: 0.9% diluted 1:1 with water for injection. MF59: diluted 1:1 with saline

GLP Compliance: Yes

Special Features: This study evaluated other test articles; for clarity only the MF59 and saline groups are presented here.

No Observed Adverse Effect Level: > 0.5 mL 1:1 MF59:saline

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Noteworthy Findings				
Died or Sacrificed Moribund	0	0	0	0
Body Weight	—	—	—	—
Food Consumption	—	—	—	—
Body Temperature	—	—	—	—
Clinical Observations	—	—	—	—
Ophthalmoscopy	—	—	—	—
Hematology				
Fibrinogen Pretest	278	199	270	234
Fibrinogen End of Treatment	320	219	477	498[+]

— No significant findings

[+] Steel-test significant at 5% level

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Fibrinogen Recovery	404	228	310	301
Serum Chemistry				
Urinalysis	—	—	—	—
Terminal Evaluation: Day 15				
Organ Weights	—	—	—	—
Macroscopic Pathology				
Injection Sites – 1 day post-last dose				
Subcutaneous Hemorrhage	1	0	4	4
Focal discoloration and/or reddening	2	2	4	4
Histopathology[a]				
Thymus				
Reduction of lymphatic tissue	1 (1.0)	0	3 (1.0)	2 (1.0)
Spleen				
Congestion	0 (1.0)	2 (1.5)	3 (1.0)	4 (1.0)
Hemosiderosis	2 (1.0)	2 (1.0)	2 (2.5)	3 (1.3)
Red pulp, neutrophils	4 (1.2)	4 (2.2)	4 (2.2)	4 (2.7)
Neutrophil precursors	2 (1.5)	3 (1.6)	4 (2.2)	2 (4.0)
Bone Marrow				
Hypercellularity	0	2 (1.0)	2 (1.0)	3 (2.6)

[a] Results are displayed as number of animals affected (mean severity).
Severity scores: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Liver				
Infiltration - mixed cells	0	1 (1.0)	1 (1.0)	1 (1.0)
Injection Sites – 1 day post-last dose				
Neutrophil infiltrates	3 (1.0)	2 (1.0)	1 (3.0)	2 (2.5)
Monocyte infiltrates	4 (1.2)	2 (1.0)	3 (1.6)	4 (1.7)
Macrophages	4 (1.5)	1 (1.0)	2 (2.0)	4 (2.0)
Muscle cell necrosis	3 (1.3)	2 (1.0)	1 (2.0)	3 (1.6)
Muscle cell regeneration	2 (1.5)	1 (1.0)	3 (1.0)	3 (1.0)
Edema	0	0	1 (1.0)	2 (2.0)
Hemorrhage	1 (1.0)	0	0	1 (1.0)
Postdose Evaluation: Day 22				
Number Evaluated	4	4	4	4
Macroscopic Pathology				
Injection Sites – 7 days post-last dose				
Subcutaneous Hemorrhage	0	0	2	2
Focal discoloration and/or reddening	2	3	2	1
Histopathology[a]				
Thymus				
Reduction of lymphatic tissue	0	2 (2.0)	2 (1.5)	1 (1.0)

[a] Results are displayed as number of animals affected (mean severity).
Severity scores: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Daily Dose	Study Number 90-6081			
	0.5 mL Saline (Control)		0.5 mL MF59	
Number of Animals	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Histopathology[a]				
Spleen				
Congestion	1 (1.0)	1 (2.0)	1 (2.0)	4 (1.7)
Hemosiderosis	1 (1.0)	1 (2.0)	1 (4.0)	3 (1.0)
Red pulp, neutrophils	4 (2.0)	4 (1.5)	4 (3.5)	4 (3.0)
Neutrophil precursors	4 (1.7)	4 (1.0)	4 (2.7)	4 (3.0)
Bone Marrow				
Hypercellularity	0	3 (2.3)	4 (1.7)	4 (2.7)
Liver				
Infiltration - mixed cells	1 (1.0)	1 (1.0)	1 (1.0)	0
Injection Sites – 7 days post-last dose				
Neutrophil infiltrates	0	0	2 (1.0)	0
Monocyte infiltrates	2 (1.0)	2 (1.0)	4 (1.0)	4 (1.0)
Macrophages	0	0	4 (1.2)	0
Muscle cell necrosis	0	2 (1.0)	0	1 (1.0)
Muscle cell regeneration	2 (1.0)	1 (1.0)	4 (1.0)	4 (1.0)
Edema	0	0	0	0
Hemorrhage	0	0	0	0

2.6.7.8 Genotoxicity: In Vitro

Reverse Mutation MF59W.1

Report Title: Bacterial Reverse Mutation Assay with MF59W.1 (Water Formulation)

Test Article: MF59

Test for Induction of: Reverse Mutation in Bacterial Cells

Strains: S. typhimurium and E. coli

Metabolizing System: Aroclor-induced Rat Liver S9, 10%

Vehicle: Saline

Treatment: Plate incorporation for 48 - 72 hr

Cytotoxic Effects: None

Genotoxic Effects: None

No. of Independent Assays: 2

No. of Replicate Cultures: 3

No. of Cells Analyzed /Culture: $\geq 0.3 \times 10^9$

Study No.: G96AQ62.502

Location 4.2.3

GLP Compliance: Yes

Date of Treatment: [redacted] 19[redacted]

Study No.: G96AQ62.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
Without Activation	MF59W.1	0	15 ± 3	110 ± 9	9 ± 2	6 ± 2	15 ± 3
		100	14 ± 4	97 ± 6	7 ± 1	3 ± 1	14 ± 3
		333	14 ± 3	109 ± 11	10 ± 5	3 ± 1	10 ± 1
		1000	13 ± 4	117 ± 16	7 ± 1	5 ± 3	17 ± 2
		3333	20 ± 2	122 ± 3	6 ± 3	6 ± 1	16 ± 4
		5000	15 ± 1	115 ± 17	10 ± 1	3 ± 4	20 ± 7
	2-nitrofluorene	1.0	124 ± 23				
	Sodium azide	1.0		534 ± 133	428 ± 51		
	9-aminoacridine	75				99 ± 24	
	Methyl methanesulfonate	1000					134 ± 19
With Activation		0	21± 6	140 ± 2	12 ± 2	7 ± 2	17 ± 7
		100	16 ± 4	133 ± 7	7 ± 1	5 ± 5	12 ± 1

Study No.: G96AQ62.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
		333	17 ± 5	139 ± 23	8 ± 1	8 ± 3	16 ± 1
		1000	11 ± 9	137 ± 8	10 ± 3	5 ± 2	12 ± 3
		3333	20 ± 8	117 ± 29	14 ± 4	5 ± 3	16 ± 5
		5000	18 ± 6	119 ± 12	12 ± 3	4 ± 1	16 ± 4
	2-aminoanthracene	1.0	774 ± 183	687 ± 45	66 ± 9	73 ± 19	87 ± 10

Reverse Mutation MF59C.1

Report Title: Bacterial Reverse Mutation Assay with MF59C.1 (Citrate Formulation)

Test Article: MF59

Test for Induction of: Reverse Mutation in Bacterial Cells

Strains: S. typhimurium and E. coli

Metabolizing System: Aroclor-induced Rat Liver S9, 10%

Vehicle: Saline

Treatment: Plate incorporation for 48 - 72 hr

Cytotoxic Effects: None

Genotoxic Effects: None

No. of Independent Assays: 2

No. of Replicate Cultures: 3

No. of Cells Analyzed /Culture: $\geq 0.3 \times 10^9$

Study No.: G96AQ61.502

Location: 4.2.3

GLP Compliance: Yes

Date of Treatment: [redacted] 19[redacted]

Study No.: G96AQ61.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
Without Activation	MF59C.1	0	13 ± 4	121 ± 8	11 ± 1	6 ± 1	22 ± 3
		100	20 ± 5	122 ± 0	9 ± 1	6 ± 0	23 ± 3
		333	18 ± 4	126 ± 10	8 ± 1	6 ± 3	15 ± 4
		1000	15 ± 3	129 ± 12	9 ± 2	7 ± 2	22 ± 4
		3333	11 ± 2	116 ± 11	11 ± 4	4 ± 3	26 ± 11
		5000	16 ± 1	131 ± 11	9 ± 3	3 ± 3	25 ± 1
	2-nitrofluorene	1.0	133 ± 40				
	Sodium azide	1.0		586 ± 87	346 ± 65		
	9-aminoacridine	75				274 ± 55	
	Methyl methanesulfonate	1000					171 ± 14
With Activation		0	24 ± 9	133 ± 9	16 ± 5	9 ± 4	17 ± 5
		100	19 ± 2	125 ± 18	10 ± 5	4 ± 2	19 ± 2
		333	20 ± 1	124 ± 7	12 ± 6	4 ± 2	19 ± 6

Study No.: G96AQ61.502							
Metabolic Activation	**Test Article**	**Concentration (µg per plate)**	**TA98 (Mean ± SD)**	**TA100 (Mean ± SD)**	**TA1535 (Mean ± SD)**	**TA1537 (Mean ± SD)**	**WP2uvrA (Mean ± SD)**
		1000	17 ± 3	128 ± 21	15 ± 3	6 ± 2	18 ± 7
		3333	27 ± 9	108 ± 4	10 ± 3	7 ± 6	16 ± 2
		5000	16 ± 1	119 ± 5	9 ± 2	9 ± 4	14 ± 5
	2-aminoanthracene	1.0	506 ± 86	572 ± 67	75 ± 17	43 ± 20	172 ± 51

2.6.7.9 Genotoxicity: In Vivo

Mouse Micronucleus MF59W.1

Report Title: Micronucleus Cytogenetic Assay in Mice With MF59W.1 (Water Formulation) **Test Article:** MF59

Test for Induction of: Bone marrow micronuclei **Treatment Schedule:** Single dose **Study No.:** G96AQ62.122

Species/Strain: Mice / ICR **Sampling Time:** 24, 48, and 72 hours post-dose

Age: 6-8 Weeks **Method of Administration:** Intraperitoneal injection, 20 mL/kg **Location:** 4.2.3

Cells Evaluated: Polychromatic erythrocytes **Vehicle Formulation:** Saline **GLP Compliance:** Yes

No. of Cells Analyzed/Animal: 1000 **Special Features:** None **Date of Dosing:** [redacted] 19[redacted]

Toxic/Cytotoxic Effects: No mortality, lethargy in all animals dosed with 5000 mg/kg MF59W.1

Genotoxic Effects: None **Evidence of Exposure:** Lethargy at 5000 mg/kg

Test Article (sampling time)	Dose (mg/kg)	No. of Animals	PCE/Total erythrocytes (Mean ± SD)		Micronucleated PCE per 1000 PCEs (Mean ± SD)	
			Males	Females	Males	Females
20 mL/kg Saline (24hr)	0	5M/5F	0.61 ± 0.08	0.60 ± 0.09	1.0 ± 0.71	1.2 ± 0.84
MF59W.1 (24hr)	1250	5M/5F	0.59 ± 0.06	0.60 ± 0.06	0.8 ± 0.84	0.8 ± 0.84
	2500	5M/5F	0.56 ± 0.03	0.57 ± 0.01	0.2 ± 0.45	0.6 ± 0.89
	5000	5M/5F	0.60 ± 0.03	0.59 ± 0.10	0.8 ± 0.84	0.2 ± 0.45
Cyclophosphamide (24hr)	60	5M/5F	0.46 ± 0.07	0.47 ± 0.09	37.2 ± 15.42	32.0 ± 7.97
20 mL/kg Saline (48hr)	0	5M/5F	0.53 ± 0.05	0.53 ± 0.07	1.0 ± 0.71	0.6 ± 0.55
MF59W.1 (48hr)	1250	5M/5F	0.56 ± 0.05	0.52 ± 0.04	1.0 ± 1.00	1.2 ± 0.84
	2500	5M/5F	0.51 ± 0.05	0.52 ± 0.02	0.4 ± 0.55	1.0 ± 1.22
	5000	5M/5F	0.53 ± 0.06	0.46 ± 0.08	0.4 ± 0.55	1.2 ± 0.84
20 mL/kg Saline (72hr)	0	5M/5F	0.51 ± 0.08	0.55 ± 0.06	0.8 ± 0.84	0.2 ± 0.45
MF59W.1 (72hr)	1250	5M/5F	0.48 ± 0.10	0.61 ± 0.13	1.0 ± 0.00	0.6 ± 0.55

Test Article (sampling time)	Dose (mg/kg)	No. of Animals	PCE/Total erythrocytes (Mean ± SD)		Micronucleated PCE per 1000 PCEs (Mean ± SD)	
			Males	Females	Males	Females
	2500	5M/5F	0.50 ± 0.07	0.59 ± 0.09	0.4 ± 0.55	0.4 ± 0.55
	5000	5M/5F	0.53 ± 0.6	0.53 ± 0.08	1.0 ± 1.00	0.6 ± 0.55

Mouse Micronucleus MF59C.1

Report Title: Micronucleus Cytogenetic Assay in Mice With MF59C.1 (Citrate Formulation)

Test Article: MF59

Test for Induction of: Bone marrow micronuclei

Treatment Schedule: Single dose

Study No.: G96AQ61.122

Species/Strain: Mice / ICR

Sampling Time: 24, 48, and 72 hours post-dose

Age: 6-8 Weeks

Method of Administration: Intraperitoneal injection, 20 mL/kg

Location: 4.2.3

Cells Evaluated: Polychromatic erythrocytes (PCE)

Vehicle Formulation: Saline

GLP Compliance: Yes

No. of Cells Analyzed/Animal: 1000

Special Features: None

Date of Dosing: [redacted] 19[redacted]

Toxic/Cytotoxic Effects: No mortality, lethargy in 9/20 males and 1/20 females dosed with 5000 mg/kg MF59C.1

Genotoxic Effects: None

Evidence of Exposure: Lethargy at 5000 mg/kg

Test Article (sampling time)	Dose (mg/kg)	No. of Animals	PCE/Total erythrocytes (Mean ± SD)		Micronucleated PCE per 1000 PCEs (Mean ± SD)	
			Male	Female	Male	Female
20 mL/kg Saline (24hr)	0	5M/5F	0.61 ± 0.08	0.60 ± 0.09	1.0 ± 0.71	1.2 ± 0.84
MF59C.1 (24hr)	1250	5M/5F	0.54 ± 0.03	0.55 ± 0.05	1.0 ± 0.71	1.0 ± 0.71
	2500	5M/5F	0.56 ± 0.03	0.62 ± 0.05	1.4 ± 1.14	1.0 ± 1.00
	5000	5M/5F	0.54 ± 0.04	0.55 ± 0.04	1.0 ± 0.71	0.6 ± 0.55
Cyclophosphamide (24hr)	60	5M/5F	0.46 ± 0.07	0.47 ± 0.09	37.2 ± 15.42	32.0 ± 7.97
20 mL/kg Saline (48hr)	0	5M/5F	0.53 ± 0.05	0.53 ± 0.07	1.0 ± 0.71	0.6 ± 0.55
MF59C.1 (48hr)	1250	5M/5F	0.55 ± 0.04	0.54 ± 0.03	0.4 ± 0.55	1.0 ± 0.71
	2500	5M/5F	0.54 ± 0.05	0.55 ± 0.04	0.8 ± 0.84	1.0 ± 0.71
	5000	5M/5F	0.51 ± 0.06	0.50 ± 0.02	0.6 ± 0.55	0.4 ± 0.55
20 mL/kg Saline (72hr)	0	5M/5F	0.51 ± 0.08	0.55 ± 0.06	0.8 ± 0.84	0.2 ± 0.45
MF59C.1 (72hr)	1250	5M/5F	0.51 ± 0.07	0.55 ± 0.08	1.4 ± 0.55	0.8 ± 0.84
	2500	5M/5F	0.55 ± 0.08	0.52 ± 0.05	0.4 ± 0.55	0.8 ± 0.45
	5000	5M/5F	0.51 ± 0.10	0.56 ± 0.09	0.4 ± 0.55	0.2 ± 0.45

2.6.7.10 Carcinogenicity

Not applicable.

2.6.7.11 Reproductive and Developmental Toxicity – Nonpivotal

Developmental Toxicity in Rabbits

Test Article: MF59

Species/ Strain	Method of Administration (Vehicle/Formulation)	Dosing Period	Doses (0.5 mL)	No. per Group)	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59 concentrations equivalent to 0.25× and 0.5× the human dose were tested. MF59 was diluted with saline and water.	Day 6 – 28 of presumed gestation Caesarean sections performed day 29	Saline, 0.25× MF59 0.5× MF59	5 females	There were no deaths. One animal (MF59, 0.5×) prematurely delivered on day 29 of gestation. Body weights and food consumption were unaffected. There were no test article-related necropsy observations. No Caesarean-sectioning or litter parameters were affected. Litter averages for corpora lutea, implantations, litter sizes, resorptions, percent male fetuses, and percent resorbed conceptuses were comparable among groups. There were no dead fetuses, and no litter consisted of only resorbed conceptuses. One late resorption occurred in a litter from a dam treated with MF59 (0.25×). There were no macroscopic external fetal alterations in this study. This study was performed to select doses for a definitive study. The definitive study did not have an MF59-alone group, therefore the data is not presented here, however the same dosing schedule with 0.5× and 1.0× MF59 combined with antigens had no effect on litter parameters and was not teratogenic in rabbits.	1303-001P GLP

2.6.7.12 Reproductive and Developmental Toxicity – Fertility and Early Embryonic Development

Not applicable.

2.6.7.13 Reproductive and Developmental Toxicity – Effects on Embryofetal Development

Not applicable.

2.6.7.14 Reproductive and Developmental Toxicity – Effects on Pre- and Post-natal Development

Developmental Toxicity in Rats

Report Title: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of a Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to Crl:CD BR VAF/Plus Female Rats

Test Article: MF59

Design See below

Species/Strain: Rat / Crl:CD BR VAF/Plus

Initial Age: Approximately 66 days

Date of First Dose: [redacted]19[redacted]

Special Features: None

No Observed Adverse Effect Level:

F_0Females: 0.5 mL MF59

F_1Litters: 0.5 mL MF59

Duration of Dosing: Days –21, 0, 6, 8 & 10 of PG[a] for C-section group, Days –21, 0, 6, 8, 10 & 20 of PG for natural delivery group

Study No.: 1303-002

Day of Mating: Day 0

Location: 4.2.3

Method of Administration: Intramuscular

GLP Compliance: Yes

Vehicle/Formulation: MF59 – water formulation. Saline – 0.9%

Litters Culled/Not Culled: Culled to 5/sex/litter where possible (naturally delivered only)

20 dams per group underwent Caesarean sections on Day 20 of presumed gestation; the litters were evaluated for gross, skeletal, or soft tissue alterations. The remaining dams were allowed to deliver naturally; weight, sex and nursing behavior of the litters were recorded. Pups were euthanized on Day 21 postpartum and examined

Note: Adjuvanted vaccine was also evaluated in this study, however for clarity only the MF59 and saline control data are presented. Saline was administered as two 0.5 mL injections to separate sites. MF59 (0.5 mL) is equivalent to 2 × the human dose.

Study No. 1303-002		
Dose	**1.0mL Saline (2 × 0.5 mL injections)**	**0.5 mL MF59**
F_0 Females	45	45
No. Pregnant	30	33
No. Died or Sacrificed Moribund	1 (day 6 L[b])	2 (day 4 PG and day 11 L)
No. Aborted or with Total Res. of Litter	0	0
Clinical Observations		

[a] PG = Presumed Gestation
[b] L = Lactation

Study No. 1303-002		
Dose	**1.0mL Saline (2 × 0.5 mL injections)**	**0.5 mL MF59**
Swollen Hindlimb (associated with IM injection)	0	40**
Localized Alopecia (limb)	0	4**
Necropsy Observations	—	—
Gestation Body Weight (%[a])	131.8 ± 21.5	2
Lactation Body Weight (%[a])	315.1 ± 24.5	4
Gestation Food Consumption (%[a])		
Days 1-20	22.4 ± 1.6	0
Days 8-10	22.5 ± 2.0	-6
Lactation Food Consumption (%[a])	41.1 ± 6.8	7
F_0 Females – Caesarean Section Day 20 PG		
Number evaluated:	20	20
Mean No. Corpora Lutea ± SD	16.8 ± 2.7	16.8 ± 3.2
Mean No. Implantations ± SD	14.5 ± 2.2	13.9 ± 3.5
Dams with any Resorptions N (%)	12 (60)	8 (40)
All Conceptuses Dead or Resorbed N (%)	0	0
Dams with Viable Fetuses N (%)	20 (100)	20 (100)
Litters (Caesarean Section Day 20 PG dams):		
No. Litters Evaluated	20	20
No. Live Fetuses	271	262

** $P \leq 0.01$ * $P \leq 0.5$
— No significant findings ** $P \leq 0.01$ * $P \leq 0.5$
[a] For controls, group means (grams ± SD) are shown. For treated groups, percent differences from controls are shown.

Study No. 1303-002		
Dose	**1.0mL Saline (2 × 0.5 mL injections)**	**0.5 mL MF59**
Litter Sizes **Mean Live Fetuses ± SD (N)**	13.6 ± 2.4	13.1 ± 3.4
Mean Dead Fetuses ± SD (N)	0	0
Resorptions **Mean Early Resorptions ± SD (N)**	0.9 ± 1.0 (18)	0.8 ± 1.2 (16)
Mean Late Resorptions ± SD (N)	0.0 ± 0.2 (1)	0.0 ± 0.0 (0)
Mean Fetal Body Weight (g/litter)	3.35 ± 0.22	3.45 ± 0.27
% Live Male Fetuses per Litter	49.3 ± 17.2	53.6 ± 15.9
Fetal Anomalies (any alteration) N (%)	9 (3.3)	20 (7.6)
Sternebrae: Incomplete Ossification		
Litter Incidence N (%)	0	5 (25.0)**
Fetal Incidence N (%)	0	5 (3.7)**
Pelvis: Incomplete Ossification of Pubes		
Litter Incidence N (%)	1 (5.0)	7 (35.0)**
Fetal Incidence N (%)	1 (0.7)	12 (8.8)**
Pelvis: Incomplete Ossification of Ischia		
Litter Incidence N (%)	0	3 (15.0)**
Fetal Incidence N (%)	0	4 (2.9)**
F_0 Females – Natural Delivery		
Number evaluated:	10	13
Mean Duration of Gestation (days)	22.7 ± 0.7	22.8 ± 0.4
F_1 Litters:		
No. Litters Evaluated	10	13
Mean No. of Implantation Sites per Litter	15.8 ± 1.8	15.2 ± 1.5
Mean No. Pups/Litter	13.4 ± 3.9	13.6 ± 2.6

— No significant findings ** $P \leq 0.01$ * $P \leq 0.5$

Study No. 1303-002		
Dose	**1.0mL Saline (2 × 0.5 mL injections)**	**0.5 mL MF59**
No. Liveborn Pups/total no. pups	129/134	172/177
No. of Litters with Stillborn Pups N (%)	0	1 (7.7)
Postnatal Mortality to Day 4[a]	2	4
Postnatal Mortality to Day 21[a]	2	5
No. of Total Litter Losses	0	0
Pup Sex Ratios – Male pups on Day 1	50.6 ± 15.0	49.9 ± 21.0
Pup Weight / Litter (grams)		
Day 1	6.0 ± 0.6	6.1 ± 1.1
Day 21	39.4 ± 5.0	42.4 ± 5.5
Pup Clinical Signs	—	—
Pup Necropsy Observations	—	—

[a] Pups were culled or euthanized for blood collection (for antibody analysis) between birth and weaning. Only unscheduled deaths are included here.
— No significant findings [**] $P \leq 0.01$ [*] $P \leq 0.5$

2.6.7.15 Studies in Juvenile Animals

Not applicable.

2.6.7.16 Local Tolerance

Test Article: MF59

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Rabbit / New Zealand White	Intramuscular MF59W	Dosed on days 1 & 8 3/sex necropsied on day 15/16, 2/sex on day 16/17	0.5 (0.25 per leg)	5/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, organ weights, clinical chemistry or hematology parameters. Injection site reactions were limited to slight erythema or scabbing, consistent with the physical introduction of a needle.	89-6280 GLP
Rabbit / New Zealand White	Intramuscular 1:1 vehicle:MF59W Vehicle consisted of saline/buffer 50µg thiomersal per 0.5 mL MF59W	Dosed on days 1, 15 & 29 All animals necropsied on day 36	0.5 (0.25 per leg)	5/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, or hematology parameters. Injection site observations included dermal bruising and reddening of the quadriceps muscle. These minimal reactions were seen across all groups and are consistent with intramuscular dosing. Microscopically, minimal to moderate focal inflammation and muscle fiber degeneration were seen.	90-6230 GLP
Dog / Beagle	Intramuscular MF59W	Dosed on days 1 & 8 1/sex necropsied on days 15 & 19/20	0.5	2/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, organ weights, hematology or serum chemistry parameters. Occasional macroscopic injection site reactions were limited to small (1-2 mm) areas of redness or scabbing consistent with the physical introduction of a needle. Microscopically, inflammation was present at injection sites.	89-6281 GLP

Species/ Strain	Method of Administration (Formulation)	Duration of Dosing	Doses (mL)	Gender and No. per Group	Noteworthy Findings	Study No.
Dog / Beagle	Intramuscular MF59W	Dosed on days 1, 16 & 29 All animals necropsied on day 36	0.5	2/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, cardiography, ophthalmoscopy, organ weights, hematology or serum chemistry parameters. Some dogs displayed a pain reaction after the first injection, which subsided quickly (within a few seconds) and did not recur. Microscopic evaluation of injection sites showed a generally minimal inflammatory response.	89-6193 GLP
Dog / Beagle	Intramuscular 1:1 MF59W:vehicle 50µg thiomersal per 0.5 mL MF59W	Dosed on days 1, 15 & 29 All animals necropsied on day 36	0.5	2/sex	No mortality. No treatment-related effects on clinical observations, body weight, food consumption, body temperature, ophthalmoscopy, cardiography, urinalysis, organ weights, hematology or serum chemistry parameters. Neurological parameters were unaffected. Macroscopic injection site reactions were limited to acute areas of redness surrounding the entry point.	90-6231 GLP

2.6.7.17 Other Toxicity Studies

2.6.7.17.1 Antigenicity (Dermal Sensitization)

Species/ Strain	Method of Administration	Duration of Dosing	Doses	Gender and No. per Group	Noteworthy Findings	Study No.
Guinea Pig / Dunkin-Hartley	Induction phase: Intradermal and topical Challenge phase: Topical	Day 1 Intradermal induction Day 7 Topical induction Day 21 Topical challenge	Dose ranging: 100, 75, 50, 25, 10, 5, 2 and 1% solutions of MF59. Main study: Intradermal induction phase – duplicate 0.1 mL injections: Test group 1 (MF59C.1) 50% aqueous FCA, 1:1 (v/v) 2% MF59C.1:0.9% saline, 1:1 (v/v) 2% MF59C.1:50% aqueous FCA Test group 2 (MF59W.1) 50% aqueous FCA, 1:1 (v/v) 2% MF59W.1:0.9% saline, 1:1 (v/v) 2% MF59W.1:50% aqueous FCA Control group 50% aqueous FCA, 0.9% saline, 1:1 (v/v) 0.9% saline:50% aqueous FCA Topical induction phase: 0.5 mL administrations of saline, undiluted MF59W.1 and MF59C.1 Challenge phase – 05mL saline, undiluted MF59W.1 and MF59C.1	Dose ranging: 4 females / group Main study: 10 females (saline) 20 females (MF59W.1) 20 females (MF59C.1)	**Induction phase** Intradermal: MF59C.1– slight reactions noted in all animals. MF59W.1 – slight reactions noted in all animals. Control – no reactions. Topical: MF59C.1 – slight reactions in 4/20 animals. MF59W.1 – slight reactions in 10/20 animals. Control – slight reactions in 2/20 animals. **Challenge phase** MF59C.1 – 3/20 animals had a positive reaction at 24 hrs, in one animal the reaction was still present at 48 hrs. MF59W.1 – no reaction in any animal. Control – no reactions. In this study, MF59 citrate and water formulations were not considered to be sensitizers in Guinea pigs.	Project No. 564278 Report No. 14465 GLP

FCA = Freund's Complete Adjuvant

2.6.7.17.2 Immunotoxicity

Not applicable.

2.6.7.17.3 Mechanistic

Not applicable.

2.6.7.17.4 Dependence

Not applicable.

2.6.7.17.5 Metabolites

Not applicable.

2.6.7.17.6 Impurities

Not applicable.

2.6.7.17.7 Other (Other) Studies

Supportive Studies – MF59 with various antigens (no MF59-alone group)

Study Title	Study Number
Repeat Dose Toxicity	
Potential of Intratracheally-Administered MF59 or LTK63 to Induce Hypersensitivity Pneumonitis or Lipoid Pneumonia in the Lungs of Sprague-Dawley Rats	L08682
4-Week Vaccine Toxicity Study With Fluad® + IC31® Vaccine by 2 Intramuscular Injections in New Zealand White Rabbits Including a 2-Week Recovery Period	486688 (report in preparation)
2-Dose Intramuscular Injection Toxicity Study with MF59-Adjuvanted Influenza Vaccine with and without CpG 7909 in Rabbits	6560-106
4-week toxicity study with Fluad, Fluad High B, and Fluad High H3+IC31 influenza vaccine formulations by three intramuscular injections in New Zealand White rabbits followed by a 2-week recovery period	488182 (report in preparation)
A 10 Week Intramuscular Vaccine Safety Study in New Zealand White Rabbits With HCV E1E2 Antigen, CpG and MF59 Adjuvant	500757
Immunogenicity Study of a Vaccine (Antigen and Adjuvant Components) in Rabbits	1303-003
Intramuscular Toxicity Study of HCV (E2+Core) Vaccine in Rabbits	3-K84
Intramuscular Toxicity Study in Rabbits with HCV E2 Vaccine	N002833A
Rabbit / Intranasal Adjuvant-HA Toxicology	93-05-025R
Biocine HIV Vaccine GCP 52 121 gp120 Antigen Combined with Biocine MF59 Emulsion Containing MTP-PE Intramuscular Tolerability Study in Rabbits	91-6009
Biocine HSV Vaccine CGP 52 120 HSVgD2/gB2 Antigen Combined with BIOCINE MF59 Emulsion Containing MTP-PE Intramuscular Tolerability Study in Rabbits	90-6306
Subchronic Intramuscular Toxicity Study of BIOCINE HIV Thai E gp120/SF2 gp120 Vaccine in Rabbits	759-001
Administration of Woodchuck Hepatitis Virus Surface Antigen (WHsAg) to Woodchucks with and without Chronic Woodchuck Hepatitis Virus (WHV) Infection.	98-07-263

Study Title	**Study Number**
HCV E2 and E2/Core Vaccines Adjuvanted with MF59-0 and Iscomatrix in Baboons.	ACR 381-PC-0
Safety and Efficacy of Chiron HBV PreS2+S/MF59C.1 Vaccine in an Adult Chimpanzee with Chronic Hepatitis B Infection	420-PT-0
Reproductive Toxicity	
Intramuscular Reproductive and Developmental Toxicity Study of Fluad H5N1 Vaccine in Rabbits, Including a Postnatal Evaluation	UBA00021
Intramuscular Dosage-Range Developmental Toxicity Study of Biocine Vaccine (HSV gD2 and gB2dTM Antigens) in Rabbits	1303-004P
Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Vaccine (Antigen and Adjuvant Components) Administered Intramuscularly to New Zealand White Rabbits	1303-001
Other Toxicity	
Biocine FLU/MF59C.1 Magnusson-Kligman Maximization Test in Guinea Pigs	564110

2.7 臨床概要

承認申請時点までに得られている臨床試験成績ついては，CTD 2.5 臨床に関する概括評価を参照のこと。